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(54) Title: T CELL RECEPTORS FOR IMMUNOTHERAPY

(57) Abstract: Provided are T cell receptors (TCR) and TCR variable regions that can selectively bind the T-cell leukemia/lymphoma 1 (TCL1) oncoprotein. The TCR may be utilized in various therapies, such as autologous TCL1-TCR adoptive T cell therapy, to treat a cancer, such as a B-cell malignancy or a solid tumor expressing TCL1. Methods for expanding a population of T cells that target TCL1 are also provided.



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DESCRIPTION

T CELL RECEPTORS FOR IMMUNOTHERAPY

BACKGROUND OF THE INVENTION

[0001] This application claims the benefit of United States Provisional Patent Application No. 62/615,342, filed January 9, 2018, the entirety of which is incorporated herein by reference.

1. Field of the Invention

[0002] The present invention relates generally to the field of immunology and medicine. More particularly, it concerns T cell receptors (TCR). In some embodiments the TCR may be used to treat a cancer.

2. Description of Related Art

[0003] Although T cell-based therapies have shown significant promise for treating a variety of cancers, relapse after administration of an immunotherapy or chemotherapeutic remains a significant clinical problem. For example, while aggressive B-cell non-Hodgkin lymphomas (NHL) and chronic lymphocytic leukemia (CLL) are often responsive to combinations of chemotherapy and anti-CD20 monoclonal antibodies (Plosker and Figgitt, 2003), relapse remains a serious clinical problem. About two-thirds of patients with aggressive NHL can be cured by chemoimmunotherapy, but a third of patients experience recurrent relapses and eventually die of their disease (Chao MP, 2013). Recent studies with chimeric antigen receptor (CAR)-modified T-cell therapy targeting CD19 demonstrate that CAR T-cell therapy induces high response rates in the majority of patients with refractory B-cell malignancies (Porter *et al.*, 2011; Kochenderfer *et al.*, 2015; Turtle *et al.*, 2016a; Neelapu *et al.*, 2017; Schuster *et al.*, 2015; Turtle *et al.*, 2016b; Locke *et al.*, 2017). A subset of these patients experience long-term remissions suggesting adoptive T-cell therapy could be an effective therapeutic strategy and possibly curative for some of these patients. However, >50% of patients relapse or progress after CD19 CAR T-cell therapy, and a major cause of failure appears to be due to loss of CD19 expression on the tumor (Sotillo *et al.*, 2015; Topp *et al.*, 2014; Neelapu *et al.*, 2017). Therefore, novel targets for adoptive T-cell therapeutic approaches are needed to further improve clinical outcome in these patients.

[0004] TCL1 is an oncoprotein with aberrant expression in > 90% of common B-cell malignancies. For example, TCL1 is hyperexpressed in multiple human B-cell lymphomas,

including follicular lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, diffuse large B-cell lymphoma and splenic marginal zone B-cell lymphoma. In addition, it is aberrantly expressed in a variety of solid tumors. In normal tissues, its expression is restricted to normal B cells. Although data suggests that TCL1 may be an important target for these
5 cancers (Weng *et al.*, 2012), specific T-cell receptors (TCR) that selectively bind this target are still needed for use in T-cell immunotherapies.

SUMMARY OF THE INVENTION

[0005] In some aspects, the present invention overcomes limitations in the art by providing new T-cell receptors (TCR) that selectively target the T-cell leukemia/lymphoma 1 (TCL1) oncoprotein encoded by the TCL1 gene. In various embodiments, these TCRs that
5 target TCL1 may be included or used in an immunotherapy to treat a cancer such as, *e.g.*, a B-cell malignancy or a solid tumor expressing TCL1. In a preferred embodiment, the TCR has antigenic specificity for a TCL1 peptide comprising, consisting of, or consisting essentially of SLLPIMWQLY (SEQ ID NO:29). As used herein, the phrase “having antigenic specificity” means that the TCR can specifically bind to and immunologically
10 recognize TCL1, such that the binding of the TCR to TCL1 elicits an immune response.

[0006] As shown in the below examples, T-cell receptors (TCR) having antigenic specificity for TCL1 were cloned from CD8⁺ T cells, and immunotherapies using T cells transduced with this TCL1-specific T-cell receptor (TCL1-TCR) were tested. Over ten TCL1-specific T-cell clones were generated from normal donor blood samples. One of the
15 T-cell clones, termed TC1 produced high levels of IFN- γ in response to TCL1-peptide pulsed T2 cells, and was strongly positive by HLA-A2-TCL1 tetramer staining. The TCR from these T cells was cloned by RT-PCR. Sequencing revealed that the TCRs was composed of TCR α (SEQ ID NO:3) and β fragments (SEQ ID NO:4). It was observed that normal donor T cells transduced with TCL1-TCR specifically killed TCL1₇₀₋₇₉ peptide-pulsed T2 cells but
20 not control T2 cells. Furthermore, these TCL1-TCR-transduced T cells specifically lysed HLA-A2⁺ TCL1-expressing lymphoma cell lines, primary lymphoma cells, and cell lines from various solid tumors including colon and liver, but not control HLA-A2⁻ lymphoma tumor cells, indicating that the TCL1 epitope is expressed by cancer cells. Collectively, these data demonstrate that the TCL1-TCR gene provided herein can be used as a specific tool to
25 develop adoptive T-cell therapy strategies against B-cell malignancies as well as solid tumors.

[0007] An aspect of the present invention relates to an engineered T cell receptor (TCR) having antigenic specificity for T-cell leukemia/lymphoma 1 (TCL1) or SEQ ID NO:29, wherein the TCR comprises the amino acid sequences of SEQ ID NO: 54, 55, 56, 57,
30 58, and 59. In some embodiments, the engineered T cell receptor (TCR) comprises: (i) an alpha chain variable region having the amino acid sequence of SEQ ID NO:1 or a sequence having at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:1; and/or

(ii) a beta chain variable region having the amino acid sequence of SEQ ID NO:2 or a sequence having at least 90%, at least 95% or at least 98% sequence identity to SEQ ID NO:2. The engineered TCR may bind HLA-A2. The engineered TCR may bind HLA-A*0201. In some embodiments, the TCR comprises an alpha chain variable region having at least 95% or at least 98% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain variable region having at least 95% or at least 98% identity to the amino acid sequence of SEQ ID NO:2. In some embodiments, the TCR comprises an alpha chain variable region having at least 99% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:2. In some embodiments, the TCR comprises an alpha chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain having at least 99% identity to the amino acid sequence of SEQ ID NO:2. In some embodiments, the TCR comprises an alpha chain variable region of SEQ ID NO:1 and/or a beta chain of SEQ ID NO:2. The TCR may comprise an alpha chain having at least 95% identity to the sequence of SEQ ID NO:3 and/or a beta chain having at least 95% identity to the sequence of SEQ ID NO:4. In some embodiments, the TCR comprises an alpha chain comprising the nucleotide sequence of SEQ ID NO:3 and/or a beta chain comprising the nucleotide sequence of SEQ ID NO:4. In some embodiments, the TCR is further defined as a soluble TCR, wherein the soluble TCR does not comprise a transmembrane domain. The TCR may further comprise a detectable label. In some embodiments, the TCR is covalently bound to a therapeutic agent such as, *e.g.*, an immunotoxin or a chemotherapeutic agent.

[0008] In some embodiments, the TCR alpha and/or beta chain variable region comprises at least one conservative amino acid substitution compared to SEQ ID NO:1 or SEQ ID NO:2. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic amino acid substituted for another acidic amino acid (*e.g.*, Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (*e.g.*, Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Val, etc.), a basic amino acid substituted for another basic amino acid (Lys, Arg, etc.), an amino acid with a polar side chain substituted for another amino acid with a polar side chain (Asn, Cys, Gln, Ser, Thr, Tyr, etc.), etc. In some aspects, the TCR alpha and/or beta chain variable regions comprise between 1 and 20, between 1 and 10,

between 1 and 5, between 1 and 3, between 2 and 20, between 2 and 10, between 2 and 5, or between 2 and 3 conservative amino acid substitutions compared to SEQ ID NO:1 or SEQ ID NO:2. Alternatively or additionally, the TCR alpha and/or beta chain variable region comprises at least one non-conservative amino acid substitution compared to SEQ ID NO:1 or SEQ ID NO:2. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the TCR. Preferably, the non-conservative amino acid substitution enhances the biological activity of the TCR, such that the biological activity of the TCR is increased as compared to the unmodified TCR. In some aspects, the TCR alpha and/or beta chain variable regions comprise between 1 and 20, between 1 and 10, between 1 and 5, between 1 and 3, between 2 and 20, between 2 and 10, or between 2 and 5 non-conservative amino acid substitutions compared to SEQ ID NO:1 or SEQ ID NO:2.

[0009] In other embodiments, an engineered TCR is provided comprising two polypeptide chains, each of which comprises a variable region comprising a complementarity determining region (CDR) 1, a CDR2, and a CDR3 of a TCR, wherein the first polypeptide chain comprises a CDR1 comprising the amino acid sequence TSGFYG (SEQ ID NO: 54; CDR1 of α chain), a CDR2 comprising the amino acid sequence NGLDGL (SEQ ID NO: 55; CDR2 of α chain) and a CDR3 comprising the amino acid sequence LLGSGAGSYQLT (SEQ ID NO: 56; CDR of α chain), and the second polypeptide chain comprises a CDR1 comprising the amino acid sequence SGHKS (SEQ ID NO: 57; CDR1 of β chain), a CDR2 comprising the amino acid sequence YYEKEE (SEQ ID NO: 58; CDR2 of β chain) and a CDR3 comprising the amino acid sequence ASSFTDGGTYEQY (SEQ ID NO: 59; CDR3 of β chain). In this regard, the engineered TCR preferably comprises the amino acid sequence of SEQ ID NOs: 54, 55, 56, 57, 58, and 59.

[0010] Another aspect of the present invention relates to a multivalent TCR complex comprising a plurality of TCRs provided herein or as described above. The multivalent TCR may comprise 2, 3, 4 or more TCRs associated with one another. The multivalent TCR may be present in a lipid bilayer, in a liposome, or is attached to a nanoparticle. In some embodiments, the TCRs are associated with one another via a linker molecule. For example, the TCRs may be expressed as single protein comprising a linker peptide linking the α chain and the β chain. The linker peptide may advantageously facilitate the expression of a recombinant TCR in a host cell. Upon expression of the construct, including the linker

peptide by a host cell, the linker peptide may be cleaved resulting in separated α and β chains.

[0011] Yet another aspect of the present invention relates to a polypeptide encoding a TCR of the present invention or as described above. Another aspect of the present invention relates to a polynucleotide or an isolated nucleic acid comprising a nucleotide sequence encoding a TCR of the present invention or the polypeptide encoding a TCR as described above or herein.

[0012] Another aspect of the present invention relates to an expression vector comprising a nucleic acid or nucleotide sequence encoding a TCR, or an alpha and/or beta chain of a TCR, of the present invention or as described above. The sequence encoding the TCR may under the control of or operably linked to an expression control sequence (*e.g.*, a promoter and/or enhancer). In some aspects, the expression control sequence is a tissue specific promoter, preferably a T cell-specific promoter such as a CD4, CD8, TCR α , or TCR β promoter. In other aspects, the expression control sequence is a constitutive or inducible promoter such as an elongation factor-1 α (EF-1 α) promoter or a cytomegalovirus (CMV) promoter. In some embodiments, the expression vector is a viral vector (*e.g.*, a retroviral vector or a lentiviral vector). In some embodiments, the vector further encodes a linker domain. The linker domain may be positioned between the alpha chain and beta chain, such that the TCR is expressed as a single protein comprising a linker peptide between the alpha and the beta chain. The linker peptide may advantageously facilitate the expression of a recombinant TCR in a host cell. Upon expression of the construct, including the linker peptide by a host cell, the linker peptide may be cleaved resulting in separated alpha and beta chains. In some embodiments, the linker domain comprises one or more cleavage sites; for example, the cleavage sites may be a furin cleavage site (*e.g.*, RAKR (SEQ ID NO: 30) or ATNFSLLKQAGDVEENPG (SEQ ID NO:31)) and/or a P2A cleavage site. The one or more cleavage sites may be separated by a spacer (*e.g.*, SGSG (SEQ ID NO:32) or GSG).

[0013] Yet another aspect of the present invention relates to a host cell engineered to express a TCR of the present invention or as described above. In some embodiments, the cell is a T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), or induced pluripotent stem (iPS) cell. The host cell may be an immune cell. The host cell may be isolated from an umbilical cord. In some embodiments, the T cell is a CD8⁺ T cell, CD4⁺ T cell, or $\gamma\delta$ T cell. In some embodiments, the T cell is a regulatory T cell (Treg). The cell

may be autologous or allogeneic. In related aspects, a population of cells is provided comprising at least one host cell engineered to express a TCR as herein described. The population of cells may be a heterogenous population comprising the host cell comprising any of the recombinant expression vectors described herein, in addition to at least one other cell (e.g., a host cell) which does not comprise any of the recombinant expression vectors. Alternatively, the population of cells can be a substantially homogenous population, in which the population comprises mainly host cells comprising the recombinant expression vector. The population can also be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector.

[0014] Another aspect of the present invention relates to a method for engineering a host cell to express a TCR as described herein comprising contacting the host (e.g. immune) cell with: (i) a TCR of the present invention or as described above or (ii) an expression vector encoding a TCR α and/or TCR β chain as herein described. In some embodiments, the immune cell is a T cell (preferably a CD4⁺ or CD8⁺ T cell), or a peripheral blood lymphocyte. In some embodiments, the contacting is further defined as transfecting or transducing. Transfecting may comprise electroporating RNA encoding a TCR of the present invention or as described above into the immune cell. The method may further comprise generating viral supernatant from the expression vector prior to transducing the immune cell. The immune cell may be a stimulated lymphocyte. The stimulated lymphocyte may be a human lymphocyte. In some embodiments, the stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2. The method may further comprise sorting the immune cells to isolate TCR engineered T cells, performing T cell cloning by serial dilution, and/or expansion of the T cell clone by the rapid expansion protocol.

[0015] Yet another aspect of the present invention relates to a method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of the present invention or as described above to a subject (e.g., a human patient), wherein the cancer expresses TCL1. In some embodiments, the subject is identified to have an HLA-A*0201 allele. In some embodiments, the TCR-engineered cell is a T cell or peripheral blood lymphocyte. The T cell may be a CD8⁺ T cell, CD4⁺ T cell, or Treg. The cancer may be a leukemia, a lymphoma, or a B-cell malignancy, or a B-cell lymphoma. In

some embodiments, the B-cell malignancy selected from the group consisting of follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), marginal zone lymphoma (MZL), Burkitt lymphoma, hairy cell leukemia, Waldenström macroglobulinemia, lymphoblastic lymphoma, and primary cutaneous B cell lymphoma. The cancer may be a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis. The cancer may be a solid tumor. The subject may be a human. The TCR engineered cells may be autologous or allogeneic. The method may further comprise lymphodepletion of the subject prior to administration of the TCL1-specific T cells. The lymphodepletion may comprise administration of cyclophosphamide and/or fludarabine. The method may further comprise administering a second anticancer therapy (*e.g.*, a chemotherapy, immunotherapy, surgery, radiotherapy, or biological therapy) to the subject. The second therapy may be an anti-CD19 immunotherapy. The cancer may be a B-cell malignancy or a lymphoma. In some embodiments, the cancer is a solid tumor. The TCR-engineered cells, and/or the at least a second therapeutic agent may be administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion. In some embodiments, the subject is determined to have cancer cells which overexpress TCL1.

[0016] Another aspect of the present invention relates to a method of treating a cancer in a mammalian subject comprising administering a therapeutically effective amount of a TCL1-targeting immunotherapy to the subject, wherein the cancer is a solid tumor. The solid tumor may be a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis. The TCL1-targeting immunotherapy may be an anti-TCL1 antibody, or a TCL1-targeting T-cell therapy.

[0017] In certain embodiments, the present disclosure provides an engineered T cell receptor (TCR) comprising an alpha chain variable region having at least 90%, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity to the amino acid sequence of SEQ ID NO: 1 and/or a beta chain variable region having at least 90%, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity to the amino acid sequence of SEQ ID NO: 2. In these embodiments, the alpha and beta chain preferably includes one or more or all of the CDR regions (*e.g.*, at least CDR3) contained in the SEQ ID NO:1 and SEQ ID

NO:2 variable regions. In some aspects, the engineered TCR binds HLA-A2 or HLA-A*0201-TCL1 peptide complexes. In particular aspects, the TCR comprises an alpha chain of SEQ ID NO: 1 and/or a beta chain of SEQ ID NO: 2.

[0018] In certain aspects, the TCR comprises an alpha chain having at least 90%,
5 such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity to the amino acid sequence of SEQ ID NO: 3 and/or a beta chain variable region having at least 90%, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity to the amino acid sequence of SEQ ID NO: 4; wherein the alpha and beta chain would preferably include one or more or all of the CDR regions (*e.g.*, at least CDR3) contained in the SEQ ID NO:1 and
10 SEQ ID NO:2 variable regions. In particular aspects, the TCR may have variation in the sequence of the variable regions of the alpha and/or beta chain while keeping the sequences of the CDR regions constant.

[0019] In some aspects, the TCR comprises an alpha chain or alpha chain variable region encoded by a nucleotide sequence having at least 90%, such as 91%, 92%, 93%, 94%,
15 95%, 96%, 97%, 98%, 99%, or 100%, identity to the nucleotide sequence of SEQ ID NOs: 5 or 7, respectively, and/or a beta chain or beta chain variable region encoded by a nucleotide sequence having at least 90%, such as 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, identity to the nucleotide sequence of SEQ ID NO: 6 or 8, respectively. In specific aspects, the TCR comprises an alpha chain or alpha chain variable region encoded by the
20 nucleotide sequence of SEQ ID NO: 5 or 7, respectively, and/or a beta chain or beta chain variable region encoded by the nucleotide sequence of SEQ ID NOs: 6 or 8, respectively.

[0020] In certain aspects, the TCR is further defined as a soluble TCR, wherein the soluble TCR does not comprise a transmembrane domain.

[0021] In some aspects, the TCR further comprises a detectable label. In certain
25 aspects, the TCR is covalently bound to a therapeutic agent. In specific aspects, the therapeutic agent is an immunotoxin or a chemotherapeutic agent.

[0022] Further provided herein is a multivalent TCR complex comprising a plurality of TCRs of the embodiments. In some aspects, the multivalent TCR comprises 2, 3, 4 or more TCRs associated with one another. In particular aspects, the multivalent TCR is present in a
30 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.

[0023] In another embodiment, there is provided a polypeptide encoding the TCR of the embodiments. Also provided herein is a polynucleotide encoding the polypeptide of the embodiments.

5 [0024] Further embodiments provide an expression vector encoding the TCR of the embodiments. In some aspects, the sequence encoding the TCR is under the control of a promoter. In particular aspects, the expression vector is a viral vector. In one specific aspect, the viral vector is a retroviral vector. In some aspects, the vector further encodes a linker domain. In some aspects, the linker domain is positioned between the alpha chain and beta chain. In certain aspects, the linker domain comprises one or more cleavage sites. In some
10 aspects, the one or more cleavage sites are a Furin cleavage site and/or a P2A cleavage site. In some aspects, the Furin cleavage site is RAKR (SEQ ID NO: 30). In other aspects, the Furin cleavage site is ATNFSLLKQAGDVEENPG (SEQ ID NO: 31). In certain aspects, the one or more cleavage sites are separated by a spacer. In specific aspects, the spacer is SGSG (SEQ ID NO: 32) or GSG.

15 [0025] In another embodiment, there is provided a host cell engineered to express the TCR of the embodiments. 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 $\gamma\delta$ T cell. In
20 particular aspects, the T cell is a regulatory T cell (Treg). In some aspects, the cell is autologous. In particular aspects, the cell is allogeneic.

[0026] A further embodiment provides a method for engineering the host cell of the embodiments comprising contacting said immune cell with the TCR of the embodiments or the expression vector of the embodiments. In some aspects, the immune cell is a T cell or a
25 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 embodiments into the immune cell.

[0027] In additional aspects, the method further comprises generating viral supernatant from the expression vector encoding the TCR of the embodiments prior to
30 transducing the immune cell.

[0028] In some aspects, the immune cell is a stimulated lymphocyte. In certain aspects, the stimulated lymphocyte is a human lymphocyte. In certain aspects, stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2.

5 [0029] In some aspects, the method further comprises sorting the immune cells to isolate TCR engineered T cells. In certain aspects, the method further comprises performing T cell cloning by serial dilution. In some aspects, the method further comprises expansion of the T cell clone by the rapid expansion protocol.

10 [0030] In another embodiment, there is provided a method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of the embodiments to the subject. In some aspects, the subject is identified to have an HLA-A*0201 allele. In some aspects, the subject is a human.

[0031] In certain aspects, the TCR-engineered cell is a T cell or peripheral blood lymphocyte. In specific aspects, the T cell is a CD8⁺ T cell, CD4⁺ T cell, or Treg.

15 [0032] In some aspects, the cancer is a melanoma. In particular aspects, the melanoma is a cutaneous melanoma, a uveal melanoma, a mucosal melanoma, or a metastatic melanoma. In certain aspects, the TCR engineered cells are autologous or allogeneic.

[0033] In additional aspects, the method further comprises lymphodepletion of the subject prior to administration of the TCL1-specific T cells. In some aspects, 20 lymphodepletion comprises administration of cyclophosphamide and/or fludarabine.

[0034] In some aspects, the method further comprises administering a second anticancer therapy. In certain aspects, the therapy is a chemotherapy, immunotherapy, surgery, radiotherapy, or biotherapy. In some aspects, the TCR-engineered cells, and/or the at least a second therapeutic agent are administered intravenously, intraperitoneally, 25 intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion. In certain aspects, the subject is determined to have cancer cells which overexpress TCL1.

[0035] In related aspects, the method comprises co-administering (sequentially, simultaneously or separately) a second anticancer therapy comprising one or more immune

checkpoint inhibitors that bind to and antagonize the activity of an immune checkpoint protein such as, *e.g.*, cytotoxic T-lymphocyte antigen-4 (CTLA4), programmed cell death protein 1 (PD-1) and its ligands PD-L1 and PD-L2, B7-H3, B7-H4, herpesvirus entry mediator (HVEM), T cell membrane protein 3 (TIM3), galectin 9 (GAL9), lymphocyte activation gene 3 (LAG3), V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA), Killer-Cell Immunoglobulin-Like Receptor (KIR), B and T lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT) or a combination thereof. In some preferred embodiments, the immune checkpoint inhibitor is an anti-PD-1, anti-PD-L1, or anti-CTLA4 antibody or antigen-binding fragment thereof or a fusion protein. In some preferred embodiments, the immune checkpoint inhibitor is a monoclonal antibody against CTLA4 such as Ipilimumab (Yervoy®; BMS) or Tremelimumab (AstraZeneca/MedImmune) and/or a monoclonal antibody against PD-1 such as Nivolumab (Opdivo®; Bristol-Myers Squibb; code name BMS-936558), Pembrolizumab (Keytruda®) or Pidilizumab.

[0036] In certain embodiments, the present disclosure provides TCR that selectively bind TCL1. In some embodiments, the alpha and beta portions of a TCR sequence provided herein may be included in a chimeric antigen receptor (CAR) that may be used in an adoptive T cell therapy. In some embodiments, the alpha and beta portions of the TCR may be encoded in a DNA that can be used, *e.g.*, to treat a lymphoma. Alternately, the alpha and beta variable regions of the TCR may be included in a protein, such as a TCR or a solubilized protein, and used in an anti-cancer therapy such as an adoptive immunotherapy. In some preferred embodiments, the TCR, CAR, or soluble peptide selectively binds TCL1 at a particular epitope, such as TCL1₆₅₋₇₉ immunogenic epitopes. It is anticipated that the TCR may result in a reduction in toxicity towards non-cancerous cells and may be particularly useful for the treatment of B-cell malignancies. In some embodiments, the cloned T cell receptors may be included in a chimeric T cell receptor (CAR) and used in an adoptive T cell transfer or immunotherapy.

[0037] In some aspects, the present disclosure provides soluble TCRs that can be used to treat HLA-A2 positive cancer patients directly. The soluble bispecific T cell-engaging molecules can be generated by linking the TCL1-TCR to CD3-specific Fab fragments. The T cell-engaging TCR can bind the tumor cell surface by presenting the respective peptide/MHC complex and the Fab fragments then crosslink TCRs on the surface of antigen-experienced

CD8⁺ T cells, resulting in cellular activation and elimination of the target cell. Thus, this soluble bispecific TCR constructs can be used for treating the cancer patients directly.

[0038] 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 a toxin, so as to direct these therapeutic molecules to the tumor sites for the treatment of cancer patients.

[0039] In certain aspects, the TCL1-specific T cells, optionally in combination with a second therapeutic agent, can be administered intravenously, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, percutaneously, subcutaneously, regionally, or by direct injection or perfusion.

[0040] Yet another aspect of the present invention relates to a pharmaceutical composition comprising the peptide of the present invention or as described above and an excipient. The pharmaceutical preparation may be formulated for parenteral administration, intravenous injection, intramuscular injection, inhalation, or subcutaneous injection. In some embodiments, the peptide is comprised in a liposome, lipid-containing nanoparticle, or in a lipid-based carrier.

[0041] Another aspect of the present invention relates to particular T cell receptor variable regions (*e.g.*, SEQ ID NO: 1 and 2).

[0042] In various aspects an immunotherapy involving a TCL1-TCR as disclosed herein may be used to treat a variety of cancers, including a leukemia, a lymphoma, or a B-cell malignancy, or a B-cell lymphoma. In some embodiments, the cancer is a B-cell malignancy selected from the group consisting of follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), and splenic marginal zone lymphoma (SMZL). As shown in the examples below, TCL1-TCR may be used to treat or specifically target a cancer or solid tumor expressing TCL1, such as a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis.

[0043] Various embodiments of the present invention can be understood by the following numbered sentences:

1. An engineered T cell receptor (TCR) comprising: (i) an alpha chain variable region having the amino acid sequence of SEQ ID NO:1 or a sequence having at least 90% sequence identity to SEQ ID NO:1; and/or (ii) a beta chain variable region having the amino acid sequence of SEQ ID NO:2 or a sequence having at least 90% sequence identity to SEQ ID NO:2.
2. The TCR of sentence 1, wherein the engineered TCR binds HLA-A2.
3. The TCR of sentence 2, wherein the engineered TCR binds HLA-A*0201.
4. The TCR of sentence 4, wherein the TCR comprises an alpha chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:2.
5. The TCR of sentence 4, wherein the TCR comprises an alpha chain variable region having at least 99% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:2.
6. The TCR of sentence 4, wherein the TCR comprises an alpha chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain having at least 99% identity to the amino acid sequence of SEQ ID NO:2.
7. The TCR of sentence 1, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:1 and/or a beta chain of SEQ ID NO:2.
8. The TCR of sentence 4, wherein the TCR comprises an alpha chain having at least 95% identity to the sequence of SEQ ID NO:3 and/or a beta chain having at least 95% identity to the sequence of SEQ ID NO:4.
9. The TCR of sentence 4, wherein the TCR comprises an alpha chain comprising the sequence of SEQ ID NO:3 and/or a beta chain comprising the sequence of SEQ ID NO:4.
10. The TCR of sentence 1, wherein the TCR is further defined as a soluble TCR, wherein the soluble TCR does not comprise a transmembrane domain.
11. The TCR of any one of sentences 1-10, further comprising a detectable label.
12. The TCR of any one of sentence 1-10, wherein the TCR is covalently bound to a therapeutic agent.
13. The TCR of sentence 12, wherein the therapeutic agent is an immunotoxin or a chemotherapeutic agent.
14. A multivalent TCR complex comprising a plurality of TCRs according to any one of sentences 1-10.

15. The complex of sentence 14, wherein the multivalent TCR comprises 2, 3, 4 or more TCRs associated with one another.

16. The complex of sentence 15, wherein the multivalent TCR is present in a lipid bilayer, in a liposome, or is attached to a nanoparticle.

5 17. The complex of sentence 15, wherein the TCRs are associated with one another via a linker molecule.

18. A polypeptide encoding the TCR of any one of sentences 1-17.

19. A polynucleotide encoding the polypeptide of sentence 18.

20. An expression vector encoding the TCR of any one of sentences 1-17.

10 21. The expression vector of sentence 20, wherein the sequence encoding the TCR is under the control of a promoter.

22. The expression vector of sentence 20, wherein the expression vector is a viral vector.

23. The expression vector of sentence 22, wherein the viral vector is a retroviral vector.

15 24. The expression vector of sentence 20, wherein the vector further encodes a linker domain.

25. The expression vector of sentence 24, wherein the linker domain is positioned between the alpha chain and beta chain.

26. The expression vector of sentence 24, wherein the linker domain comprises one or more cleavage sites.

20 27. The expression vector of sentence 26, wherein the one or more cleavage sites are a furin cleavage site and/or a P2A cleavage site.

28. The expression vector of sentence 27, wherein the furin cleavage site is RAKR (SEQ ID NO: 30).

25 29. The expression vector of sentence 27, wherein the Furin cleavage site is ATNFSLLKQAGDVEENPG (SEQ ID NO:31).

30. The expression vector of sentence 24, wherein the one or more cleavage sites are separated by a spacer.

31. The expression vector of sentence 30, wherein the spacer is SGSG (SEQ ID NO:32) or GSG.

30 32. A host cell engineered to express the TCR of any one of sentences 1-10.

33. The host cell of sentence 32, wherein the cell is a T cell, NK cell, invariant NK cell, NKT cell, mesenchymal stem cell (MSC), or induced pluripotent stem (iPS) cell.

34. The host cell of sentence 32, wherein the host cell is an immune cell.

35. The host cell of sentence 32, wherein the host cell is isolated from an umbilical cord.

36. The host cell of sentence 33, wherein the T cell is a CD8⁺ T cell, CD4⁺ T cell, or $\gamma\delta$ T cell.

37. The host cell of sentence 33, wherein the T cell is a regulatory T cell (Treg).

38. The host cell of sentence 32, wherein the cell is autologous.

5 39. The host cell of sentence 32, wherein the cell is allogeneic.

40. A method for engineering the host cell of sentence 32 comprising contacting said immune cell with the TCR of any one of sentences 1-10 or the expression vector of any one of sentences 20-31.

10 41. The method of sentence 40, wherein the immune cell is a T cell, or a peripheral blood lymphocyte.

42. The method of sentence 40, wherein the contacting is further defined as transfecting or transducing.

43. The method of any one of sentences 40-42, wherein transfecting comprises electroporating RNA encoding the TCR of any one of sentences 1-10 into the immune cell.

15 44. The method of any one of sentences 42, further comprising generating viral supernatant from the expression vector of sentence 20 prior to transducing the immune cell.

45. The method of any one of sentences 40-44, wherein the immune cell is a stimulated lymphocyte.

20 46. The method of sentence 45, wherein the stimulated lymphocyte is a human lymphocyte.

47. The method of sentence 45, wherein the stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2.

48. The method of any one of sentences 40-47, further comprising sorting the immune cells to isolate TCR engineered T cells.

25 49. The method of sentence 48, further comprising performing T cell cloning by serial dilution.

50. The method of sentence 49, further comprising expansion of the T cell clone by the rapid expansion protocol.

30 51. A method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of any one of sentences 32-38 to a subject, wherein the cancer expresses TCL1.

52. The method of sentence 51, wherein the subject is identified to have an HLA-A*0201 allele.

53. The method of sentence 51, wherein the TCR-engineered cell is a T cell or peripheral blood lymphocyte.

54. The method of sentence 51, wherein the T cell is a CD8⁺ T cell, CD4⁺ T cell, or Treg.

55. The method of sentence 51, wherein the cancer is a leukemia, a lymphoma, or a B-cell malignancy, or a B-cell lymphoma.

56. The method of sentence 55, wherein the B-cell malignancy selected from the group consisting of follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), and marginal zone lymphoma (MZL).

57. The method of sentence 51, wherein the cancer is a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis.

58. The method of sentence 51, wherein the cancer is a solid tumor.

59. The method of sentence 51, wherein the subject is a human.

60. The method of sentence 51, wherein the TCR engineered cells are autologous or allogeneic.

61. The method of sentence 51, further comprising lymphodepletion of the subject prior to administration of the TCL1-specific T cells.

62. The method of sentence 61, wherein the lymphodepletion comprises administration of cyclophosphamide and/or fludarabine.

63. The method of any one of sentences 51-62, further comprising administering a second anticancer therapy to the subject.

64. The method of sentence 63, wherein the second therapy is a chemotherapy, immunotherapy, surgery, radiotherapy, or biological therapy.

65. The method of sentence 64, wherein the second therapy is an anti-CD19 immunotherapy.

66. The method of sentence 65, wherein the cancer is a B-cell malignancy or a lymphoma.

67. The method of sentence 65, wherein the cancer is a solid tumor.

68. The method of any one of sentences 51-63, 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.

69. The method of any one of sentences 51-68, wherein the subject is determined to have cancer cells which overexpress TCL1.

70. A method of treating a cancer in a mammalian subject comprising administering a therapeutically effective amount of a TCL1-targeting immunotherapy to the subject, wherein
5 the cancer is a solid tumor.

71. The method of sentence 70, wherein the solid tumor is a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis.

72. The method of any one of sentences 70-71, wherein the TCL1-targeting
10 immunotherapy is an anti-TCL1 antibody, or a TCL1-targeting T-cell therapy.

[0044] 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
15 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,
20 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
25 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 CD3 ζ , FcR, CD27, CD28, CD137, DAP10, and/or OX40. In some cases, molecules can be co-expressed with the CAR, including co-stimulatory molecules, reporter genes for imaging (*e.g.*, for positron emission tomography), gene products that conditionally ablate the T cells
30 upon addition of a pro-drug, homing receptors, chemokines, chemokine receptors, cytokines, and cytokine receptors.

[0045] 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.

[0046] HLA-A2 refers to the human leukocyte antigen serotype A2 and is also referred to as HLA-A*02. Several serotypes of the gene products of many HLA-A*02 alleles are well known, including HLA-A*0201, *0202, *0203, *0206, *0207, and *0211 gene products.

[0047] The terms “inhibiting,” “reducing,” or “prevention,” or any variation of these terms, when used in the claims and/or the specification includes any measurable decrease or complete inhibition to achieve a desired result.

[0048] The term “effective,” as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result.

[0049] The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0050] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0051] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0052] 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.”

[0053] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and

“include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

5 [0054] 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

[0055] 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.

[0056] **FIGS. 1A-C:** Characterization of TCL1-specific T cell clone and cloning of the TC1 TCR α and β genes. **FIG. 1A**, IFN- γ ELISA assay of TC1 T clone against TCL1 peptide pulsed T2 cells. **FIG. 1B**, Tetramer staining of TC1 T clone. **FIG. 1C**, Information of TC1 TCR $\alpha\beta$ chain sequence.

[0057] **FIGS 2A-D:** Characterization of TCL1-TCR in Jurkat 76 cells. **FIG. 2A**, Vector design for expression of TCL1-TCR TRB and TRA by lentivirus. **FIG. 2B**, TRBV antibody and tetramer staining of TCL1-TCR-transduced J76 cells by flow assay. **FIG. 2C**, IL-2 ELISA assay of TCL1-TCR-transduced or un-transduced J76 cells. **FIG. 2D**, Avidity assay of TCL1-TCR-transduced J76 cells by IL-2 ELISA.

[0058] **FIGS. 3A-D:** Characterization of TCL1-TCR-transduced primary T cells. **FIG. 3A**, TRBV5-5 and tetramer staining of TCL1-TCR-transduced primary T cells by flow cytometry. **FIG. 3B**, IL-2 and IFN- γ production by TCL1-TCR-transduced primary T cells against TCL1₇₀₋₇₉ peptide pulsed T2 cells. **FIG. 3C**, Cytokine production by TCL1-TCR-transduced CD8 or CD4 T cells against TCL1₇₀₋₇₉ peptide pulsed T2 cells. **FIG. 3D**, Avidity assay of TCL1-TCR-transduced primary T cells against different concentration TCL1₇₀₋₇₉ peptide pulsed T2 cells by IL-2 ELISA.

[0059] **FIGS. 4A-C:** Cytotoxicity assay of TCL1-TCR-transduced primary T cells. **FIG. 4A**, TCL1-TCR-transduced primary T cells against TCL1₇₀₋₇₉ peptide pulsed T2 cells. **FIG. 4B**, Cytotoxicity assay of TCL1-TCR-transduced primary T cells against TCL1-expressing, HLA A2+/A2- lymphoma cell lines. **FIG. 4C**, Cytotoxicity assay of TCL1-TCR-transduced primary T cells against TCL1-expressing, HLA A2+ primary lymphoma cells or normal B cells obtained from the same patients.

[0060] **FIGS. 5A-D:** In vivo assay of TCL1-TCR-transduced T cells against lymphoma cells. **FIG. 5A**, In vivo luciferase assay of Mino cells in Winn assay. **FIG. 5B**, TCL1-TCR-transduced T cells significantly extended the survival of experimental mice in

Winn assay. **FIG. 5C**, In vivo luciferase assay of TCL1-TCR-transduced T cells against Mino cells in day 12 adoptive transfer assay. **FIG. 5D**, TCL1-TCR-transduced T cells significantly extended the survival of experimental mice in adoptive transfer assay.

[0061] FIGS. 6A-C: The expression of TCL1 in embryonic cell line, hematological, and non-hematological tumor cells. The expression of TCL1 protein in embryonic cell line by intracellular staining (**FIG. 6A**) and real time PCR (**FIG. 6B**) are shown. **FIG. 6C**, the expression of TCL1 in various tissues and tumors from TCGA database is shown.

[0062] FIGS. 7A-C: The cytotoxicity of TCL1-TCR-T against solid tumors. The expression of TCL1 in solid tumor cell line HCT-15, SW480, HepG2 by intracellular staining (**FIG. 7A**) and real time PCR (**FIG. 7B**) are shown. **FIG. 7C**, Cytotoxicity assay of TCL1-TCR-transduced primary T cells against HCT-15, SW480, HepG2 solid tumor cell lines.

[0063] FIGS. 8A-C: Safety assay of TCL1-TCR-T. **FIG. 8A**, Alanine and Glycine replacement of TCL1 70-79 epitope. **FIG. 8B**, The IL-2 ELISA assay of TCL1-TCR-transduced J76 against T2 cells pulsed with Alanine or Glycine replaced TCL1 70-79 peptide. **FIG. 8C**, the IL-2 ELISA assay of TCL1-TCR-transduced primary T cells against T2 cell pulsed with Alanine or Glycine replaced TCL1 70-79 peptide.

[0064] FIGS. 9A-B: **FIG. 9A**, The expression of TCL1 in lymphoma, solid tumor cell lines and normal T cells. Intracellular staining was performed to measure the TCL1 expression in lymphoma, solid tumor cells and normal T cells. C33A, Cervix cancer; A498, renal cancer; KATO-III, stomach cancer; TccSUP, bladder cancer; LnCap, prostate cancer; Mel624, skin cancer; SW480, SW620, colon cancer; U87, U373, brain cancer; MDA-231, breast cancer; WM793, skin cancer; HCT-15, colon cancer; MCF-7, breast cancer; Tera, testis cancer; A498, Kidney cancer; OVCAR3, ovary cancer; Panc-1, pancreas cancer; HepG2, liver cancer; OE33, esophagus cancer; H1650, lung cancer; FB41 T, FB50 T, D403 T, KM19 T, normal donor T cells. Daudi, Jeko-1, Mino, Raji, B-cell lymphoma cells. The relative expression was calculated as: Relative expression=TCL1 MFI- Isotype MFI. **FIG. 9B**, Overexpression of TCL1 in lymphoma cells. TCL1 protein level was analyzed by intracellular staining assay in lymphoma cell lines (Daudi, Mino, Jeko-1 and Raji). Normal donor-derived T cells (T1 and T2) were used as negative control. Data showed that TCL1 is overexpressed in lymphoma cells but not normal donors' T cells.

[0065] **FIG. 10:** Avidity assay of TCL1-TCR-transduced J76, J76CD8 α , J76CD8 $\alpha\beta$. TCL1-TCR-transduced J76, J76CD8 α , J76CD8 $\alpha\beta$ cells were incubated with T2 cells pulsed with different concentration of TCL1 70-79 peptide overnight, and the level of IL-2 was measured by ELISA assay.

5 [0066] **FIG. 11:** TCL1-TCR alpha and beta DNA sequences are shown. The variable regions in the alpha and beta chains are underlined. (SEQ ID NO: 5 = encoding the alpha variable region; SEQ ID NO: 6 = encoding the beta variable region; SEQ ID NO: 7 = encoding the entire alpha region of TCR; SEQ ID NO: 8 = encoding the entire beta region of TCR).

10 [0067] **FIG. 12:** TCL1-TCR alpha and beta protein sequences are shown. The variable regions in the alpha and beta chains are underlined. (SEQ ID NO: 1 = alpha variable region; SEQ ID NO: 2 = beta variable region; SEQ ID NO: 3 = entire alpha region of TCR; SEQ ID NO: 4 = entire beta region of TCR).

15 [0068] **FIG. 13:** TCL1-TCR alpha and beta sequences are shown. CDR1, CDR2, CDR3 sequences of TCL1-TCR alpha and beta sequence are underlined.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. ENGINEERED ANTIGEN RECEPTORS

[0069] In various aspects, T cell receptors (TCR) are provided that specifically bind TCL1 or a TCL1₇₀₋₇₉ peptide (SEQ ID NO:29). The antigen binding region of the TCR may be included in a chimeric antigen receptor (CAR) as the extracellular domain comprising an antigen binding region. In some aspects, the TCR is an isolated or purified TCR. 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).

[0070] T-cell leukemia/lymphoma Antigen1 (TCL1) is a B-cell oncoprotein that is overexpressed in multiple forms of B-cell lymphoma. As shown in the below examples, the TCL1-specific TCR gene when transduced into T cells re-directed the T cells to specifically lyse TCL1₇₀₋₇₉ peptide-pulsed but not control peptide-pulsed T2 cells. The TCR-transduced CD8⁺ T cells efficiently lysed HLA-0201⁺, TCL1-expressing human lymphoma cell lines and primary lymphoma cells, but not normal B cells. Adoptive transfer of TCR-transduced T cells into the tumor-engrafted mouse model inhibited the tumor growth and significantly extended the survival of mice. In addition, it was observed that TCL1 is expressed in the human embryonic stem cells and multiple forms of solid tumor cells. The TCR-transduced T cells can successfully lyse the solid tumor cells but not control tumor cells as observed by *in vitro* assays. Collectively, the data demonstrate that the TCL1-specific TCR-based immunotherapies using the TCR provided herein may be used to treat human B-cell malignancies such as lymphomas and other non-hematological tumors that express TCL1.

[0071] The TCL1 oncoprotein exhibits restricted expression in embryonic and normal adult tissues but aberrant expression in >90% of CLL, MCL, follicular lymphoma (FL), DLBCL, and other B-cell lymphomas (Herling *et al.*, 2007; Weng *et al.*, 2012; Aggarwal *et al.*, 2008). It has been shown to promote malignant cell proliferation and survival by co-activating Akt (Teitell, 2005). Moreover, high levels of TCL1 expression were associated with adverse clinical outcome in CLL, MCL, and DLBCL suggesting that TCL1 may be pathogenetically causal in these malignancies (Herling *et al.*, 2007; Aggarwal *et al.*, 2008; Herling *et al.*, 2009; Ramuz *et al.*, 2005). In direct analysis of the consequence of TCL1 overexpression, transgenic (Tg) mice that ectopically express human TCL1 in B cells (*Eμ*-

TCL1^{Tg}) develop a lymphoproliferative disease similar to human CLL as well as other forms of B-cell lymphomas which strongly implicates a tumorigenic role for TCL1 (Bichi *et al.*, 2002; Hoyer *et al.*, 2002). To test whether TCL1 can be a target for B-cell lymphoma immunotherapy, an immunogenic HLA-A2-binding T-cell epitope derived from TCL1 that
5 can induce cytotoxic T cells in both HLA-A2⁺ normal donors and lymphoma patients was identified (Weng *et al.*, 2012). The TCL1₆₅₋₇₉ peptide-specific CTLs specifically lysed the HLA-A2⁺, TCL1-expressing B-cell lymphomas but not normal B cells, indicating the epitope can be used as a valid target for the immunotherapy of B-cell lymphomas.

[0072] In some embodiments, host cells such as, *e.g.*, T cells (*e.g.*, CD4⁺ T cells,
10 CD8⁺ T cells, $\gamma\delta$ 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 chimeric antigen receptors (CARs). For example, the autologous or allogeneic cells (*e.g.*, isolated from an umbilical cord) are modified to express a T cell receptor (TCR) having antigenic
15 specificity for a cancer antigen. In particular embodiments, the antigen receptors have antigenic specificity for TCL1 or a TCL1₇₀₋₇₉ peptide (SLLPIMWQLY; SEQ ID NO:29). In certain embodiments, the engineered TCR has an alpha chain comprising an amino acid sequence having least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO:1 and/or a beta chain comprising an amino acid sequence having at least 90, 91,
20 92, 93, 94, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO:2. In some embodiments, the TCR has an alpha chain with at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO:3 and/or a beta chain with at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sequence identity to SEQ ID NO:4. Suitable methods of modification are known in the art. See, for instance, Sambrook and Ausubel, *supra*. For
25 example, the T cells may be transduced to express a T cell receptor (TCR) having antigenic specificity for a cancer antigen using transduction techniques described in Heemskerk *et al.*, 2008 and Johnson *et al.*, 2009.

[0073] 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
30 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 typically 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.

5 **[0074]** 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 WO2000/14257, WO2013/126726, WO2012/129514, WO2014/031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960,
10 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.*, 2013; Davila *et al.*, 2013; Turtle *et al.*, 2012; Wu *et al.*, 2012. In some aspects, the genetically engineered antigen receptors include a CAR as described in
15 U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO2014/055668 A1.

A. T Cell Receptor (TCR)

[0075] In some embodiments, the genetically engineered antigen receptors include recombinant T cell receptors (TCRs) and/or TCRs cloned from naturally occurring T cells. A
20 "T cell receptor" or "TCR" refers to a molecule that contains a variable α and β chains (also known as TCR α and TCR β , respectively) or a variable γ and δ chains (also known as TCR γ and TCR δ , 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 $\alpha\beta$ form. In certain embodiments, the engineered TCR has an alpha chain variable region of SEQ ID NO:1
25 and/or a beta chain variable region of SEQ ID NO:2. In some embodiments, the TCR has an alpha chain of SEQ ID NO:3 and a beta chain of SEQ ID NO:4, respectively.

[0076] Typically, TCRs that exist in $\alpha\beta$ and $\gamma\delta$ forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found, *e.g.*, on the surface of a cell or in soluble form. Generally, a TCR is found
30 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 $\alpha\beta$ form or $\gamma\delta$ form.

[0077] 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 α 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.

[0078] 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.*, 1990; Chothia *et al.*, 1988; see also Lefranc *et al.*, 2003). In some embodiments, CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the α chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the β 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.

[0079] In some embodiments, the TCR chains contain a constant domain. For example, like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α -chain, β -

chain) can contain two immunoglobulin domains, a variable domain (*e.g.*, V_α or V_β; 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.*,
5 a-chain constant domain or C_α, typically amino acids 117 to 259 based on Kabat, β-chain constant domain or C_β, 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
10 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.

[0080] In some embodiments, the TCR chains can contain a transmembrane domain.

15 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.

20 [0081] 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
25 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
30 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.

[0082] 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 alpha and a TCR beta chain comprise a disulfide bond between the alpha and beta chains, said disulfide bond being one which has no equivalent in native alpha-beta T cell receptors (*i.e.*, a non-naturally occurring disulfide bond). In some embodiments, a TCR for a target antigen (*e.g.*, a cancer antigen) is identified and introduced into the cells. In some embodiments, a nucleic acid encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of a TCR DNA sequences. 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

[0083] In some embodiments, the engineered antigen receptors include chimeric antigen receptors (CARs), including activating or stimulatory CARs, costimulatory CARs (see WO2014/055668), and/or inhibitory CARs (iCARs, see Fedorov *et al.*, 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).

[0084] 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 oligomerization. In some embodiments, the hinge portion of may comprise or consist of an 8-14 amino acid peptide (*e.g.*, a 12 AA peptide), a portion of CD8 α , 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).

[0085] 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.

[0086] 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 CD3 ζ 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 Fc γ RIII and Fc ϵ RI. 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 by reference in their entirety. In some embodiments, an endodomain may comprise the human CD3 ζ intracellular domain.

[0087] The antigen-specific extracellular domain and the intracellular signaling-
 5 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 CD3 ζ domain, or a cysteine mutated human CD3 ζ domain, or a transmembrane domain from a human transmembrane signaling protein such as, *e.g.*, the CD16 and CD8 and
 10 erythropoietin receptor. Examples of transmembrane domains are provided, *e.g.*, in Table 1.

[0088] 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
 15 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
 20 receptor signaling domain may comprise the zeta chain of CD3 alone or in combination with an Fc γ 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, Fc ϵ RI γ , ICOS/CD278, IL-2R β /CD122, IL-2R α /CD132, DAP10, DAP12, and CD40. In some embodiments,
 25 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.

[0089] 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
 30 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, EF1 α 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.

[0090] 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).

[0091] 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. For example, the TCR (*e.g.*, an alpha chain comprising SEQ ID NO: 1 or 3, and a beta chain comprising SEQ ID NO:2 or 4) may be included in a CAR with a hinge region, a transmembrane region, and an endodomain as described in Table 1 below.

Table 1. Regions that may be included in an anti-TCL1 targeting CAR

Hinge/Scaffold
12 AA (peptide) (<i>e.g.</i> , SEQ ID NO:34)
t-20 AA (peptide) (<i>e.g.</i> , SEQ ID NO:35)
CD8 α (<i>e.g.</i> , SEQ ID NO:36)
IgG4 Fc (<i>e.g.</i> , SEQ ID NO:37)
2D3 (<i>e.g.</i> , SEQ ID NO:38)
IgG4 Fc Δ EQ (IgG4Fc N40Q) (<i>e.g.</i> , SEQ ID NO:39)
IgG4 Fc Δ Q (IgG4Fc L18E N40Q) (<i>e.g.</i> SEQ ID NO:40)
t-12AA + t-20AA
mKate (<i>e.g.</i> , SEQ ID NO:41)

phiLov (<i>e.g.</i> , SEQ ID NO:42)
dsRed (<i>e.g.</i> , SEQ ID NO:43)
Venus (<i>e.g.</i> , SEQ ID NO:44)
eGFP (<i>e.g.</i> , SEQ ID NO:45)
CH3 HA (<i>e.g.</i> , SEQ ID NO:46)
mTFP-1 (<i>e.g.</i> , SEQ ID NO:47)
CD8 α + t-20AA
Double t-20 AA
t-20AA + CD8 α
CD8 α + Leucine Zipper Basep1 (<i>e.g.</i> , SEQ ID NO:48)
CD8 α + Leucine Zipper Acid1 (<i>e.g.</i> , SEQ ID NO:49)

Transmembrane domain
CD28 (<i>e.g.</i> , SEQ ID NO:50)
CD137 (4-1BB) (<i>e.g.</i> , SEQ ID NO:51)
CD8 α (<i>e.g.</i> , SEQ ID NO:52)
CD3 ζ (<i>e.g.</i> , SEQ ID NO:53)

Endo-domain (signaling domain)
CD28 + CD3 ζ
CD28 + CD27 + CD3 ζ
CD28 + OX40 + CD3 ζ
CD28 + 4-1BB + CD3 ζ
CD28 + CD27 + OX40 + CD3 ζ
CD28 + 4-1BB + CD27 + CD3 ζ
CD28 + 4-1BB + OX40 + CD3 ζ
4-1BB + CD3 ζ
4-1BB + OX40 + CD3 ζ
4-1BB + CD27 + CD3 ζ
CD27 + CD3 ζ
CD27 + OX 40 + CD3 ζ

CD28Δ +CD3ζ
CD28Δ +CD27 + CD3ζ
CD28Δ + OX40 + CD3ζ
CD28Δ + 4-1BB + CD3ζ
CD28Δ + 4-1BB + OX40 + CD3ζ
CD28Δ + CD27 + OX40 + CD3ζ
CD28Δ + 4-1BB + CD27 + CD3ζ
4-1BB + ICOS + CD3ζ
CD28 + ICOS + CD3ζ
ICOS + CD3ζ
CD3ζ
CD28 only

ζ - zeta; Δ- mutant; Note = 4-1BB is also referred to as CD137; "+" refers to the fusion of the different regions.

II. Soluble TCRs

5 [0092] In some embodiments, the present disclosure provides soluble TCRs, such as a TCL1-TCR provided herein. Soluble TCRs are useful, not only for the purpose of investigating specific TCR-pMHC interactions, but also potentially as a diagnostic tool to detect infection, or to detect autoimmune disease markers. Soluble TCRs also have applications in staining, for example to stain cells for the presence of a particular peptide
10 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.

[0093] In the context of this application, "solubility" is defined as the ability of the
15 TCR to be purified as a mono disperse heterodimer in phosphate buffered saline (PBS) (KCL 2.7 mM, KH₂PO₄ 1.5 mM, NaCl 137 mM and Na₂PO₄ 8 mM, pH 7.1-7.5. Life Technologies, Gibco BRL) at a concentration of 1 mg/ml and for more than 90% of said TCR to remain as a mono disperse heterodimer after incubation at 25° C for 1 hour.

[0094] 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 NO:1 or 3), except the transmembrane domain thereof, and (ii) all or part of a TCR β chain (*e.g.*, SEQ ID NO:2 or 4), 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 disulfide bond between constant domain residues which is not present in native TCR.

[0095] 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 (International Patent Publication No. WO 99/60120; U.S. Patent No. 7,666,604).

[0096] A soluble TCR, which may be human or produced in human cells, 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.

[0097] 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 T cell receptor (TCR) complex, which comprises a plurality of soluble T cell receptors as described herein. Each of the plurality of soluble TCRs is preferably identical.

[0098] A multivalent TCR complex generally 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 T cell receptors 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.

[0099] 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.

[00100] 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.

[00101] A label or another moiety, such as a toxic or therapeutic moiety, may be included in a multivalent TCR complex of the present invention. 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 may be achieved by mixing the TCR and the enzyme at a molar ratio of about 3:1 to generate tetrameric complexes, and isolating the desired complex from any complexes not containing the correct ratio of molecules. These mixed molecules may 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.

[00102] 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 invention may have enhanced binding capability for a TCR ligand compared to a non-multimeric T cell receptor heterodimer. Thus, the multivalent TCR complexes may be used in some embodiments 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*.

[00103] 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 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.

[00104] In some embodiments, the soluble TCR or multivalent TCR complex can be used to deliver therapeutic agents to the location of cells presenting a particular antigen. This can be useful, *e.g.*, for the treatment of tumors. A therapeutic agent could be delivered such that it would exercise its effect locally and not only on the cell it binds (*e.g.*, a chemotherapeutic, radioactive, or enzymatic agent may result in a local effect near or on a tumor). Thus, one particular strategy envisages anti-tumor molecules linked to T cell receptors or multivalent TCR complexes specific for tumor antigens.

[00105] Many therapeutic agents can be employed for this use, for instance radioactive compounds, enzymes (*e.g.*, perforin) or chemotherapeutic agents (*e.g.*, cisplatin). To improve limiting toxic effects in the desired location the toxin may be provided inside a liposome linked to streptavidin so that the compound is released slowly. This may reduce damaging effects during the transport in the body and help to limit toxic effects until after binding of the TCR to the relevant antigen presenting cells.

[00106] Other suitable therapeutic agents include:

- small molecule cytotoxic agents, *i.e.* compounds with the ability to kill mammalian cells having a molecular weight of less than 700 daltons. Such compounds could also contain toxic metals capable of having a cytotoxic effect. Furthermore, it is to be understood that these small molecule cytotoxic agents also include pro-drugs, *i.e.* compounds that decay or are converted under physiological conditions to release cytotoxic agents. Examples of such agents include cis-platin, maytansine derivatives, rachelmycin, calicheamicin, docetaxel, etoposide, gemcitabine, ifosfamide, irinotecan, melphalan, mitoxantrone, sorfimer sodiumphotofrin II, temozolmide, topotecan, trimetreate glucuronate, auristatin E vincristine and doxorubicin;
- peptide cytotoxins, *i.e.* proteins or fragments thereof with the ability to kill mammalian cells. Examples include ricin, diphtheria toxin, pseudomonas bacterial exotoxin A, DNAase and RNAase;

- radio-nuclides, *i.e.* unstable isotopes of elements which decay with the concurrent emission of one or more of α or β particles, or γ rays. Examples include iodine 131, rhenium 186, indium 111, yttrium 90, bismuth 210 and 213, actinium 225 and astatine 213;

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- prodrugs, such as antibody directed enzyme pro-drugs; and

- immuno-stimulants, *i.e.* moieties which stimulate immune response. Examples include cytokines such as IL-2, chemokines such as IL-8, platelet factor 4, melanoma growth stimulatory protein, *etc.*, antibodies or fragments thereof such as anti-CD3 antibodies or fragments thereof, complement

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activators, xenogeneic protein domains, allogeneic protein domains, viral/bacterial protein domains and viral/bacterial peptides.

[00107]

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.

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[00108]

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.

20

[00109]

Also provided is a method of treatment of cancer (*e.g.*, a leukemia, lymphoma, or B-cell malignancy, or other cancer that expresses TCL1 as described herein) 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 invention.

[00110]

As is common in anti-cancer and autoimmune therapy the TCRs of the present disclosure may be used in combination with other agents for the treatment of cancer and autoimmune disease, and one or more additional therapeutic or therapy may be administered to treat other related condition(s) found in the patient groups.

25

III. Adoptive Cell Transfer Therapies

[00111] 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, mesenchymal stem cell (MSC)s, or induced pluripotent stem (iPS) cells) therapy, such as a TCL1-specific cell therapy. Adoptive T cell therapies with genetically engineered TCR-transduced T cells (*e.g.*, expressing a TCR comprising one or more of SEQ ID NOs:1-4, such as SEQ ID NOs: 1 and 3, or SEQ ID NOs: 2 and 4) are also provided herein. In further
5 embodiments, methods are provided for the treatment of cancer (*e.g.*, melanoma) comprising immunizing a subject with a purified tumor antigen or an immunodominant tumor antigen-specific peptide. 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.

[00112] 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 (*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, mesenchymal stem cell (MSC)s, or induced pluripotent stem (iPS) cells) cells are
15 antigen-specific cells (*e.g.*, TCL1-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
20 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
25 which can be used in the methods described herein.

A. T Cell Preparation and Administration

[00113] In some embodiments, T cells are autologous. However, the cells can be allogeneic. In some embodiments, the T cells are isolated from the patient, 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.

[00114] In some embodiments, the T cells are derived from the blood, bone marrow, lymph, umbilical cord, 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); for example, the stem cells or iPSC may be differentiated into various T cell populations. 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.

[00115] 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.

[00116] 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).

5 **[00117]** 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
10 expressed to a relatively higher degree on one or more naive, memory, and/or effector T cell subpopulations. A variety of methods may be used for separation of cells based on expression of markers, including magnetic activated cell sorting (MACS) and fluorescence activated cell sorting (FACS).

[00118] In some embodiments, CD8⁺ T cells are further enriched for or
15 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
20 sub-populations (*e.g.*, see Terakura *et al.*, 2012; Wang *et al.*, 2012).

[00119] 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,
25 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 2×10⁶ lymphocytes), *e.g.*, from about 5 to about 21 days, preferably from about 10 to about 14 days. For example, the cells may be cultured from 5 days, 5.5 days, or 5.8 days to 21 days, 21.5 days, or 21.8 days, such as from 10 days, 10.5 days, or 10.8 days to 14
30 days, 14.5 days, or 14.8 days.

[00120] 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.*,
5 200-, 300-, 400-, 500-, 600-, 700-, 800-, 900-, or greater) over a period of about 10 to about 14 days.

[00121] 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
10 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
15 optionally expressed from a vector, such as an human leukocyte antigen A2 (HLA-A2) binding peptide, in the presence of a T cell growth factor, such as 300 IU/ml IL-2 or IL-15, with IL-2 being preferred. The *in vitro*-induced T cells are rapidly expanded by re-stimulation with the same antigen(s) of the cancer pulsed onto HLA-A2-expressing antigen-presenting cells. Alternatively, the T cells can be re-stimulated with irradiated, autologous
20 lymphocytes or with irradiated HLA-A2+ allogeneic lymphocytes and IL-2, for example.

[00122] 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 including, *e.g.*, Sambrook *et al.*, 2001; and
25 Ausubel *et al.*, 1994. In some embodiments, 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.

[00123] In certain embodiments, a T cell growth factor that promotes the
30 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.

5 **[00124]** The T cell may be administered intravenously, intramuscularly, subcutaneously, transdermally, intraperitoneally, intrathecally, parenterally, intrathecally, intracavitary, intraventricularly, intra-arterially, or via the cerebrospinal fluid, or by any implantable or semi-implantable, permanent or degradable device. The appropriate dosage of the T cell therapy may be determined based on the type of disease to be treated, severity and
10 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.

[00125] 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
15 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 (*e.g.*, 3 ml). Multiple injections delivered as single dose may comprise about 0.1 to about 0.5 ml volumes.

[00126] In some embodiments, naked DNA or a suitable vector encoding a CAR can be introduced into a subject's T cells (*e.g.*, T cells obtained from a human patient
20 with cancer or other disease). Methods of stably transfecting T cells by electroporation using naked DNA are known in the art. See, *e.g.*, U.S. Pat. No. 6,410,319. Naked DNA generally refers to the DNA encoding a chimeric receptor of the present invention contained in a plasmid expression vector in proper orientation for expression. In some embodiments, the use of naked DNA may reduce the time required to produce T cells expressing a CAR
25 generated via methods of the present invention.

[00127] Alternatively, a viral vector (*e.g.*, a retroviral vector, adenoviral vector, adeno-associated viral vector, or lentiviral vector) can be used to introduce the TCR or chimeric construct into T cells. Generally, a vector encoding a TCR or CAR that is used for transfecting a T cell from a subject should generally be non-replicating in the subject's T
30 cells. A large number of vectors are known that are based on viruses, where the copy number of the virus maintained in the cell is low enough to maintain viability of the cell. Illustrative

vectors include the pFB-neo vectors (STRATAGENE®) as well as vectors based on HIV, SV40, EBV, HSV, or BPV.

[00128] Once it is established that the transfected or transduced T cell is capable of expressing a TCR or CAR as a surface membrane protein with the desired regulation and at a desired level, it can be determined whether the TCR or chimeric receptor is functional in the host cell to provide for the desired signal induction. Subsequently, the transduced T cells may be reintroduced or administered to the subject to activate anti-tumor responses in the subject. To facilitate administration, the transduced T cells may be made into a pharmaceutical composition or made into an implant appropriate for administration *in vivo*, with appropriate carriers or diluents, which are preferably pharmaceutically acceptable. The means of making such a composition or an implant have been described in the art (see, for instance, Remington's Pharmaceutical Sciences, 16th Ed., Mack, ed., 1980). Where appropriate, transduced T cells expressing a TCR or CAR can be formulated into a preparation in semisolid or liquid form, such as a capsule, solution, injection, inhalant, or aerosol, in the usual ways for their respective route of administration. Means known in the art can be utilized to prevent or minimize release and absorption of the composition until it reaches the target tissue or organ, or to ensure timed-release of the composition. Generally, a pharmaceutically acceptable form is preferably employed that does not significantly adversely affect the cells expressing the TCR or chimeric receptor. In some embodiments, the transduced T cells can be made into a pharmaceutical composition containing a balanced salt solution such as Hanks' balanced salt solution, or normal saline.

B. Antigen-Presenting Cells

[00129] Antigen-presenting cells, which include macrophages, B lymphocytes, and dendritic cells, are distinguished by their expression of a particular MHC molecule. APCs internalize antigen and re-express a part of that antigen, together with the MHC molecule on their outer cell membrane. The major histocompatibility complex (MHC) is a large genetic complex with multiple loci. The MHC loci encode two major classes of MHC membrane molecules, referred to as class I and class II MHCs. T helper lymphocytes generally recognize antigen associated with MHC class II molecules, and T cytotoxic lymphocytes recognize antigen associated with MHC class I molecules. In humans the MHC is referred to as the HLA complex and in mice the H-2 complex.

[00130] In some cases, artificial antigen presenting cells (aAPCs) are useful in preparing TCR or CAR-based therapeutic compositions and cell therapy products. For general guidance regarding the preparation and use of antigen-presenting systems, see, *e.g.*, U.S. Pat. Nos. 6,225,042, 6,355,479, 6,362,001 and 6,790,662; U.S. Patent Application Publication Nos. 2009/0017000 and 2009/0004142; and International Publication No. WO2007/103009).

[00131] aAPCs may be used to expand T Cells expressing a TCR or CAR. During encounter with tumor antigen, the signals delivered to T cells by antigen-presenting cells can affect T cell programming and their subsequent therapeutic efficacy. This has stimulated efforts to develop artificial antigen-presenting cells that allow optimal control over the signals provided to T cells (Turtle *et al.*, 2010). In addition to antibody or antigen of interest, the aAPC systems may also comprise at least one exogenous assisting molecule. Any suitable number and combination of assisting molecules may be employed. The assisting molecule may be selected from assisting molecules such as co-stimulatory molecules and adhesion molecules. Exemplary co-stimulatory molecules include CD70 and B7.1 (also called B7 or CD80), which can bind to CD28 and/or CTLA-4 molecules on the surface of T cells, thereby affecting, *e.g.*, T cell expansion, Th1 differentiation, short-term T cell survival, and cytokine secretion such as interleukin (IL)-2 (see Kim *et al.*, 2004). Adhesion molecules may include carbohydrate-binding glycoproteins such as selectins, transmembrane binding glycoproteins such as integrins, calcium-dependent proteins such as cadherins, and single-pass transmembrane immunoglobulin (Ig) superfamily proteins, such as intercellular adhesion molecules (ICAMs) that promote, for example, cell-to-cell or cell-to-matrix contact. Exemplary adhesion molecules include LFA-3 and ICAMs, such as ICAM-1. Techniques, methods, and reagents useful for selection, cloning, preparation, and expression of exemplary assisting molecules, including co-stimulatory molecules and adhesion molecules, are exemplified in, *e.g.*, U.S. Pat. Nos. 6,225,042, 6,355,479, and 6,362,001.

C. Nucleic Acids

[00132] In an aspect, the present disclosure provides a nucleic acid encoding an isolated TCR, CAR, or soluble peptide that selectively binds TCL1 (*e.g.*, at the TCL1₇₀₋₇₉ immunogenic epitopes) and has at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a TCR variable region disclosed herein (*e.g.*, SEQ ID NO:1-4), or the peptide may have 1, 2, 3, or 4 point mutations (*e.g.*, substitution

mutations) as compared to SEQ ID NO:1-4. As stated above, peptide may be, *e.g.*, from 8 to 35 amino acids in length, or any range derivable therein. In some embodiments, the tumor antigen-specific peptide corresponds to a portion of the tumor antigen protein such as TCL1₇₀₋₇₉ of TCL1. The term "nucleic acid" is intended to include DNA and RNA and can be either double stranded or single stranded.

[00133] Accordingly, a nucleic acid encoding a TCR, CAR, or soluble peptide that selectively binds TCL1 may be operably linked to an expression vector and the peptide produced in the appropriate expression system using methods well known in the molecular biological arts. A nucleic acid encoding a tumor antigen-specific peptide disclosed herein may be incorporated into any expression vector which ensures good expression of the peptide. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (*e.g.* replication defective retroviruses, adenoviruses and adeno-associated viruses), so long as the vector is suitable for transformation of a host cell.

[00134] A recombinant expression vector being "suitable for transformation of a host cell" means that the expression vector contains a nucleic acid molecule of the invention and regulatory sequences selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid molecule. The terms, "operatively linked" or "operably linked" are used interchangeably, and are intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid.

[00135] Accordingly, the present invention provides a recombinant expression vector comprising nucleic acid encoding a TCR, CAR, or soluble peptide that selectively binds TCL1, and the necessary regulatory sequences for the transcription and translation of the inserted protein-sequence. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, or viral genes (*e.g.*, see the regulatory sequences described in Goeddel, 1990).

[00136] Selection of appropriate regulatory sequences is generally dependent on the host cell chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed,

other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. It will also be appreciated that the necessary regulatory sequences may be supplied by the native protein and/or its flanking regions.

5 **[00137]** A recombinant expression vector may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with the TCR, CAR, or soluble peptide that selectively binds TCL1 disclosed herein. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, β -galactosidase, chloramphenicol acetyltransferase, or
10 firefly luciferase. Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β -galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. If the selectable marker gene encodes a protein conferring antibiotic resistance such as neomycin resistance transformant cells can be selected with G418. Cells that have incorporated the selectable marker gene will survive,
15 while the other cells die. This makes it possible to visualize and assay for expression of a recombinant expression vector, and in particular, to determine the effect of a mutation on expression and phenotype. It will be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

[00138] Recombinant expression vectors can be introduced into host cells to
20 produce a transformant host cell. The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector of the invention. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (*e.g.* a vector) into a cell by one of many possible techniques known in the art. Suitable
25 host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells.

[00139] A nucleic acid molecule of the invention may also be chemically synthesized using standard techniques. Various methods of chemically synthesizing
30 polydeoxy-nucleotides are known, including solid-phase synthesis which, like peptide synthesis, has been fully automated in commercially available DNA synthesizers (See *e.g.*, U.S. Patent Nos. 4,598,049; 4,458,066; 4,401,796; and 4,373,071).

II. PHARMACEUTICAL PREPARATIONS

[00140] 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 invention 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 B-cell malignancy or a solid tumor that expresses TCL1. 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.

[00141] The phrases "pharmaceutical," "pharmaceutically acceptable," 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, for example, a human, as appropriate. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington: The Science and Practice of Pharmacy, 21st edition, Pharmaceutical Press, 2011, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the vaccine compositions of the present invention is contemplated.

[00142] 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.*

[00143] 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.

[00144] A therapeutic composition disclosed herein can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularly, 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 foregoing 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).

[00145] 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 intravenous, intratumoral or subcutaneous injection, the carrier may comprise water, saline, alcohol, a fat, a wax or a buffer. Biodegradable microspheres (*e.g.*, polylactic galactide) may also be employed as carriers in some embodiments. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patents 4,897,268 and 5,075,109.

[00146] 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 (*e.g.*, U.S. Patents 5,797,898, 5,770,219 and 5,783,208, and U.S. Patent Application 2005/0065463). 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.

[00147] 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 are known and may be used in various embodiments (*e.g.*, U.S. Patents 5567434, 5552157, 5565213, 5738868, and 5795587).

[00148] 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.

[00149] 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.

[00150] 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.

A. Combination Therapies

[00151] In certain embodiments, the compositions and methods of the present embodiments involve 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 5 NK cells, NKT cells, mesenchymal stem cell (MSC)s, or induced pluripotent stem (iPS) cells) population in combination with at least one additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, a conditioning chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or 10 a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy.

[00152] 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 15 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 a chemotherapy 20 such as, *e.g.*, dacarbazine, or temozolomide. The additional therapy may be one or more of the chemotherapeutic agents known in the art.

[00153] 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 or conditioning chemotherapy. The administrations may be in intervals ranging from 25 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 30 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.

[00154] Various combinations may be employed. For the example below an antigen-specific T cell therapy, peptide, or TCR is “A” and an anti-cancer therapy is “B”:

5 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

[00155] 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.

III. EXAMPLES

[00156] 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

Materials and Methods

Patient Samples

[00157] The Institutional Review Board of The University of Texas M. D. Anderson Cancer Center approved the study. An informed consent was obtained in accordance with the Declaration of Helsinki prior to collection of patient samples. Peripheral blood mononuclear cells (PBMC) were isolated from blood samples by density gradient separation. Tissue samples were processed into single-cell suspension and cryopreserved in aliquots.

Cell lines

[00158] MCL cell lines, Mino and Jeko-1; Burkitt's lymphoma cell line, Daudi; non-hematological tumor cell line, HCT-15, SW480, SW620, and T2 hybridoma cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 mM HEPES, 5 1× Glutamax, 50 μM β-mercaptoethanol, 1 mM sodium pyruvate, 100 U/mL penicillin + 100 μg/mL streptomycin, and 10 μg/mL gentamicin (all from Invitrogen, Carlsbad, CA) at 37 °C and 5% CO₂ in air.

Tumor and immune cell subset isolation

[00159] Primary lymphoma cells, normal peripheral blood B and T cells were 10 isolated by magnetic cell separation (MACS, Miltenyi Biotec, Auburn, CA) and the purity confirmed by flow cytometry as previously described (Weng *et al.*, 2016a). The procedure yielded >90% purity of lymphoma cells, normal B and T cells.

Reagents

[00160] Mouse anti-human antibodies against CD3, CD4, CD8, CD20, IFN-γ 15 and CD19 were obtained from BD Biosciences, San Jose, CA. Enzyme-linked immunosorbent assay (ELISA) kits for IFN-γ were obtained from R&D Systems, Minneapolis, MN. All peptides were synthesized by Sigma-Aldrich, St Louis, MO to greater than 70% purity and dissolved in dimethyl sulfoxide (Sigma-Aldrich). APC-conjugated tetramers were synthesized by MHC Tetramer Lab, Protein Chemistry Core of Baylor 20 College of Medicine, Houston, TX. APC-conjugated anti-TCL1 and isotype antibodies were obtained from Cell Signaling Technology, Beverly, MA.

Real-time PCR

[00161] Total RNA was extracted from purified solid tumor cell lines, or peripheral blood B and T cells using Trizol (Invitrogen). About 3 μg of total RNA from each 25 source was reverse transcribed into cDNA with Superscript III kit (Invitrogen). Quantitative PCR was performed with Taqman real-time PCR kit (Applied Biosystems), and TCL1 (Applied Biosystems, Cat # Hs00951350_m1) and β-actin primers (Applied Biosystems, Cat# Hs99999903_m1) using the following conditions: 50°C for 2 min, 94°C for 10 min, followed by 94°C for 15 sec, 60°C for 60 sec for 40 cycles on Applied Biosystems

StepOne™/ StepOnePlus™ Real-Time PCR System. The expression of TCL1 mRNA relative to the β -actin mRNA was calculated in each sample.

Flow cytometry

- [00162] For intracellular staining, cells were fixed and permeabilized using BD Biosciences Cytofix/Cytoperm™ Plus Fixation/Permeabilization kit as per manufacturer's instructions. Cells were then stained with mouse anti-human-TCL1 antibody (eBioscience) or Alexa Fluor® 647 Mouse IgG2b isotype control antibody (eBiosciences) for 30 min at 4°C. After two washes, samples were acquired on a FACS Calibur (BD Biosciences) and analyzed using Cell Quest Pro (BD Biosciences) or FlowJo (Tree Star, Inc., Ashland, OR) software.
- 10 Intracellular cytokine staining was performed as previously described (Weng *et al.*, 2016b). For tetramer staining, APC-conjugated TCL1₇₁₋₇₈ tetramer or HIV Gag₇₇₋₈₅ tetramer and FITC-conjugated mouse anti-human CD8 antibody were mixed with the cells in 50 μ l volume for 30 minutes at room temperature, washed twice, and analyzed by a flow cytometry.

Cloning by Limiting dilution

- 15 [00163] TCL1-specific T cell lines were cultured at one cell/well in 96-well round bottom plate, supplied with 30ng/ml anti-CD3, 180IU/ml IL-2 and 1×10^5 feeder cells. The T clones were grown for 2 weeks and analyzed for antigen-specificity by IFN- γ ELISA. T cell clones that were tested positive were further expanded by rapid expansion protocol (REP).

20 Generation of TCL1-specific TCR-T cells by lentivirus

- [00164] Total RNA purified from T cell clone was analyzed by nano-string for TCR α and β subfamilies. The full TCR $\alpha\beta$ sequences of TC1 T cell clone was obtained by RT-PCR and codon-optimized. The constant regions of α and β chains were cystein-mutated and the TCR $\alpha\beta$ chains were ligated with P2A and cloned into a lentivirus producing vector.
- 25 TCR-containing lentivirus were produced in 293T cells, filtered, concentrated and stored at -80°C. HLA A2+ normal donors' T cells were activated by OKT3 antibody for 72 hours and the transduction with lentivirus were carried out at 1000g centrifuge at 32°C for 2 hour followed by overnight incubation. The expression of antigen specific TCR was analyzed by TCR Vb specific antibody and tetramer 48 hour later. The tetramer positive T cells were

sorted by flow and further expanded by REP for additional functional assays as previously described (Lee *et al.*, 2008).

Cytotoxicity assay

[00165] T2 cells were cultured overnight with 40 µg/ml peptide in the presence of 3 µg/ml β₂-microglobulin, washed twice, and used as targets in non-radioactive cytotoxicity assay (Promega). Primary tumor cells were isolated from PBMC or biopsy samples of lymphoma patients by MACS (Miltenyi). Primary lymphoma cells, lymphoma cell lines and non-hematological tumor cell line (1×10⁴ cells/well) were incubated with the effector T cells at the indicated ratios in 96-well round-bottom plates at 37°C for 4-16 hours, and target cell lysis was determined by non-radioactive cytotoxicity assay or by flow cytometry (Weng *et al.*, 2016a). All assays were performed in triplicate wells and repeated at least two times.

In vivo assay of TCL1-specific TCR-T cells against xenograft tumors

[00166] For the Winn assay, 0.5 × 10⁶ Mino-Luc cells and 5 × 10⁶ TCL1-specific TCR gene-transduced or non-gene-modified T cells were suspended in 300 µL PBS and injected intravenously into the NSG mice (Non-Obese Diabetic/Severe Combined Immuno-Deficiency/IL-2 receptor γ-chain allelic mutation; NOD/Shi-scid/IL-2R γnull) aged 6-7 weeks (Central Institute for Experimental Animals). The growth of tumor was measured every 7 days until the mice died or were euthanized because of tumor progression. For adoptive transfer experiments, NSG mice aged 7 weeks were similarly inoculated with 0.5 × 10⁶ of Mino-luc cells on day 0. Twelve days later, 10 × 10⁶ TCL1-specific TCR gene-transduced or non-gene-modified T cells were intravenously administrated. The growth of tumor was measured as in Winn assay.

Statistical Analysis

[00167] The Student t test was used to compare various experimental groups. P values <0.05 were considered statistically significant. Unless otherwise indicated, mean and standard deviations are shown.

Example 2

TCL1-Specific T-cell Receptor redirects T Cells Against B Cell Lymphoma and Non-Hematological Tumor Cells

[00168] To further explore the potential of TCL1 T-cell epitope as a therapeutic target for clinical immunotherapy, the above TCR was transduced into the HLA A2+ normal donors' PBMCs by lentivirus. It was observed that the TCR-transduced T cells specifically recognized TCL1 peptide-pulsed T2 cells at high avidity (1-10 nM) and lyse the HLA-A2+, TCL1-expressing B-cell lymphoma cell lines and primary lymphoma cells, but not normal B cells. Adoptive transfer of TCR-transduced T cells inhibited the growth of lymphoma cells *in vivo* and significantly extended the survival of mice. In addition, it was observed that TCL1 is expressed in the human embryonic stem cells and multiple types of solid tumor cells. TCL1-specific TCR-transduced T cells can recognize these solid tumor cells but not control tumor cells. These results suggest that TCL1-derived peptides are expressed in the context of HLA-A2 molecules on tumor cells and TCL1-TCR transduced T cells can be used for immunotherapy of B-cell malignancies as well as non-hematological tumors.

Characterization of HLA-A2-restricted TCL1-specific T cell clone and cloning of the TCR α and β genes

[00169] In order to obtain the TCL1-specific T cell receptor gene, several TCL1-specific T clones were first generated by limiting dilution. One of the clones, termed TC1 showed high reactivity to the TCL1 peptide pulsed T2 cells by IFN- γ ELISA assay (FIG. 1A). TCL1₇₀₋₇₉ peptide-specific tetramer staining revealed the TC1 T clone is tetramer-positive (FIG. 1B). The RNA was then extracted from T clone and nano-string analysis of TCR α and β subfamilies was performed. It revealed the TC1 TCRs (hereafter referred to as TCL1-TCR) were composed of TRAV1-1, TRAJ57*01 F (b), TRAC and TRBV5-5, TRBJ2-7*01 F, TRBD2*01 F, TRBC2 subfamily sequences (FIG. 1C).

The expression and function of TCL1-TCR in the TCR $\alpha\beta$ (-) J76 cells

[00170] In order to determine the expression of TCL1-specific TCR, a P2A vector was generated that expressed TCR α and β in the same vector (FIG. 2A). The TCL1-TCR was transfected into the TCR $\alpha\beta$ (-) J76 cells using a lentivirus. 48 hours after the transfection, the expression of TCL1-TCR was measured by flow cytometry. The inventors

found the TCL1-TCR can be detected in TCR $\alpha\beta$ (-) J76 cells by TC1-specific TRBV antibody and tetramer staining (FIG. 2B). In order to determine if the TCL1-TCR transfected J76 can specifically recognize the TCL1₇₀₋₇₉ peptide, the TCL1-TCR-transduced J76 cells were incubated with T2 cell pulsed with TCL1₇₀₋₇₉ peptide. The TCL1-TCR-transduced J76 cells but not untransduced J76 cells were observed to specifically secrete a large amount of IL-2 after incubation with TCL1₇₀₋₇₉ peptide-pulsed T2 cells, indicating the TCL1-TCR is functional (FIG. 2C). In order to determine the avidity of TCR, the TCL1-TCR-transduced J76 cells were incubated with T2 cell pulsed with different concentration of TCL1₇₀₋₇₉ peptide, it was found that the TCL1-TCR-transduced J76 can recognize the peptide at concentration of 1-10 nM, indicating that TCL1-TCR has moderate to high avidity (FIG. 2D)(23). Moreover, the inventors found that the TCL1-TCR is CD8-independent and the addition of CD8 $\alpha\alpha$ and CD8 $\alpha\beta$ did not further increase the TCR avidity (FIG. 10).

The expression and function of TCL1-TCR in HLA A2+ normal donor T cells

[00171] In order to determine if the TCL1-TCR can be expressed and function in primary T cells, the TCL1-TCR was transduced by lentivirus into OKT3-activated primary T cells, and it was observed that about 40-50% of T cells can express the TCL1-TCR by tetramer and TRBV antibody staining (FIG. 3A). In order to determine if TCL1-TCR is functional in the primary T cells, the TCL1-TCR-transduced T cells were incubated with T2 cells pulsed with TCL1₇₀₋₇₉ peptide. The TCL1-TCR-transduced T cells were observed to specifically secrete IFN- γ and IL-2 against peptide-pulsed T2 cells (FIG. 3B). Interestingly, both CD4 and CD8 T cells acquired the specific function with the TCL1-TCR, confirming that the TCR is CD8 $\alpha\beta$ -independent (FIG. 3C). The TCL1-TCR-transduced CD8 or CD4 T cells specifically secreted Th1/Tc1-type cytokines but not Th2/Tc2-type cytokines. Avidity assay revealed that the TCL1-TCR-transduced primary T cells recognized the peptide at 1-10 nM concentration, confirming that the TCR avidity is moderate to high (FIG. 3D).

The recognition of lymphoma cell lines and primary lymphoma cells by TCL1-TCR-transduced T cells

[00172] In order to determine if the TCL1-TCR-transduced primary T cells have cytotoxic function, the TCL1-TCR-transduced T cells were incubated with T2 cells pulsed with TCL1₇₀₋₇₉ peptide. The TCL1-TCR-transduced T cells were observed to specifically kill the TCL1₇₀₋₇₉ peptide-pulsed T2 cells but not control HIV peptide-pulsed T2

cells, indicating that the TCL1-TCR-transduced T cells have cytotoxic activity (FIG. 4A). The control untransduced T cells did not exhibit this function.

[00173] In order to determine if the TCL1-TCR-transduced T cells can specifically lyse lymphoma cells, the TCL1-TCR-transduced T cells were incubated with HLA A2+, TCL1-expressing lymphoma cell lines or primary lymphoma tumor cells derived from patients with CLL, MCL, FL, DLBCL and SMZL. The inventors found the TCL1-TCR-transduced T cells can specifically lysed the lymphoma cell lines and primary lymphoma cells, but not HLA A2- lymphoma cell lines, or normal B cell derived from the same patient, indicating that the TCL1-TCR-transduced T cells can specifically target B-cell lymphoma tumor cells (FIGS. 4B-C).

TCL1-TCR-transduced T cell exhibit anti-lymphoma activity in vivo

[00174] In order to determine if the TCL1-TCR-transduced T cell can inhibit tumor growth *in vivo*, the effect of TCL1-TCR-transduced T cells against luciferase-transduced Mino cells was tested in two experiments. In one setting (Winn assay), 5×10^6 TCL1-TCR-transduced T cells were co-injected with 0.5×10^6 luciferase-transduced Mino cells into immune-deficient mice, and it was observed that the TCL1-TCR-transduced T cells significantly inhibited tumor growth *in vivo* (FIG. 5A). In the second setting, 0.5×10^6 luciferase-transduced Mino cells were injected into immune deficient mice to establish tumor. After 12 days, mice were treated with 10×10^6 TCL1-TCR-transduced T cell. The inventors found TCL1-TCR transduced T cells but not control T cells significantly inhibited tumor growth and extended survival of mice (FIG. 5B).

The expression of TCL1 in embryonic cells and non-hematological tumor cells

[00175] Previous studies have found that TCL1 is expressed in embryonic cells but not in adult tissues except B cells (Narducci *et al.*, 2002). Consistent with this, we observed TCL1 expression in embryonic cell lines (ESI-049, SKU: ES-702 ; www.esibio.com/index.php/products/product-category/cell-lines/esi-049-human-embryonic-stem-cell-line-46-xy/, from ESI BIO) by intracellular staining and real-time PCR assay (FIGS. 6A-B). Moreover, analysis of TCGA database showed that TCL1 is overexpressed in multiple non-hematological tumors but not in the normal tissues except B-cell-rich organs like lymph node, tonsil, and spleen, which is consistent with previous reports (FIG. 6C).

Cytotoxic activity of TCL1-TCR-transduced T cells against non-hematological tumor cell lines

[00176] In order to determine if TCL1-TCR-transduced T cells can target TCL1-expressing solid tumor cancer cells, 3 HLA A2+, human solid tumor cell lines (SW480, HCT-15, HepG2) were collected. TCL1 was expressed in all of these solid tumor cell lines by intracellular staining and real-time PCR assay (FIGS. 7A-B). In co-culture assays, TCL1-TCR-transduced T cells specifically lysed these tumor cells *in vitro*, but not HLA A2- control tumor cells (FIGS. 7C-D).

Safety of TCL1-TCR-transduced T cells.

[00177] Alanine and glycine replacement assays of the TCL1₇₀₋₇₉ peptide (FIG. 8A) demonstrated that the middle 8 amino acids “LLPIMWQL” (SEQ ID NO: 33) of TCL1₇₀₋₇₉ epitope (SLLPIMWQLY; SEQ ID NO: 29) were critical residues for recognition by TCL1-TCR-transduced T cells as replacement of any of these 8 amino acids abrogated their reactivity. The first (S) and last (Y) amino acids of the epitope were found to be not critical for recognition by TCL1-TCR-transduced J76 or primary (ND, normal donor) T cells (FIGS. 8B-C). Blast search of LLPIMWQL (SEQ ID NO: 33) sequence against known proteins in the reference database did not reveal any homology with proteins other than TCL1, indicating that TCL1-TCR-transduced T cells are unlikely to have on-target off-tumor effect and could be safe for adoptive T cell therapy.

[00178] Adoptive T cell transfer with CD19 CAR T cells have shown promising clinical responses in lymphoma patients, however, relapse is common. Most of the relapsed lymphomas are CD19 negative suggesting antigen loss as a mechanism of immune escape (Sotillo *et al.*, 2015; Topp *et al.*, 2014; Neelapu *et al.*, 2017). Therefore, novel T cell therapy strategies targeting other tumor antigens are needed to further improve outcomes in patients with B-cell malignancies. In the above experiments, TCL1-TCR-transduced T cells targeting B-cell oncoprotein TCL1 were shown to be effective against multiple types of B-cell malignancies. In addition, they were effective against TCL1-expressing solid tumor cells, but did not target normal B cells or control tumor cells that lack TCL1 or HLA-A2. Collectively, these results indicate that TCL1-TCR-transduced T cells can be used as an immunotherapy strategy to treat TCL1-expressing cancers such as B-cell malignancies and non-hematological tumors.

[00179] Genetic modification of normal T cells with T cell receptor has been an effective way to redirect the specificity of T cells against tumors (Fesnak *et al.*, 2016). Compared to the popular CAR T therapy, there are some limitations that hindered the success of TCR-T immunotherapy. For example, the avidity of TCRs is generally lower than CARs, there is potential for mismatched pairing of α and β chains of transduced TCRs with endogenous TCRs, and there may be cross-recognition of degenerate peptides derived from other proteins that may result in toxicity (Kunert *et al.*, 2013). We used several strategies to minimize these limitations. To minimize mismatch pairing, we introduced cysteine mutations into the constant regions of TRAC and TRBC (Cohen *et al.*, 2007) and also codon optimized the TCL1-TCR (van Loenen *et al.*, 2011). High avidity TCRs have been reported to be associated with better tumor killing ability than low avidity TCRs (Stone *et al.*, 2015; Johnson *et al.*, 2009). However, very high avidity TCRs may be associated with cross-reactivity and off-target toxicity (Linette *et al.*, 2013; Morgan R *et al.*, 2013; Holler *et al.*, 2003). TCRs of 10 μ M avidity are capable of mediating killing of tumor cells without inducing GVHD (Zhong *et al.*, 2013). The inventors have isolated a TCL1-TCR of 1-10 nM avidity, which is considered to be moderate to high (Kunert *et al.*, 2013). In the above experiments, the inventors found that the TCL1-TCR of this avidity can mediate significant tumor lysis without causing auto-reactivity, indicating that this TCR is likely to be effective against tumor. Furthermore, the absence of homology of the critical amino acid sequence, LLPIMWQL (SEQ ID NO: 33), to other human proteins supports the idea that this TCL1-TCR is likely to be safe in clinic (Cameron *et al.*, 2013).

* * *

[00180] 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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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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- 30 Weng *et al.*, Targeting B-cell malignancies through human B-cell receptor specific CD4+ T cells. Oncoimmunology; 5(11):e1232220, 2016a.
- Weng *et al.*, TCL1: a shared tumor-associated antigen for immunotherapy against B-cell lymphomas. Blood; 120(8):1613-23, 2012.
- Wu *et al.*, Cancer, March 18(2): 160-75, 2012.
- 35 Zhong *et al.*, T-cell receptor affinity and avidity defines antitumor response and autoimmunity in T-cell immunotherapy. Proc Natl Acad Sci U S A. 2013;110(17):6973-8.

CLAIMS

What is Claimed is:

1. An engineered T cell receptor (TCR) having antigenic specificity for T-cell leukemia/lymphoma 1 (TCL1) or SEQ ID NO:29, wherein the TCR comprises the amino acid
5 sequences of SEQ ID NO: 54, 55, 56, 57, 58, and 59.
2. The TCR of claim 1, wherein the engineered TCR comprises:
 - (i) an alpha chain variable region having the amino acid sequence of SEQ ID NO:1 or a sequence having at least 90% sequence identity to SEQ ID NO:1; and/or
 - (ii) a beta chain variable region having the amino acid sequence of SEQ ID NO:2 or a
10 sequence having at least 90% sequence identity to SEQ ID NO:2.
3. The TCR of claim 2, wherein the engineered TCR binds HLA-A2 or HLA-A*0201, or has antigenic specificity for TCL1 in the context of HLA-A2 or HLA-A*0201.
4. The TCR of claim 4, wherein the TCR comprises an alpha chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain
15 variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:2.
5. The TCR of claim 4, wherein the TCR comprises an alpha chain variable region having at least 99% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain variable region having at least 95% identity to the amino acid sequence of SEQ ID NO:2.
6. The TCR of claim 4, wherein the TCR comprises an alpha chain variable region
20 having at least 95% identity to the amino acid sequence of SEQ ID NO:1 and/or a beta chain having at least 99% identity to the amino acid sequence of SEQ ID NO:2.
7. The TCR of claim 1, wherein the TCR comprises an alpha chain variable region of SEQ ID NO:1 and/or a beta chain of SEQ ID NO:2.
8. The TCR of claim 4, wherein the TCR comprises an alpha chain having at least 95%
25 identity to the amino acid sequence of SEQ ID NO:3 and/or a beta chain having at least 95% identity to the amino acid sequence of SEQ ID NO:4.

9. The TCR of claim 4, wherein the TCR comprises an alpha chain comprising the amino acid sequence of SEQ ID NO:3 and/or a beta chain comprising the amino acid sequence of SEQ ID NO:4.
10. The TCR of claim 1, wherein the TCR is further defined as a soluble TCR, wherein
5 the soluble TCR does not comprise a transmembrane domain.
11. The TCR of any one of claims 1-10, further comprising a detectable label.
12. The TCR of any one of claim 1-10, wherein the TCR is covalently bound to a therapeutic agent.
13. The TCR of claim 12, wherein the therapeutic agent is an immunotoxin or a
10 chemotherapeutic agent.
14. A multivalent TCR complex comprising a plurality of TCRs according to any one of claims 1-10.
15. The complex of claim 14, wherein the multivalent TCR comprises 2, 3, 4 or more TCRs associated with one another.
16. The complex of claim 15, wherein the multivalent TCR is present in a lipid bilayer, in
15 a liposome, or is attached to a nanoparticle.
17. The complex of claim 15, wherein the TCRs are associated with one another via a linker molecule or a non-naturally occurring disulfide bond.
18. A nucleic acid comprising or consisting of a nucleotide sequence encoding the TCR
20 of any one of claims 1-17.
19. An expression vector comprising the nucleic acid according to claim 18.
20. The expression vector of claim 19, wherein the sequence encoding the TCR is under the control of a promoter.
21. The expression vector of claim 19, wherein the expression vector is a viral vector.
22. The expression vector of claim 21, wherein the viral vector is a retroviral vector or a
25 lentiviral vector.

23. The expression vector of claim 19, wherein the vector further encodes a linker domain.
24. The expression vector of claim 23, wherein the linker domain is positioned between the alpha chain and beta chain.
- 5 25. The expression vector of claim 23, wherein the linker domain comprises one or more cleavage sites.
26. The expression vector of claim 25, wherein the one or more cleavage sites are a furin cleavage site and/or a P2A cleavage site.
27. The expression vector of claim 26, wherein the furin cleavage site is RAKR (SEQ ID
10 NO: 30).
28. The expression vector of claim 26, wherein the Furin cleavage site is ATNFSLLKQAGDVEENPG (SEQ ID NO:31).
29. The expression vector of claim 23, wherein the one or more cleavage sites are separated by a spacer.
- 15 30. The expression vector of claim 29, wherein the spacer is SGSG (SEQ ID NO:32) or GSG.
31. A host cell engineered to express the TCR of any one of claims 1-10, preferably wherein the host cell comprises an expression vector according to any one of claims 18-30.
32. The host cell of claim 31, wherein the cell is a T cell, NK cell, invariant NK cell,
20 NKT cell, mesenchymal stem cell (MSC), or induced pluripotent stem (iPS) cell.
33. The host cell of claim 31, wherein the host cell is an immune cell.
34. The host cell of claim 31, wherein the host cell is isolated from an umbilical cord.
35. The host cell of claim 32, wherein the T cell is a CD8⁺ T cell, CD4⁺ T cell, or $\gamma\delta$ T
25 cell.
36. The host cell of claim 32, wherein the T cell is a regulatory T cell (Treg).

37. The host cell of claim 31, wherein the cell is autologous.
38. The host cell of claim 31, wherein the cell is allogeneic.
39. A method for engineering the host cell of claim 31 comprising contacting said immune cell with the nucleic acid of claim 18 or the expression vector of any one of claims 19-30.
40. The method of claim 39, wherein the immune cell is a T cell, or a peripheral blood lymphocyte.
41. The method of claim 39, wherein the contacting is further defined as transfecting or transducing.
42. The method of any one of claims 39-41, wherein transfecting comprises electroporating RNA encoding the TCR of any one of claims 1-10 into the immune cell.
43. The method of any one of claims 41, further comprising generating viral supernatant from the expression vector of claim 20 prior to transducing the immune cell.
44. The method of any one of claims 39-43, wherein the immune cell is a stimulated lymphocyte.
45. The method of claim 44, wherein the stimulated lymphocyte is a human lymphocyte.
46. The method of claim 44, wherein the stimulating comprises contacting the immune cell with or incubating the immune cell in OKT3 and/or IL-2.
47. The method of any one of claims 39-46, further comprising sorting the immune cells to isolate TCR engineered T cells.
48. The method of claim 47, further comprising performing T cell cloning by serial dilution.
49. The method of claim 48, further comprising expansion of the T cell clone by the rapid expansion protocol.

50. A method of treating cancer in a subject comprising administering an effective amount of the TCR-engineered cells of any one of claims 31-37 to a subject, wherein the cancer expresses TCL1.
51. The method of claim 50, wherein the subject is identified to have an HLA-A*0201 allele.
52. The method of claim 50, wherein the TCR-engineered cell is a T cell or peripheral blood lymphocyte.
53. The method of claim 50, wherein the T cell is a CD8⁺ T cell, CD4⁺ T cell, or Treg.
54. The method of claim 50, wherein the cancer is a leukemia, a lymphoma, or a B-cell malignancy, or a B-cell lymphoma.
55. The method of claim 54, wherein the B-cell malignancy selected from the group consisting of follicular lymphoma (FL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), marginal zone lymphoma (MZL), Burkitt lymphoma, hairy cell leukemia, Waldenström macroglobulinemia, lymphoblastic lymphoma, and primary cutaneous B cell lymphoma.
56. The method of claim 50, wherein the cancer is a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis.
57. The method of claim 50, wherein the cancer is a solid tumor.
58. The method of claim 50, wherein the subject is a human.
59. The method of claim 50, wherein the TCR engineered cells are autologous or allogeneic to the subject.
60. The method of claim 50, further comprising lymphodepletion of the subject prior to administration of the TCL1-specific T cells.
61. The method of claim 60, wherein the lymphodepletion comprises administration of cyclophosphamide and/or fludarabine.

62. The method of any one of claims 50-61, further comprising administering a second anticancer therapy to the subject.

63. The method of claim 62, wherein the second therapy is a chemotherapy, immunotherapy, surgery, radiotherapy, or biological therapy.

5 64. The method of claim 63, wherein the second therapy is an anti-CD19 immunotherapy, or an immune checkpoint inhibitor therapy, preferably wherein the second therapy is an antibody or antigen-binding fragment thereof that specifically binds to an antigen selected from the group consisting of: PD-1, PD-L1 and CTLA4.

65. The method of claim 64, wherein the cancer is a B-cell malignancy or a lymphoma.

10 66. The method of claim 64, wherein the cancer is a solid tumor.

67. The method of any one of claims 50-62, 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.

15 68. The method of any one of claims 50-67, wherein the subject is determined to have cancer cells which overexpress TCL1.

69. A method of treating a cancer in a mammalian subject comprising administering a therapeutically effective amount of a TCL1-targeting immunotherapy to the subject, wherein the cancer is a solid tumor.

20 70. The method of claim 69, wherein the solid tumor is a cancer of the brain, colon, liver, pancreas, skin, breast, ovarian, prostate, renal, stomach, bladder, lung, esophagus, cervix, or testis.

71. The method of any one of claims 69-70, wherein the TCL1-targeting immunotherapy is an anti-TCL1 antibody, or a TCL1-targeting T-cell therapy.

25

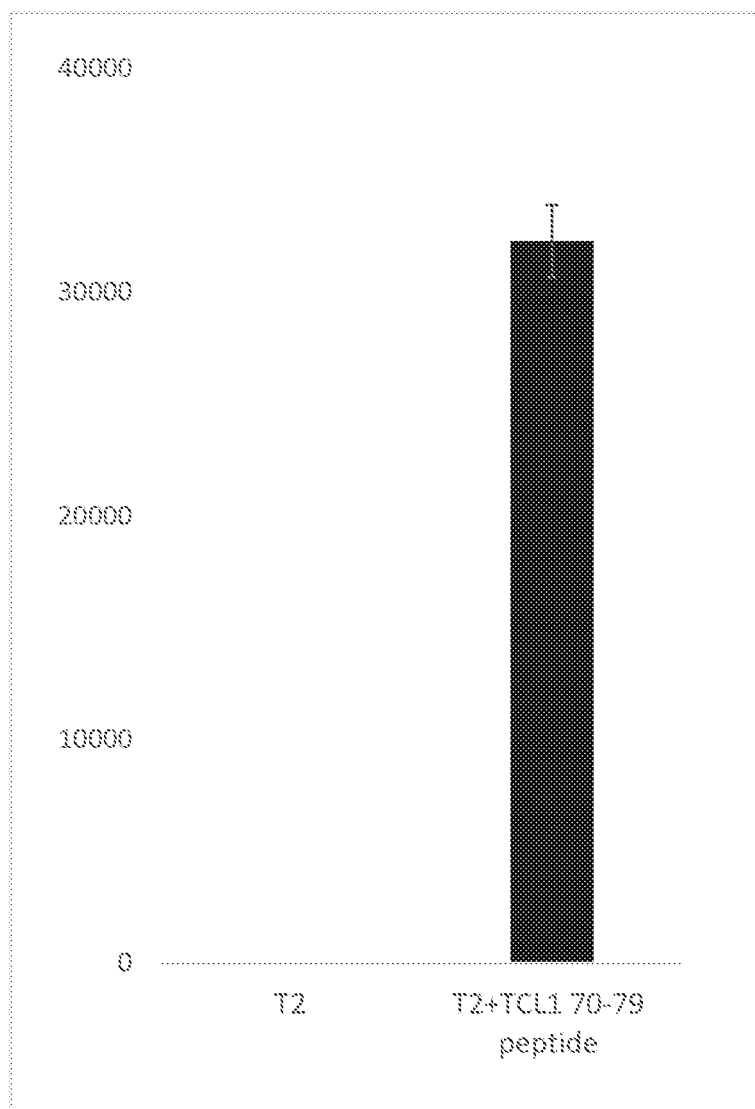


FIG. 1A

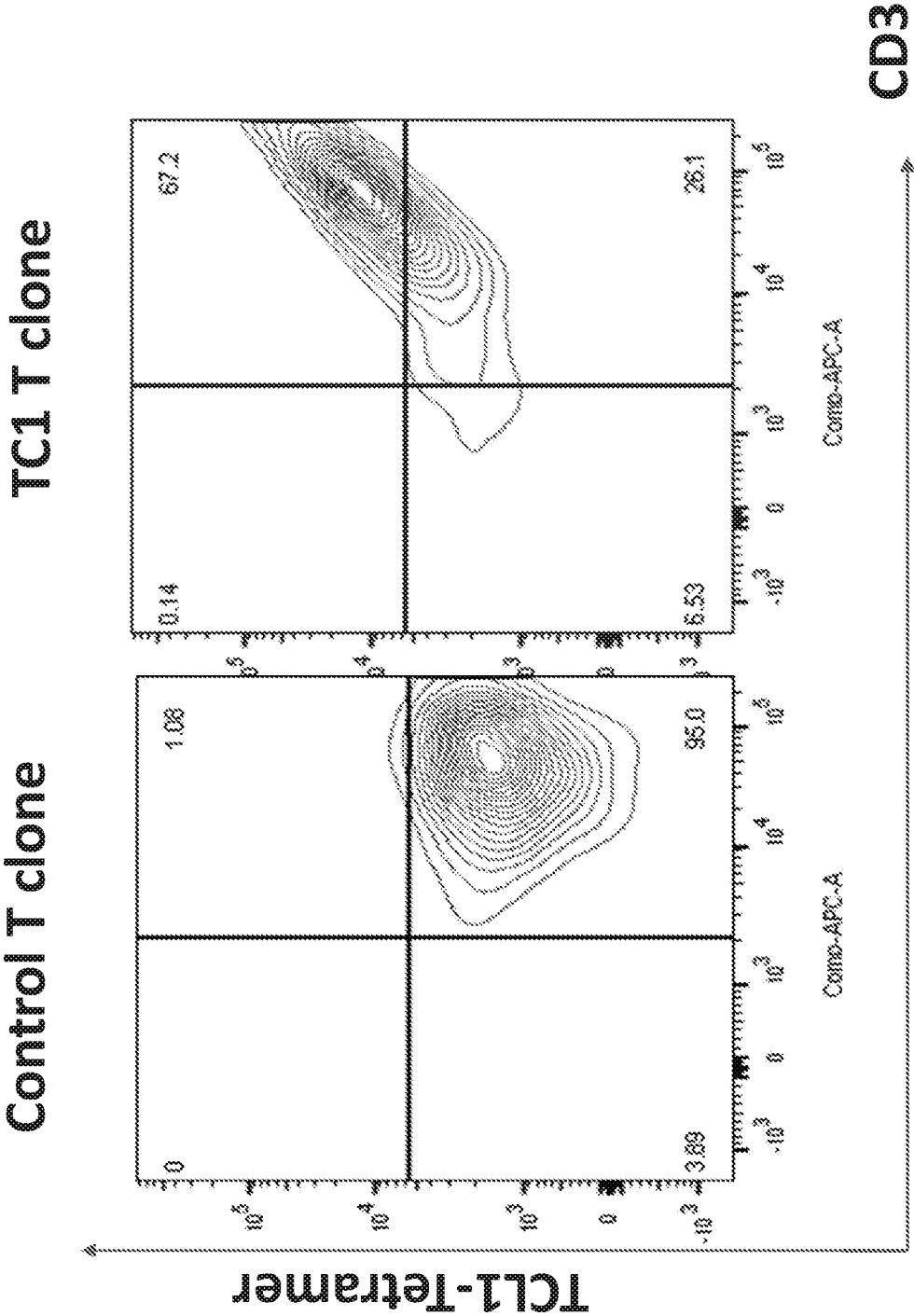


FIG. 1B

TCL1-T clone	TCR α			TCR β			
	V α	J α	C α	V β	J β	D	C β
TC1	TRAV1-1*02	TRAJ28*01	C α 1	TRBV5-5*01	TRBJ2-7*01	TRBD2*01	C β 2

FIG. 1C

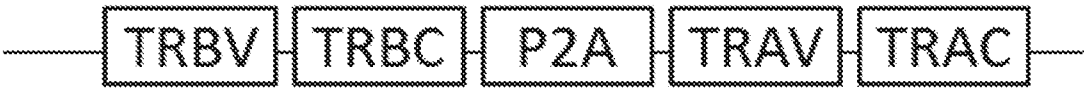


FIG. 2A

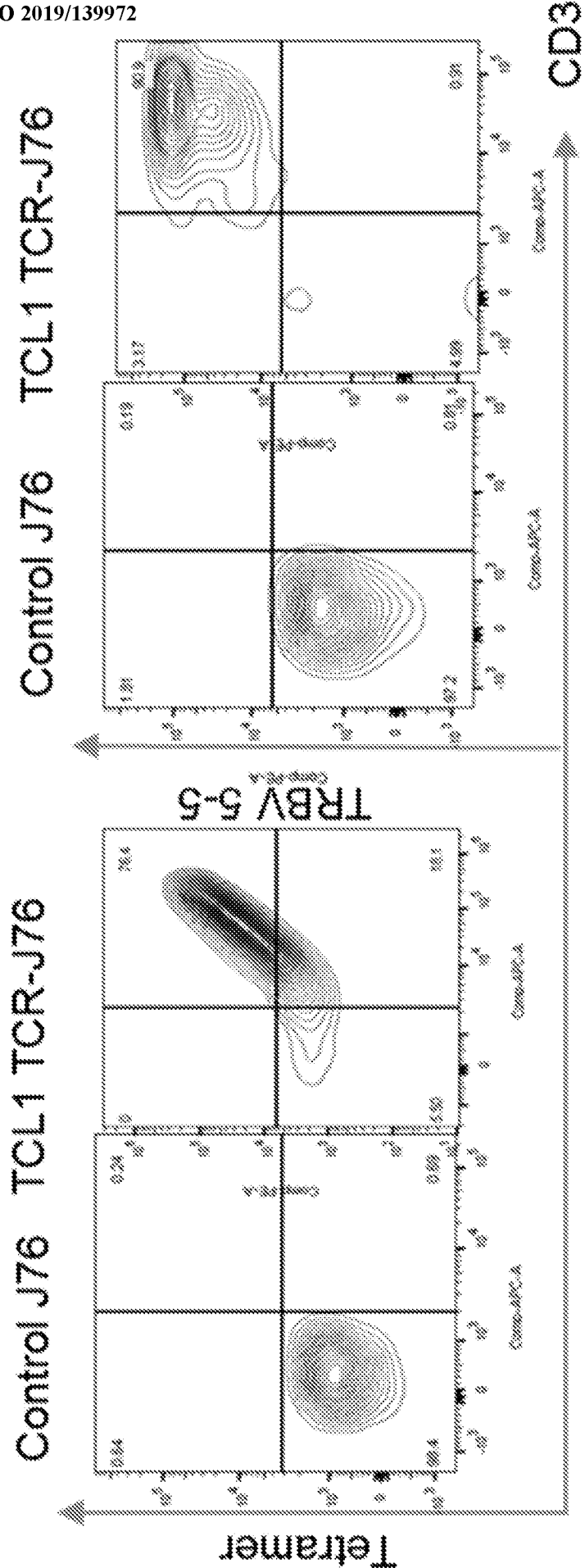


FIG. 2B

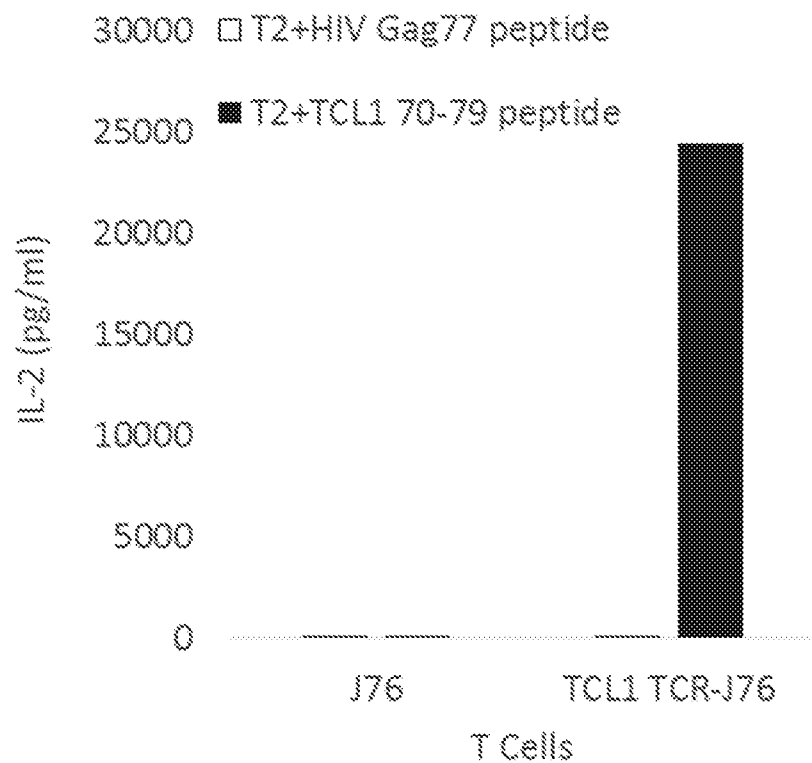


FIG. 2C

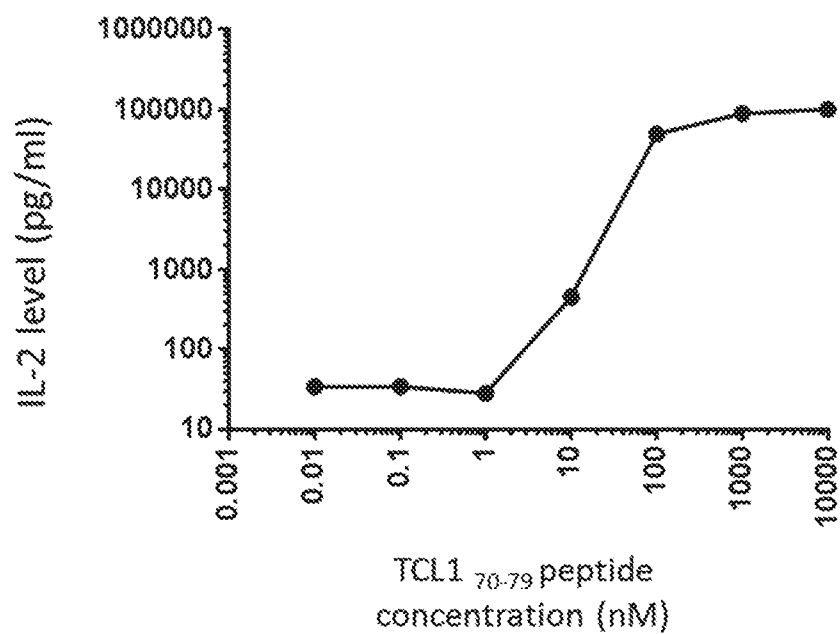


FIG. 2D

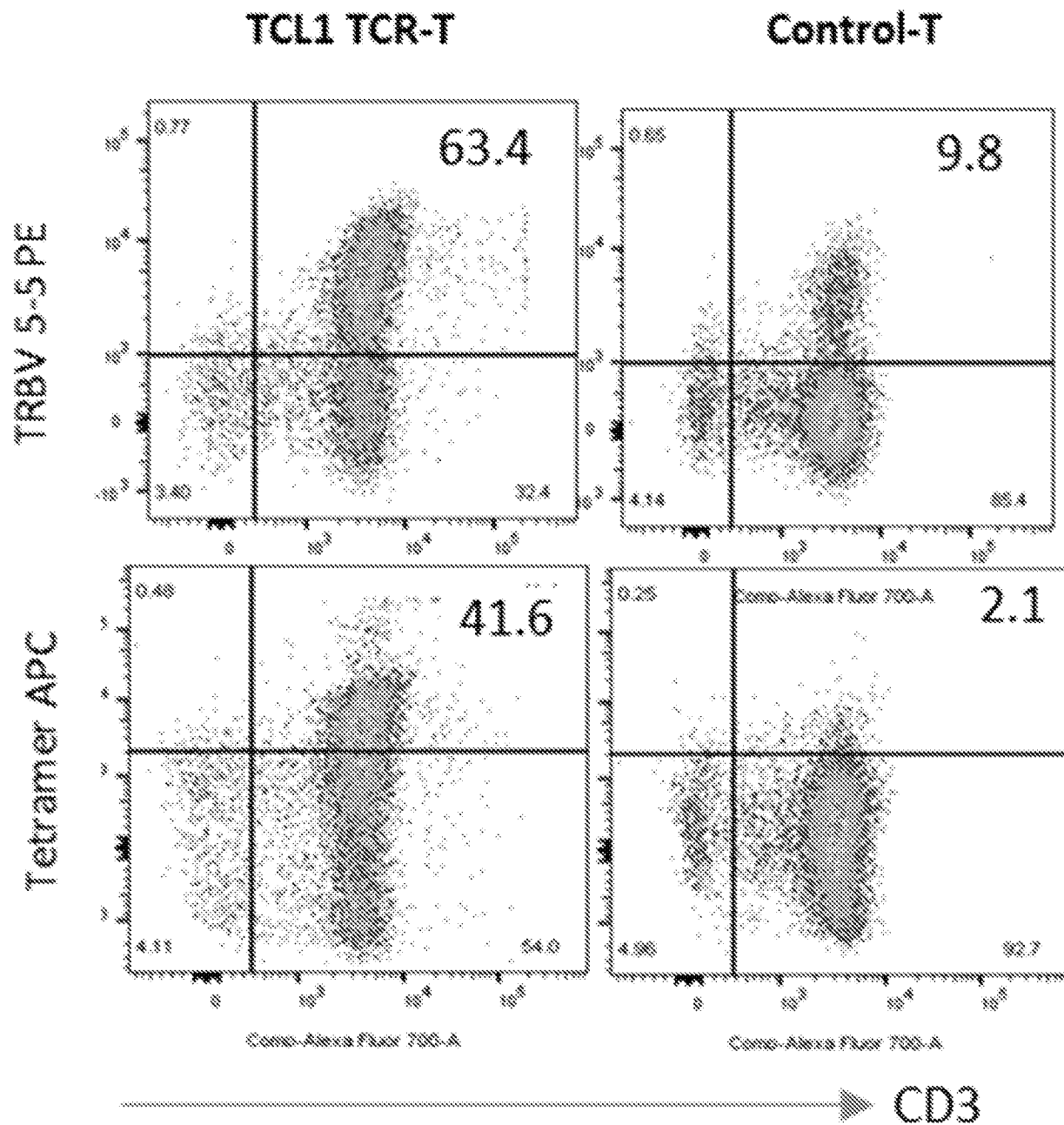


FIG. 3A

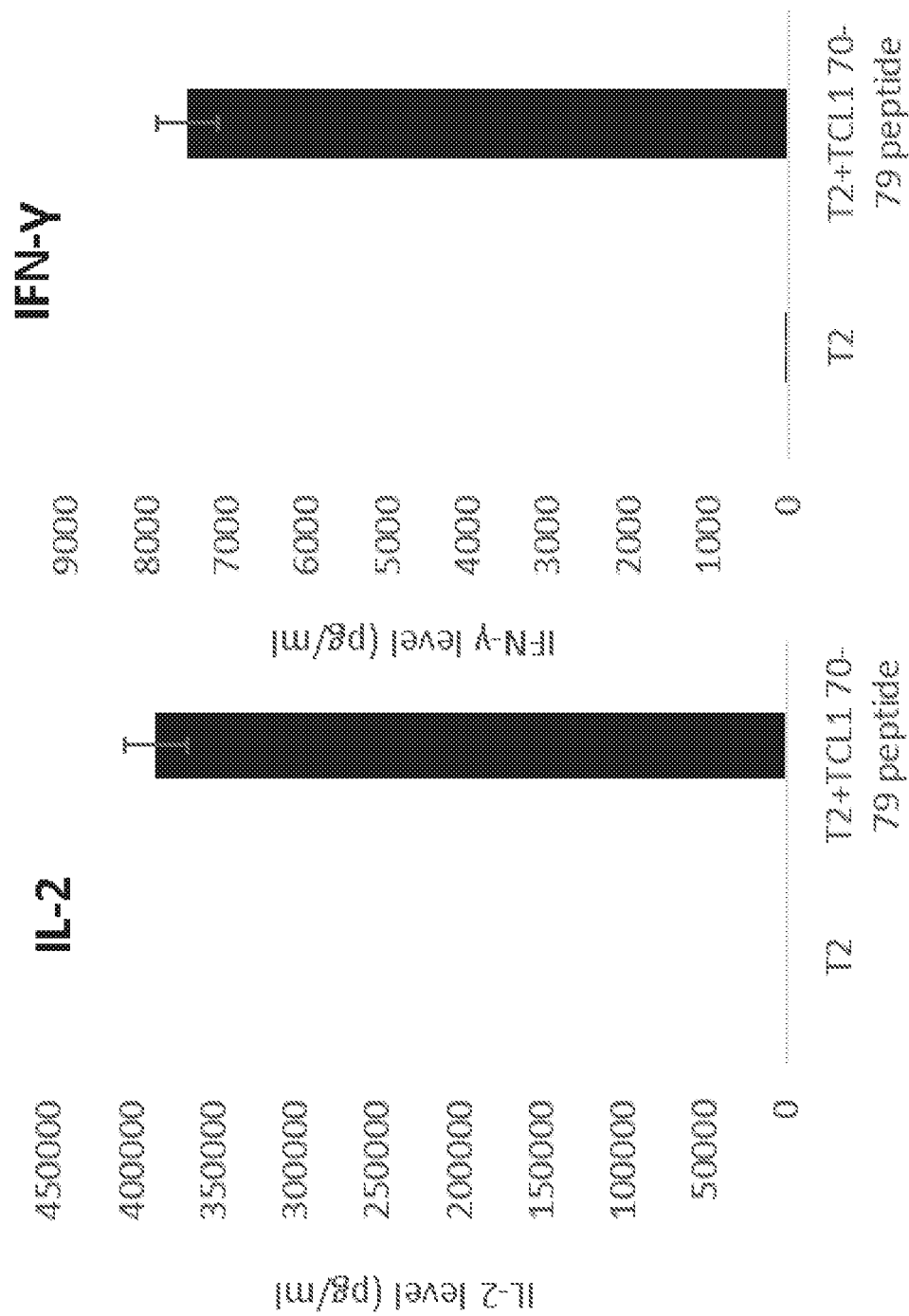
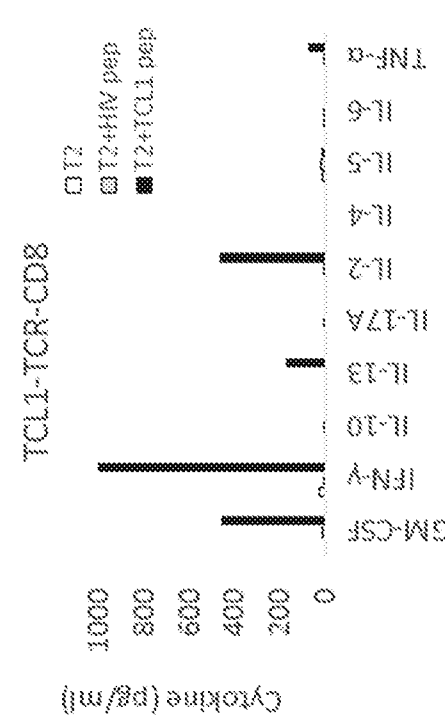


FIG. 3B



FIG. 3C



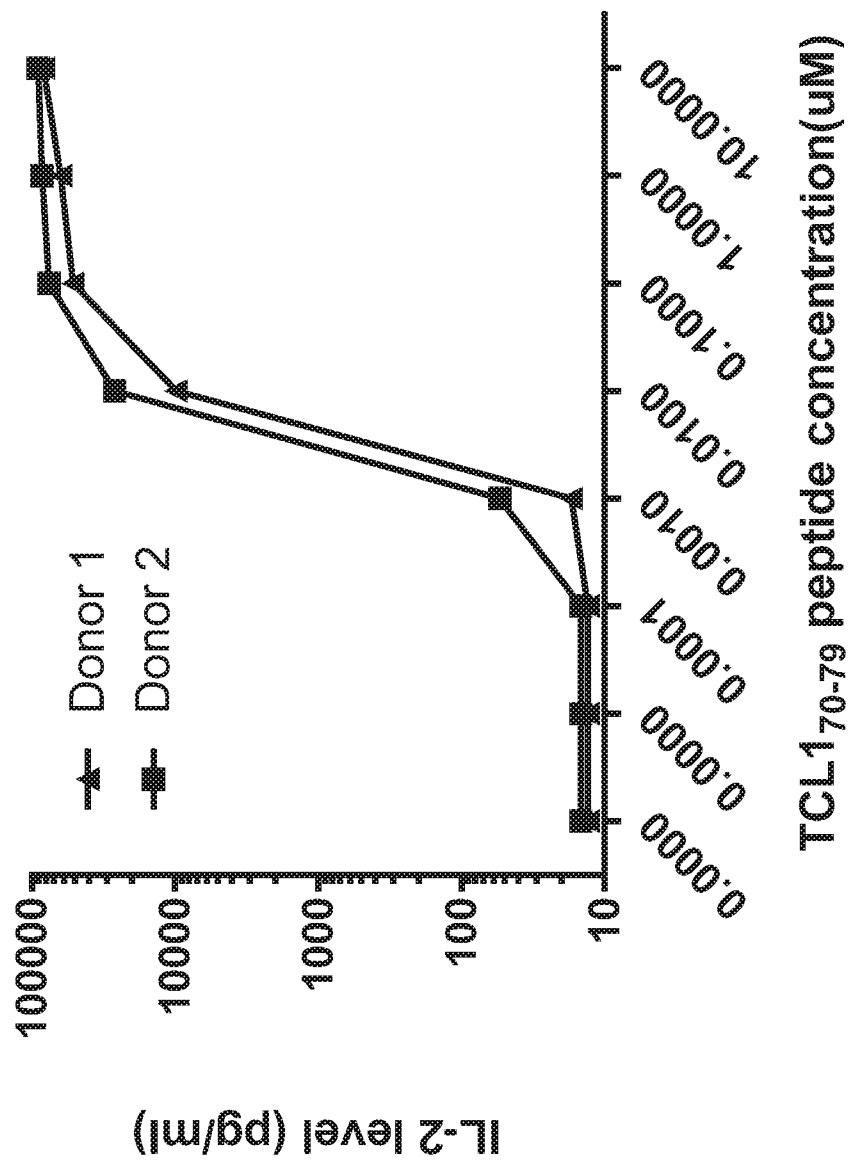


FIG. 3D

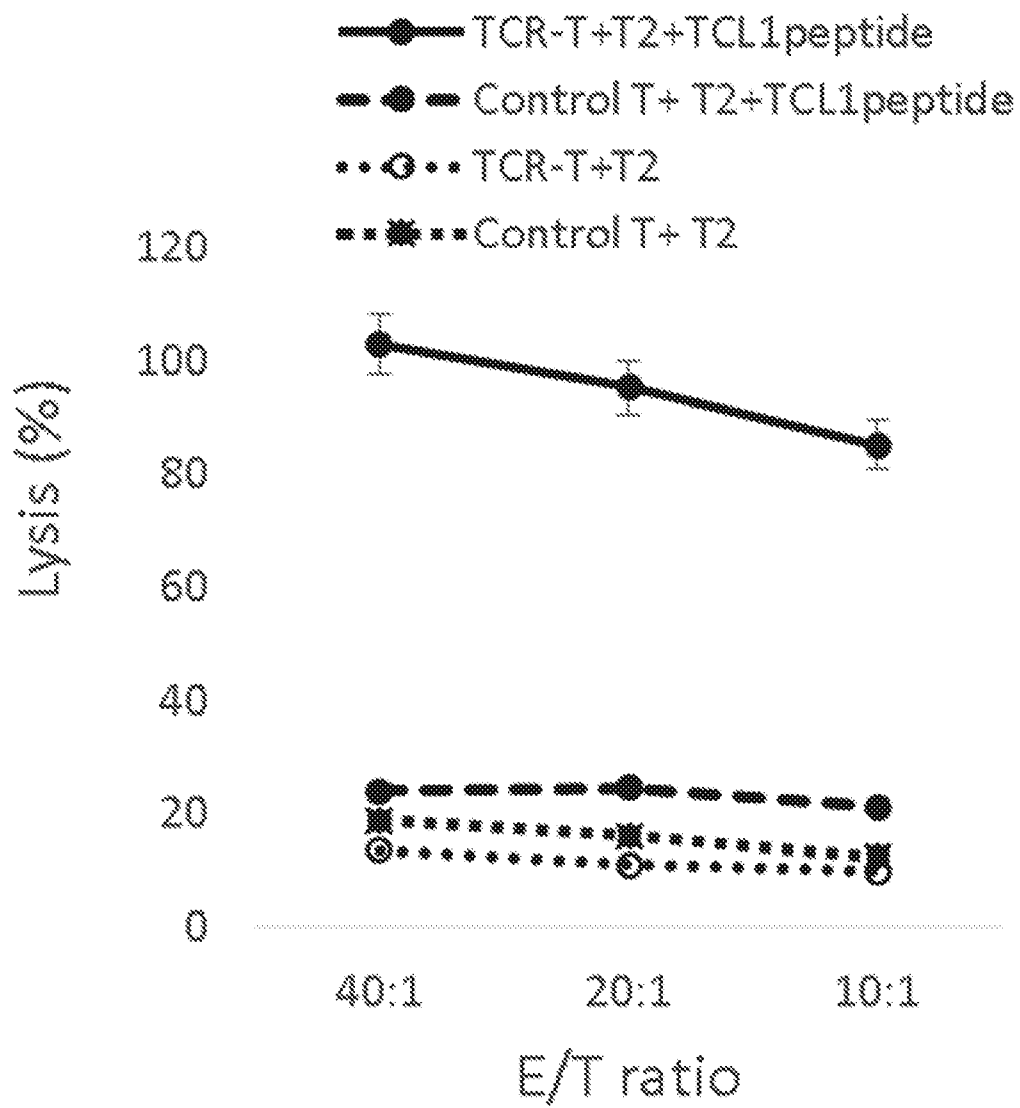


FIG. 4A

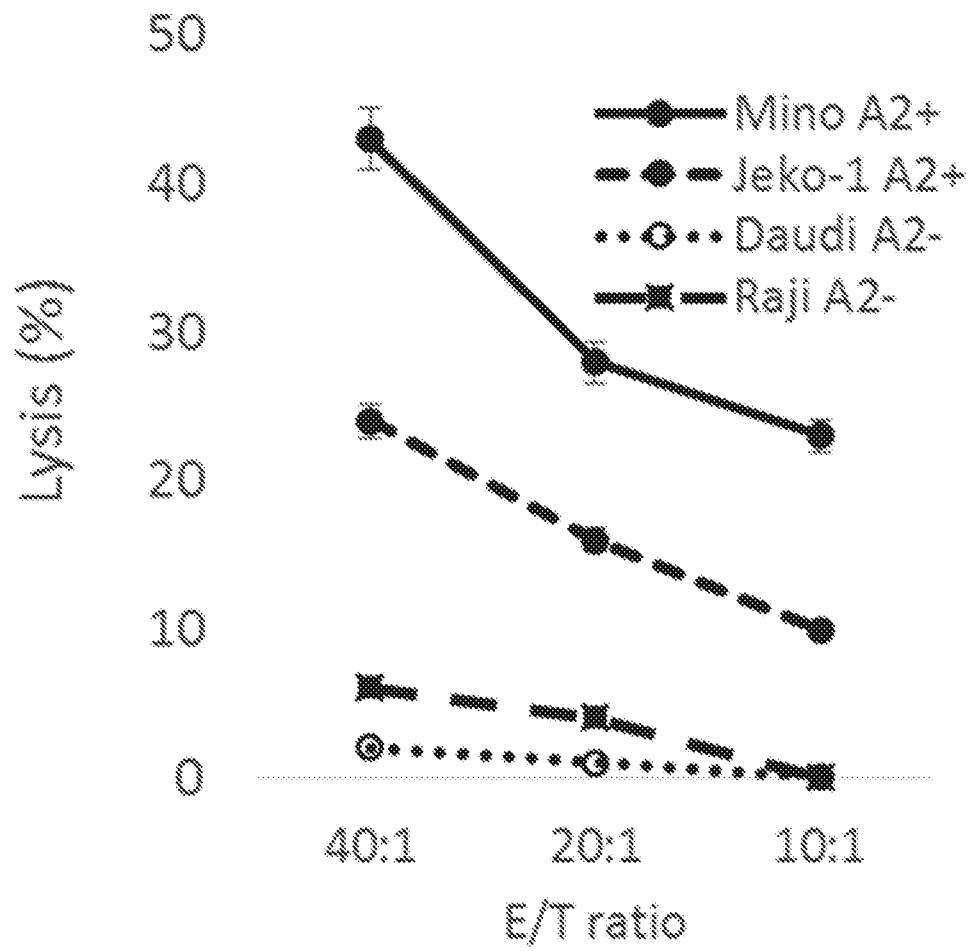


FIG. 4B

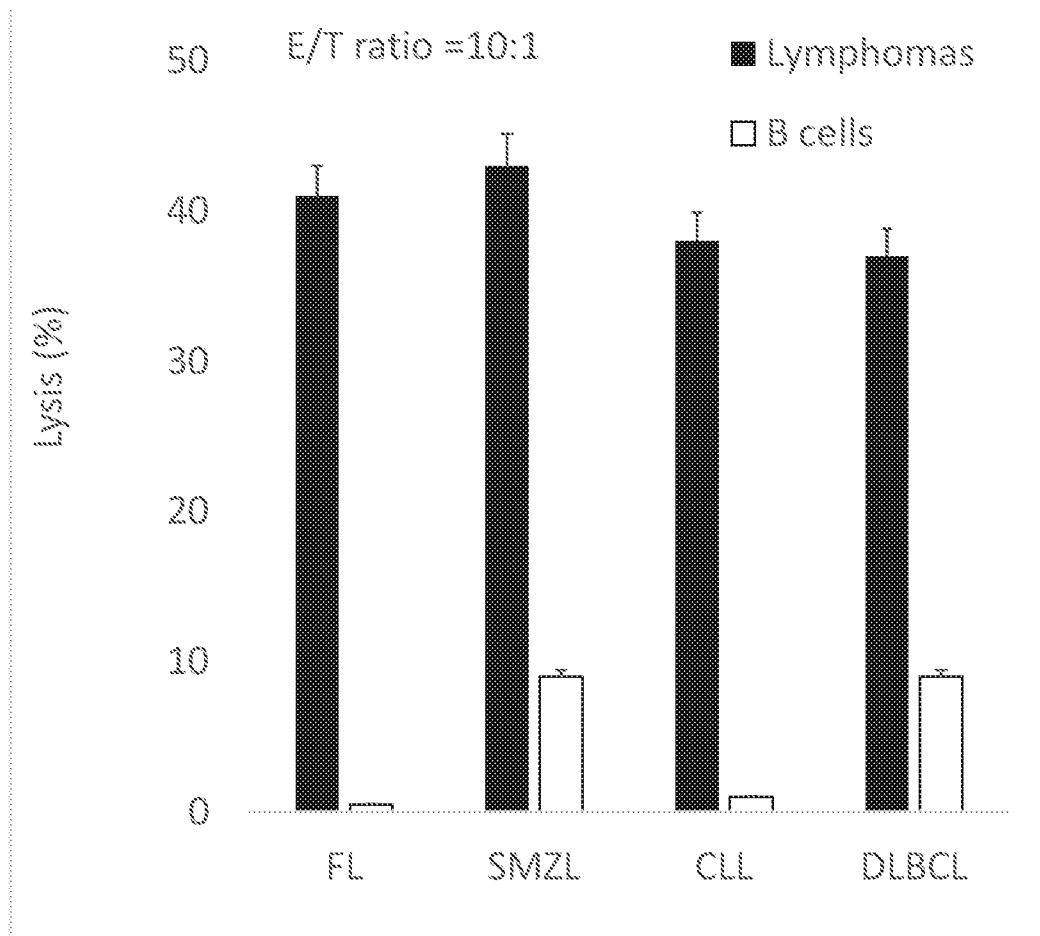


FIG. 4C

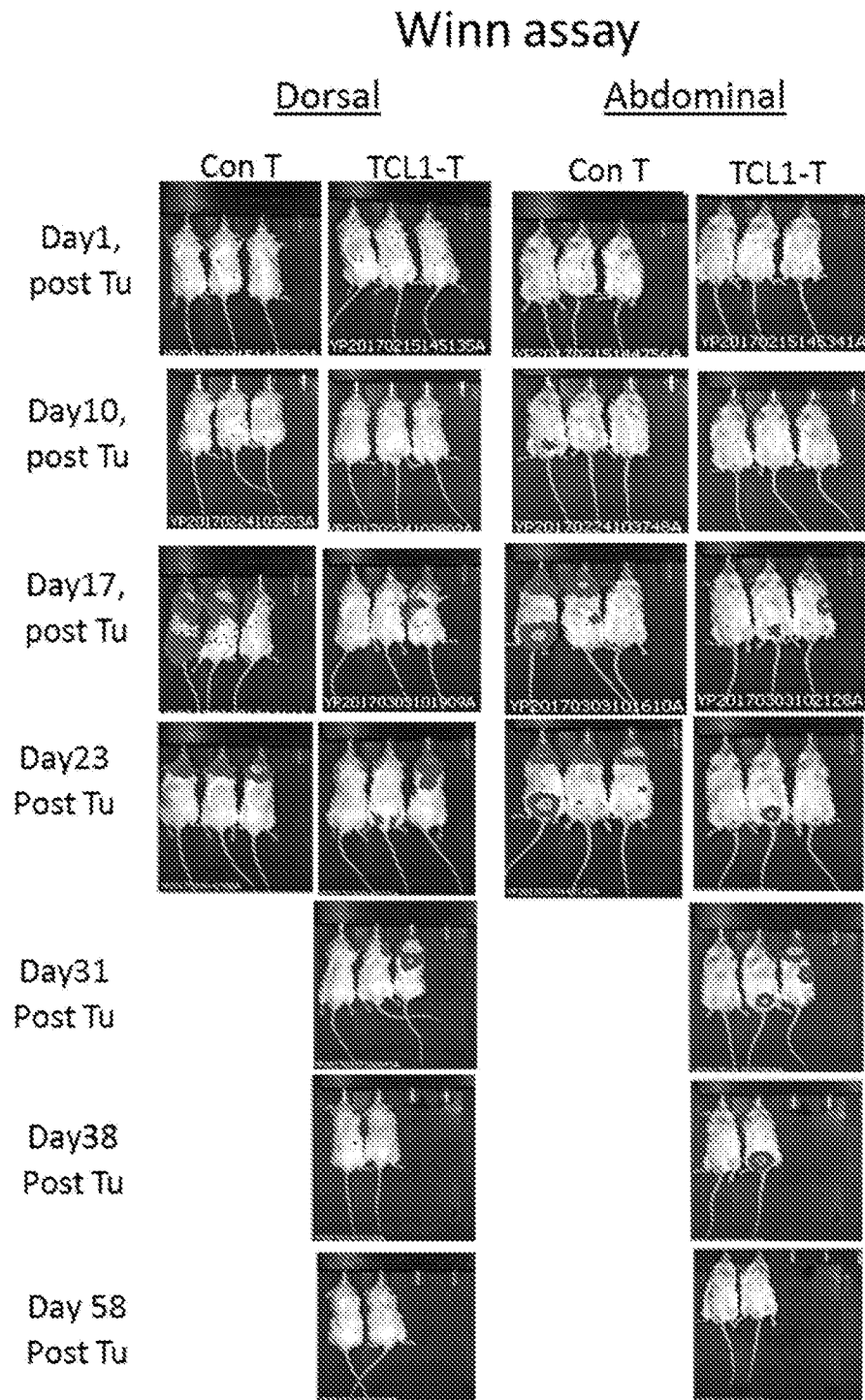


FIG. 5A

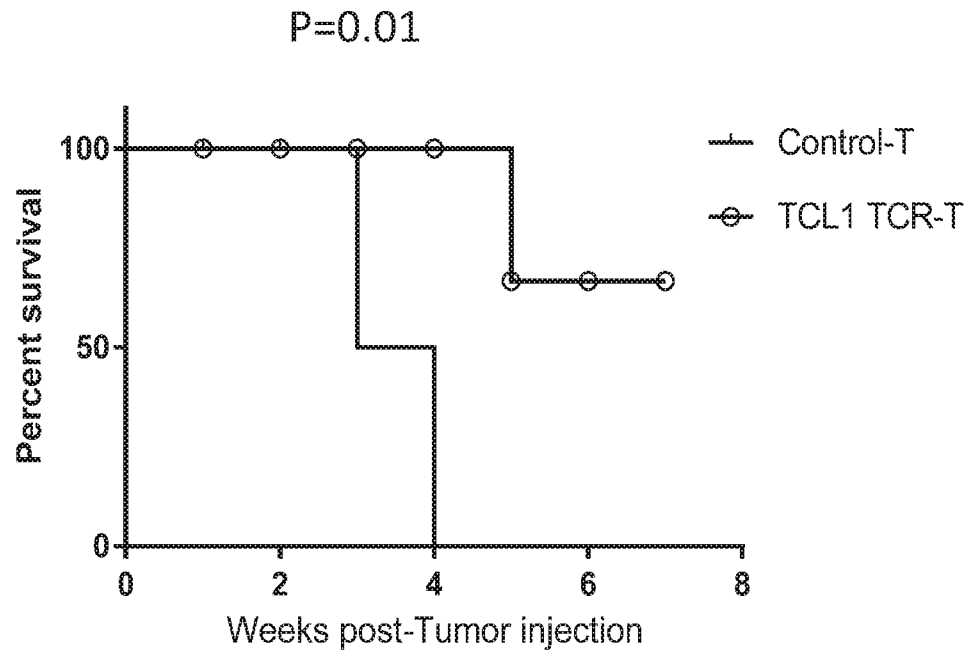


FIG. 5B

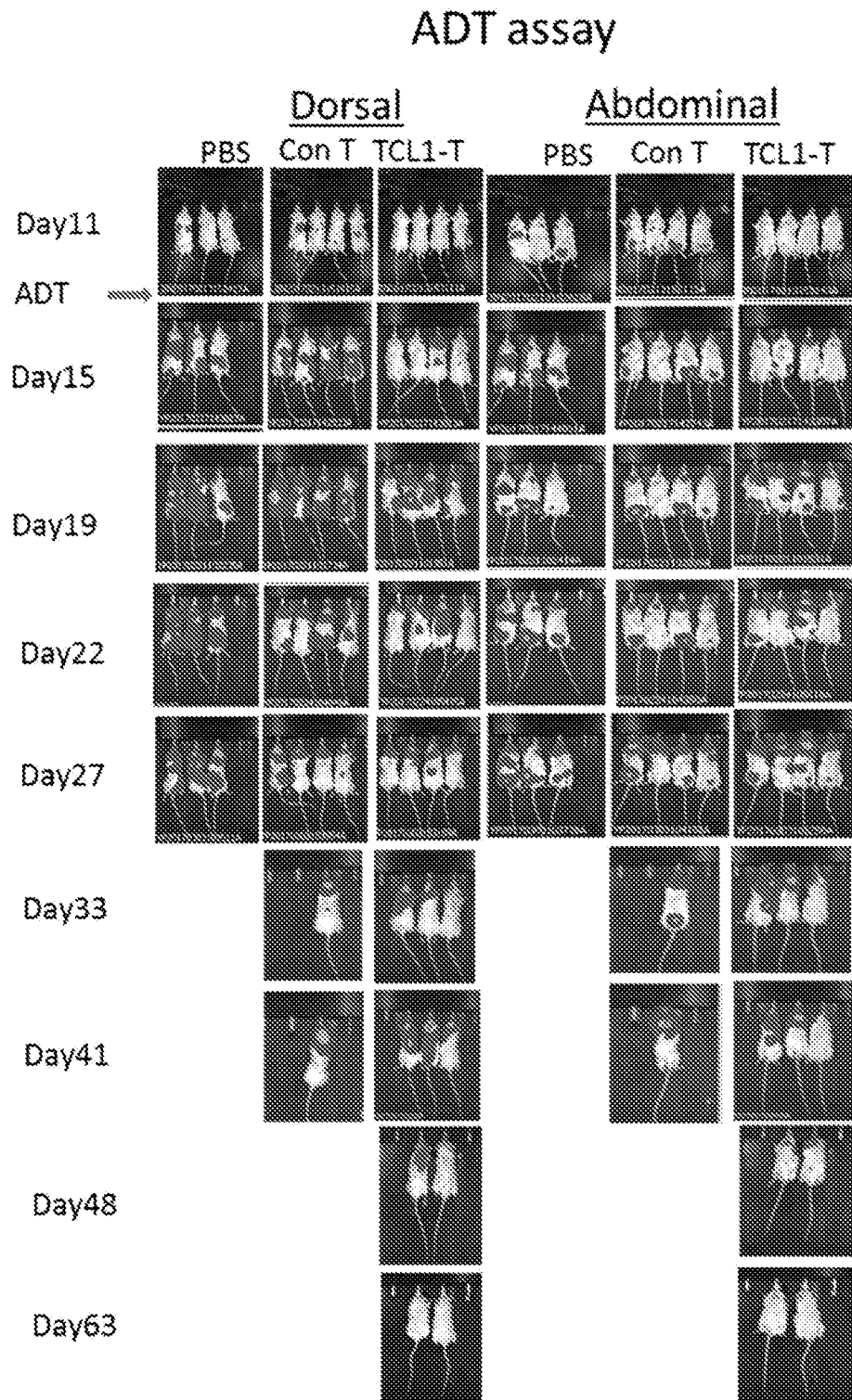


FIG. 5C

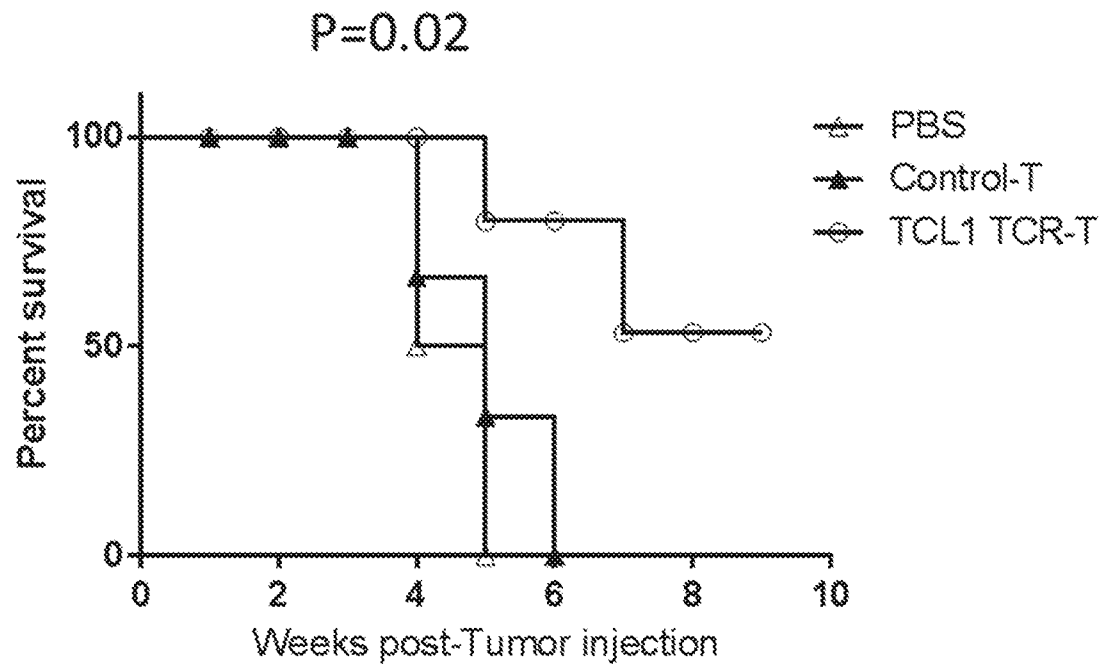


FIG. 5D

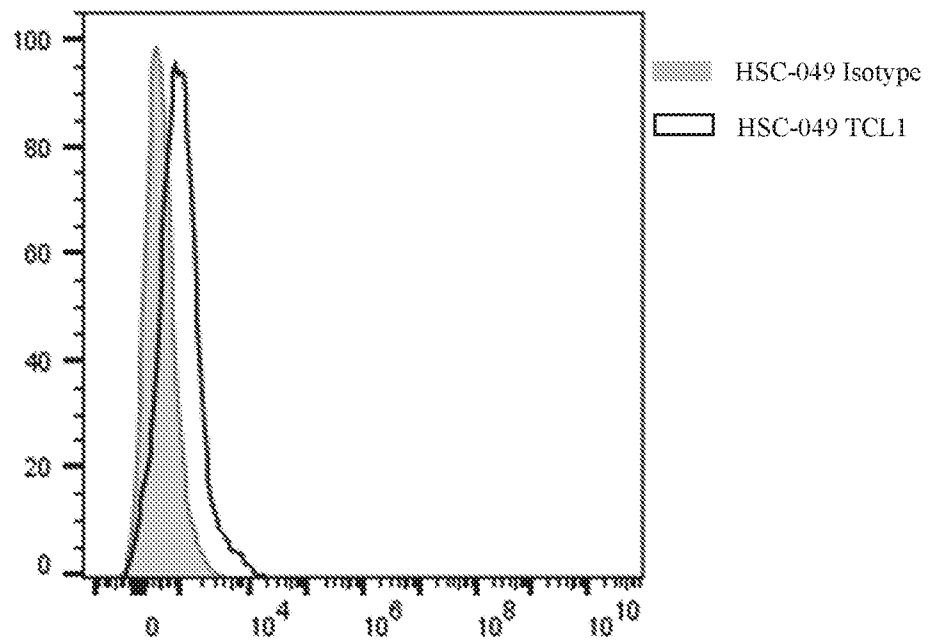


FIG. 6A

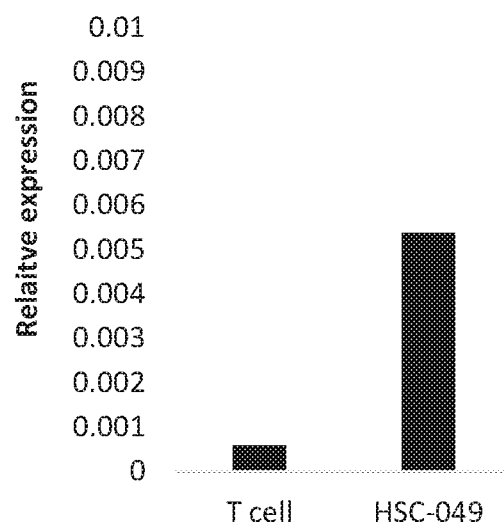


FIG. 6B

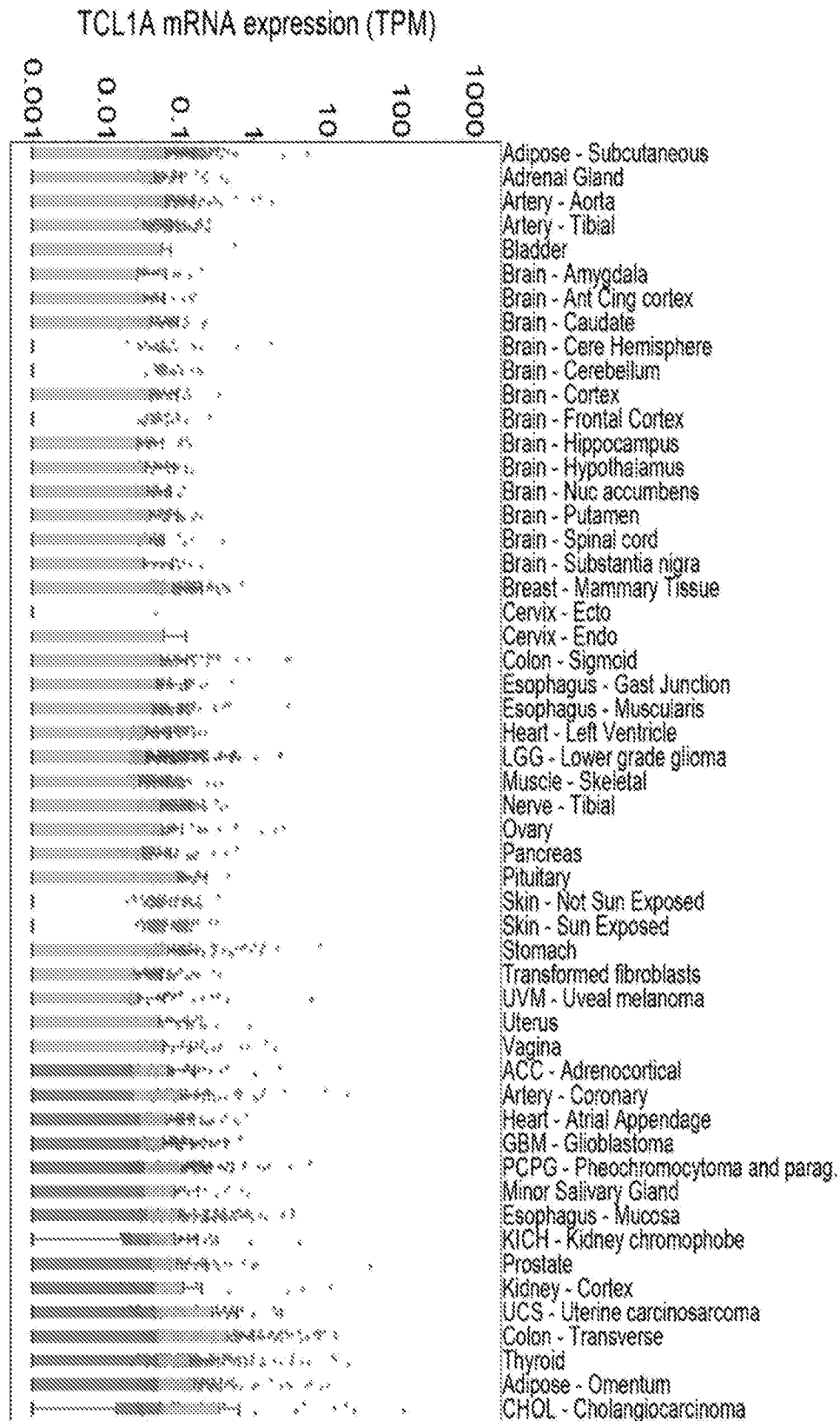


FIG. 6C

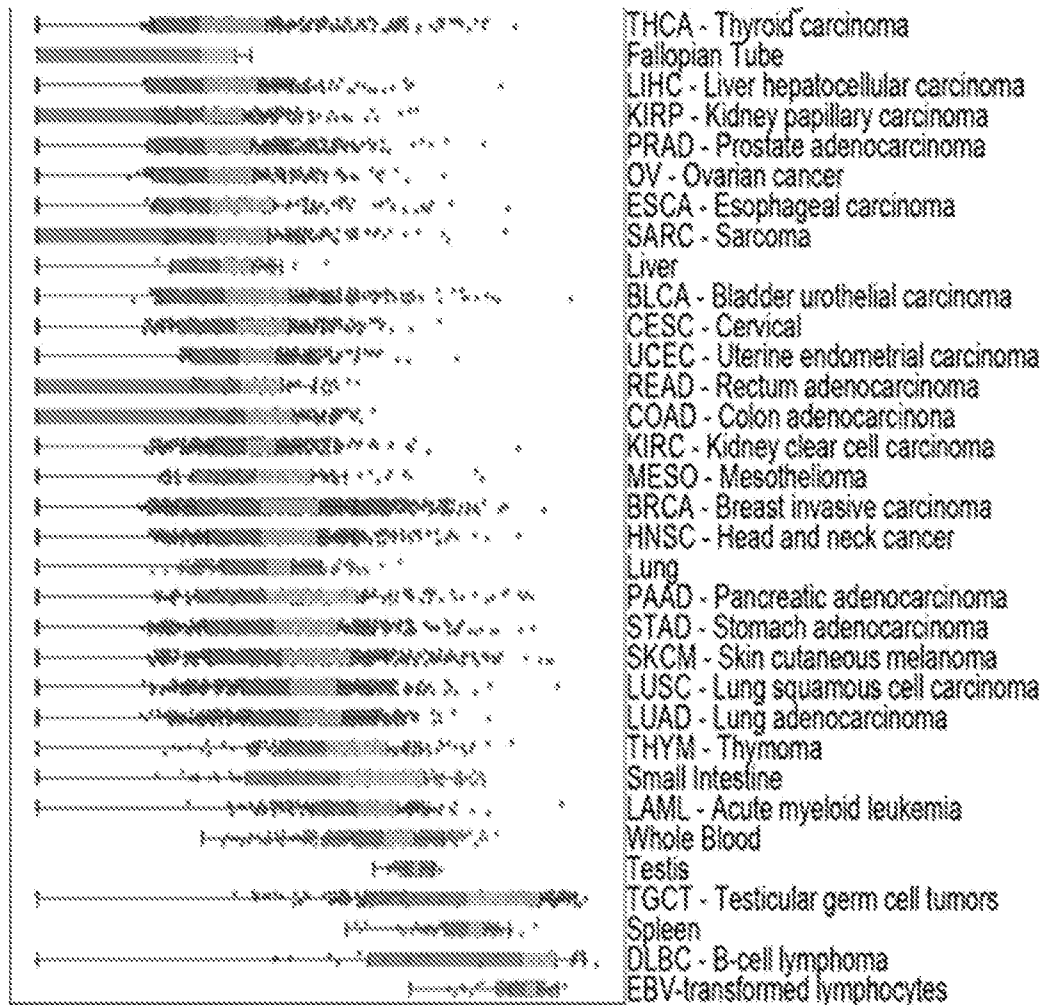


FIG. 6C (cont'd)

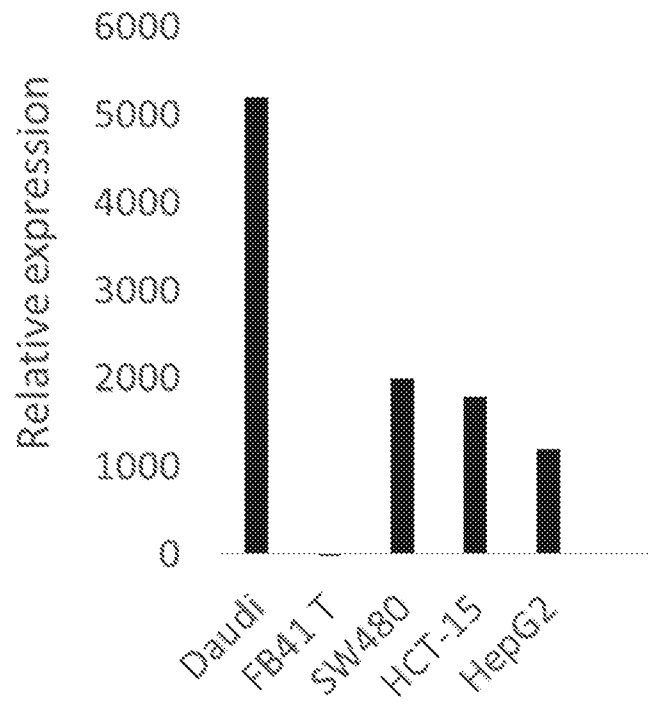


FIG. 7A

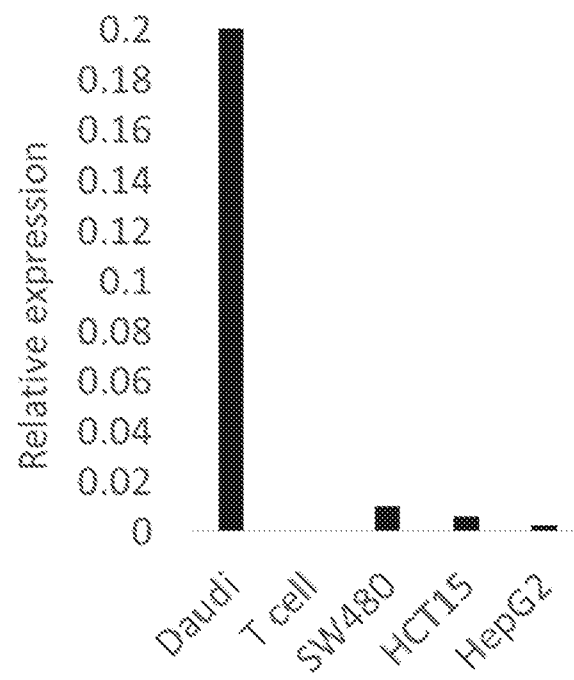


FIG. 7B

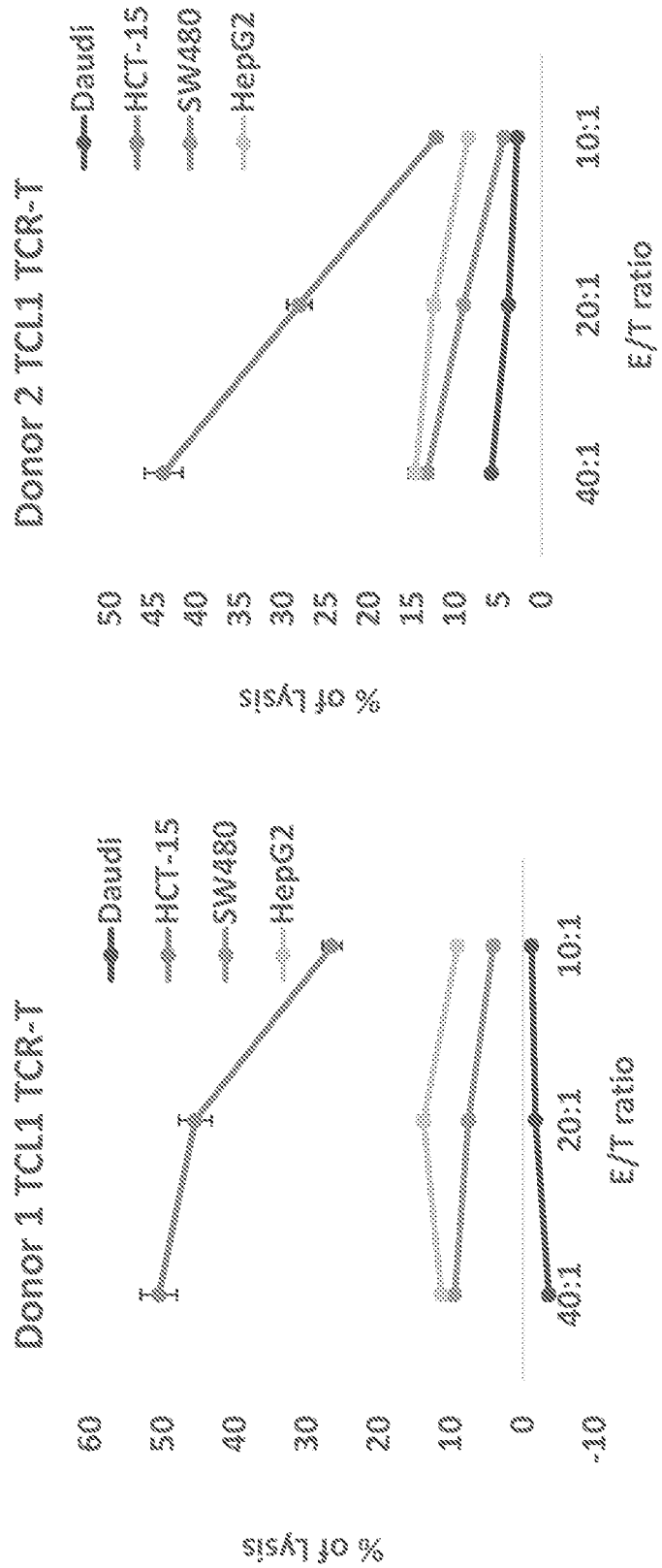


FIG.7C

TCL1 10mer	TCL1 70-70 peptides sequences	SEQ ID NO
A1	ALLPIMWQLY	9
A2	SALPIMWQLY	10
A3	SLAPIMWQLY	11
A4	SLLAIMWQLY	12
A5	SLLPAMWQLY	13
A6	SLLPIAWQLY	14
A7	SLLPIMAQLY	15
A8	SLLPIMWALY	16
A9	SLLPIMWQAY	17
A10	SLLPIMWQLA	18
G1	GLLPIMWQLY	19
G2	SGLPIMWQLY	20
G3	SLGPIMWQLY	21
G4	SLLGIMWQLY	22
G5	SLLPGMWQLY	23
G6	SLLPIGWQLY	24
G7	SLLPIMGQLY	25
G8	SLLPIMWGLY	26
G9	SLLPIMWQGY	27
G10	SLLPIMWQLG	28
10mer	SLLPIMWQLY	29
T2		

FIG. 8A

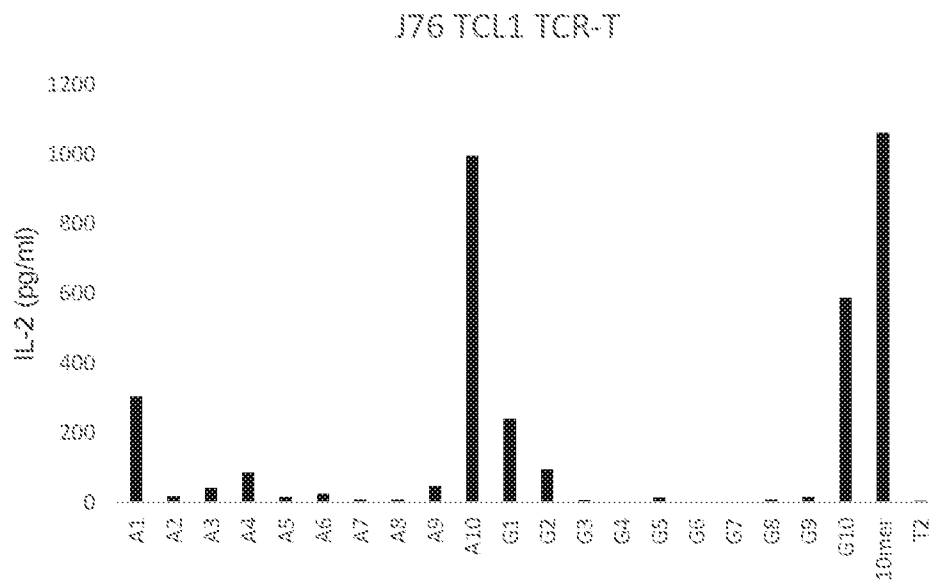


FIG. 8B

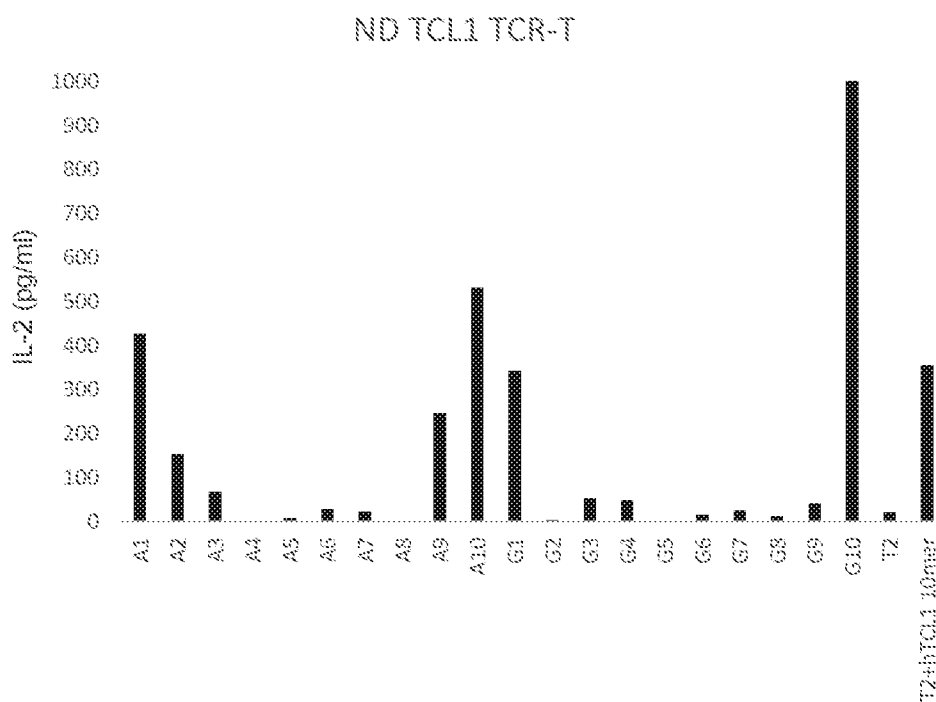


FIG. 8C

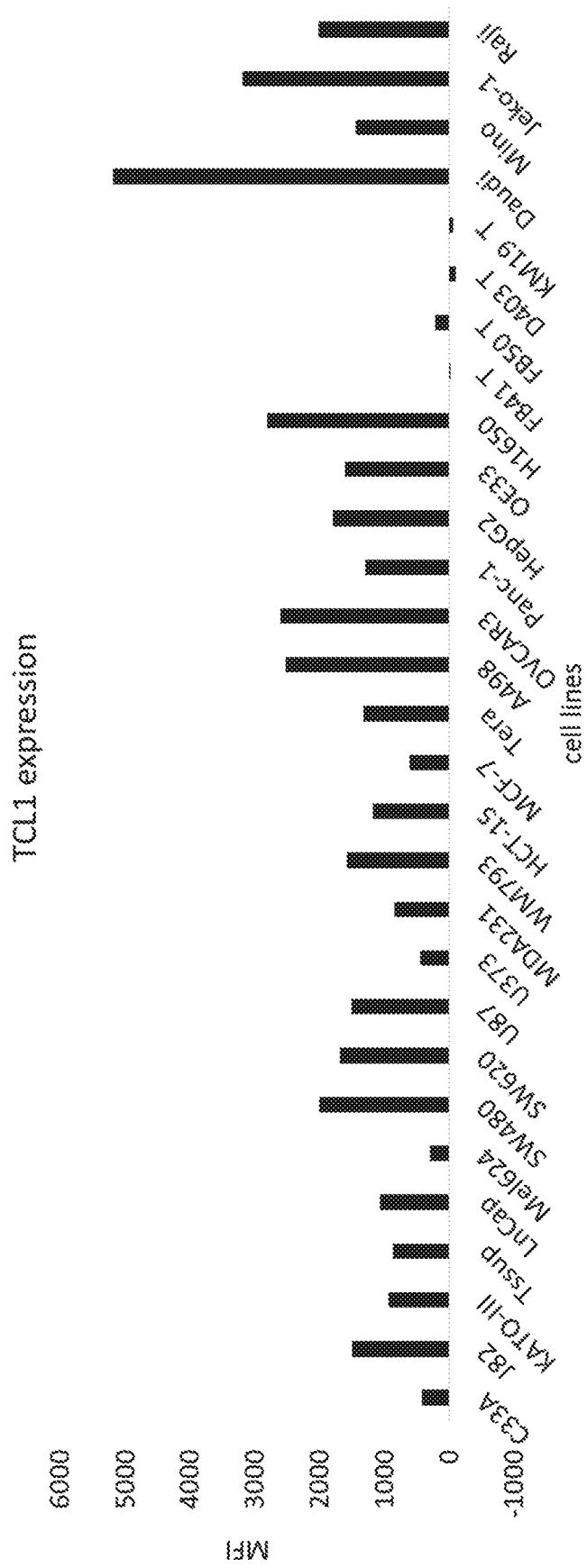


FIG. 9A

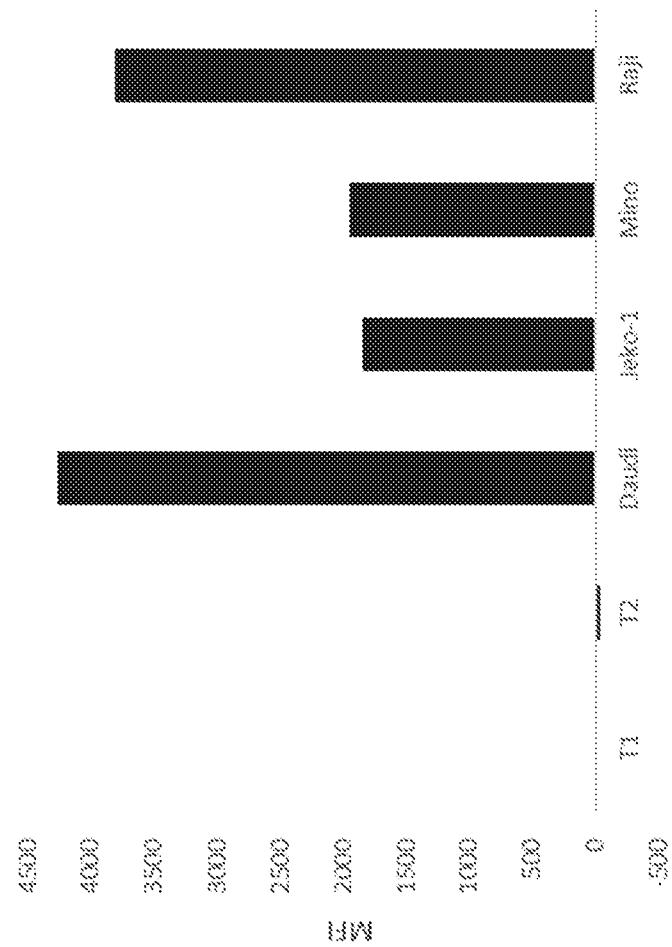


FIG. 9B

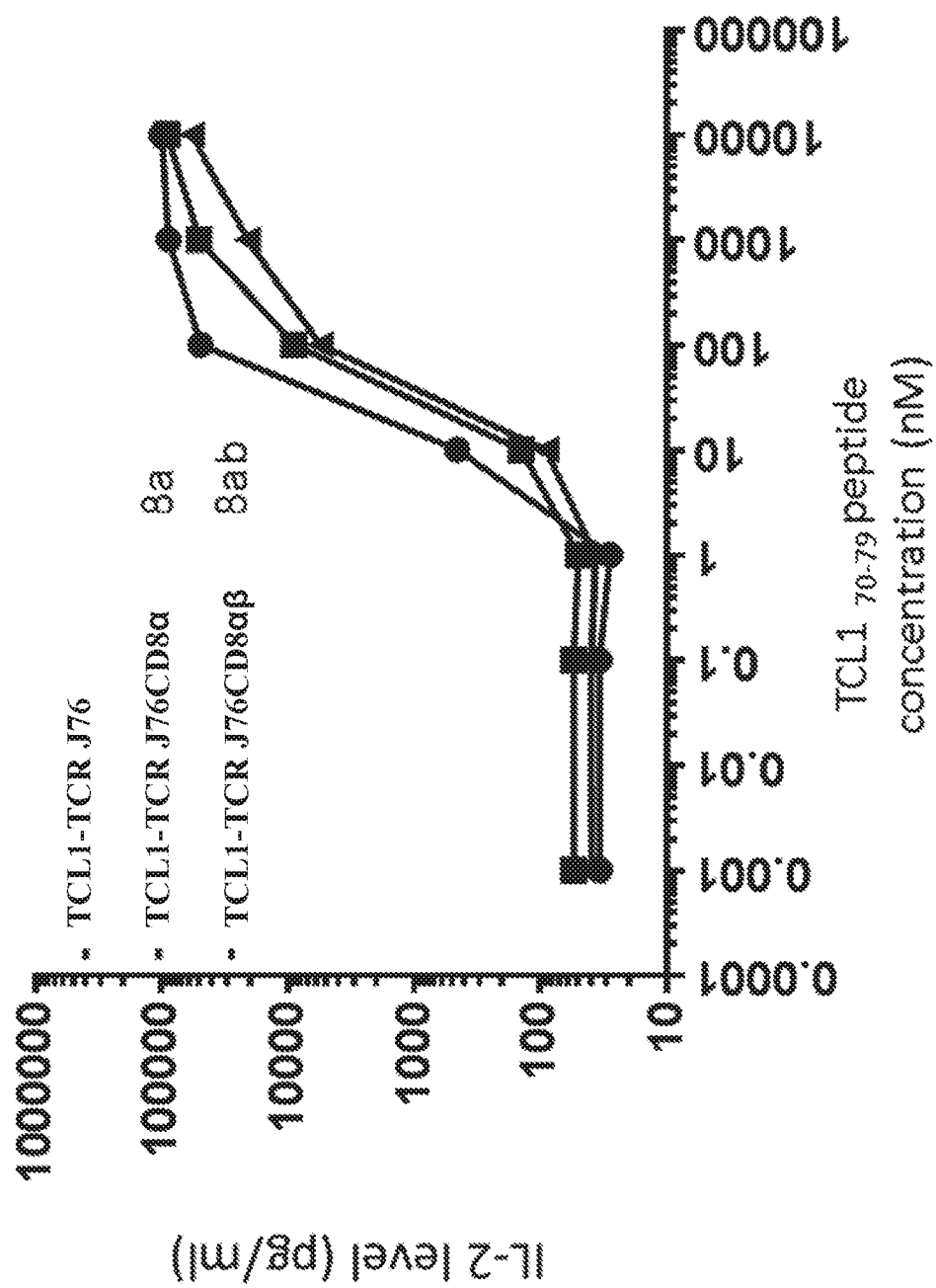


FIG. 10

TCL1-TCR alpha DNA sequence

ATGTGGGGCGCCTTCCTGCTGTACGTGTCCATGAAGATGGGAGGGAACCGCAGGACAGTCTCTGGAGCAGCCAAGC
GAGGTGACAGCAGTGGAGGGAGCAATCGTGCAGATCAACTGCACCTACCAGACAAGCGGCTTTTACGGCCTGTCC
TGGTATCAGCAGCACGACGGAGGAGCACCCACCTTCCTGAGCTATAATGGCCTGGATGGCCTGGAGGAGACAGGC
CGGTTTCACTCCTTTCTGTCTAGAAGCGACTCCTACGGCTATCTGCTGCTGCAGGAGCTGCAGATGAAGGATTCT
GCCAGCTACTTTTGTCTGCTGGGAAGCGGAGCAGGATCCTATCAGCTGACCTTCGGCAAGGGCACAAAGCTGTCC
GTGATCCCTAACATCCAGAACCCCCGACCCTGCCGTGTACCAGCTGCCGGACAGCAAGAGCAGCGACAAGAGCGTG
 TG

CCTGTTACCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTA
 CATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGT
 GGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCC
 CGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAACTGGTGAAAAAGAG
 CTTTCGAGACAGACACCAACCTGAACTTCCAGAACCTGAGCGTGATCGGCTTCCGGATCCT
 GCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCCGCTGTGGTCCAGCTGA

TCL1-TCR beta DNA sequence

ATGGGACCAGGCCTGCTGTGCTGGGTGCTGCTGTGCCTGCTGGGAGCAGGACCTGTGGATGCCGGCGTGACCCAG
AGCCCAACACACCTGATCAAGACCAGGGGACAGCACGTGACACTGAGGTGCTCCCCAATCTCTGGCCACAAGTCC
GTGTCTTGGTACCAGCAGGTGCTGGGACAGGGACCACAGTTTCTCTTTCAGTACTATGAGAAGGAGGAGCGGGGC
AGAGGCAACTTCCCCGACAGGTTTTCCGCCCCGCCAGTTTCTTAATTACTCTAGCGAGCTGAACGTGAATGCCCTG
CTGCTGGGCGACAGCGCCCTGTATCTGTGCGCCTCCTCTTTTACCGATGGCGGCACATACGAGCAGTATTTTCGGC
CCTGGCACCCAGGCTGACCGTGACAGAGGACCTGAAGAACGTGTTCCCCCTGAGGTGGCCGTGTTTGAGCCTTCC
 GAGGCCGAGATC
 TCTCACACCCAGAAGGCCACCCCTGGTGTGCCTGGCAACCGGCTTCTACCCAGATCACGTGGAGCTGTCTTGGTGG
 GTGAACGGCAAGGAGGTGCACAGCGGCGTGTGCACAGACCCACAGCCCCTGAAGGAGCAGCCCGCCCTGAATGAT
 TCCCGGTACTGTCTGAGCTCCAGGCTGCGCGTGTCTGCCACCTTTTGGCAGAACCCTCGGAATCACTTCAGATGC
 CAGGTGCAGTTTTTATGGCCTGTCCGAGAACGATGAGTGGACCCAGGACAGGGCAAAGCCAGTGACACAGATCGTG
 TCTGCCGAGGCATGGGGAAGAGCAGACTGTGGCTTCAACAGCGAGTCCATATCAGCAGGGCGTGTGAGCGCCACC
 ATCCTGTACGAGATCCTGCTGGGCAAGGCCACACTGTATGCCGTGCTGGTGTCTGCCCTGGTGTGATGGCCATG
 GTGAAGAGGAAGGATAGCCGCGGCTGA

FIG. 11

TCL1-TCR alpha protein sequence

MWGAFLLYVSMKMGGTAGQSLEQPSEVTAVEGAIVQIN
CTYQTSGFYGLSWYQQHDGGAPTFLSYNGLDGLEETGR
FSSFLSRSDSYGYLLLQELQMKDSASYFCLLGSGAGSY
QLTFGKGTKLSVIPNIQNPDPAVYQLRDSKSSDKSVCL
FTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSNSA
VAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLV
EKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRL
WSS

TCL1-TCR beta protein sequence

MGPGLLCWVLLCLLGAGPVDAGVTQSPTHLIKTRGQHV
TLRCSPISGHKSVSWYQQVLGQGPQFIFQYYEKEERGR
GNFPDRFSAROFPNYSSELNVNALLLGDSALYLCASSF
TDGGTYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEA
EISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVC
TDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR
CQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGF
TSESYQQGVLSATILYEILLGKATLYAVLVSALV
LMAMVKRKDSRG

Underlined: Highly variable region sequence of TCL1-TCR alpha and beta chain

FIG. 12

TCL1-TCR alpha DNA sequence

ATGTGGGGCGCCTTCCTGCTGTACGTGTCCATGAAGATGGGAGGAACCGCAGGACAGTCTCTGGAGCAGCCAAGC
 GAGGTGACAGCAGTGGAGGGAGCAATCGTGCAGATCAACTGCACCTACCAGACAAGCGGCTTTTACGGCCTGTCC
 TGGTATCAGCAGCACGACGGAGGAGCACCCACCTTCCTGAGCTATAATGGCCTGGATGGCCTGGAGGAGACAGGC
 CGGTTTCAGCTCCTTTCTGTCTAGAAGCGACTCCTACGGCTATCTGCTGCTGCAGGAGCTGCAGATGAAGGATTCT
 GCCAGCTACTTTTGTCTGCTGGGAAGCGGAGCAGGATCCTATCAGCTGACCTTCGGCAAGGGGCACAAAGCTGTCC
 GTGATCCCTAACATCCAGAACCCCGACCTGCCGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTG
 TG
 CCTGTTCACCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTA
 CATCACCGATAAGTGCGTGTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGT
 GGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCC
 CGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAACTGGTGGAAAAGAG
 CTTTCGAGACAGACACCAACCTGAACTTCCAGAACCTGAGCGTGATCGGCTTCCGGATCCT
 GCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA (SEQ ID NO:7)

TCL1-TCR beta DNA sequence

ATGGGACCAGGCCTGCTGTGCTGGGTGCTGCTGTGCCTGCTGGGAGCAGGACCTGTGGATGCCGGCGTGACCCAG
 AGCCCAACACACCTGATCAAGACCAGGGGACAGCACGTGACACTGAGGTGCTCCCCAATCTCTGGCCACAAGTCC
 GTGTCTTGGTACCAGCAGGTGCTGGGACAGGGACCACAGTTTCATCTTTCAGTACTATGAGAAGGAGGAGCGGGGC
 AGAGGCAACTTCCCCGACAGGTTTTCCGCCCCGCCAGTTTCCTAATTACTCTAGCGAGCTGAACGTGAATGCCCTG
 CTGCTGGGCGACAGCGCCCTGTATCTGTGCGCCTCCTCTTTTACCGATGGCGGCACATACGAGCAGTATTTCCGGC
 CCTGGCACCAGGCTGACCGTGACAGAGGACCTGAAGAACGTGTTCCCCCCTGAGGTGGCCGTGTTTGAGCCTTCC
 GAGGCCGAGATC
 TCTCACACCCAGAAGGCCACCCCTGGTGTGCCTGGCAACCGGCTTCTACCCAGATCACGTGGAGCTGTCTTGGTGG
 GTGAACGGCAAGGAGGTGCACAGCGGCGTGTGCACAGACCCACAGCCCCTGAAGGAGCAGCCCGCCCTGAATGAT
 TCCCGGTACTGTCTGAGCTCCAGGCTGCGCGTGTCTGCCACCTTTTGGCAGAACCCTCGGAATCATTTCAGATGC
 CAGGTGCAGTTTTATGGCCTGTCCGAGAACGATGAGTGGACCCAGGACAGGGCAAAGCCAGTGACACAGATCGTG
 TCTGCCGAGGCATGGGGAAGAGCAGACTGTGGCTTACCAGCGAGTCCTATCAGCAGGGCGTGCTGAGCGCCACC
 ATCCTGTACGAGATCCTGCTGGGCAAGGCCACACTGTATGCCGTGCTGGTGTCTGCCCTGGTGTGATGGCCATG
 GTGAAGAGGAAGGATAGCCGCGGCTGA (SEQ ID NO:8)

Underlined: CDR1, CDR2, CDR3 sequence of TCL1-TCR alpha and beta sequence

FIG. 13

TCL1-TCR alpha protein sequence

MWGAFLLYVSMKMGGTAGQSLEQPSSEVTAVEGAIVQIN
CTYQTSGGFYGLSWYQQQHDGGAPTFLSYNGLDGLEETGR
FSSFLSRSDSYGYLLLLQELQMKDSAS YFC LLGSGAGSY
QLTFGKGTKLSVIPN IQNPDPAVYQLRDSKSSDKSVCL
FTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSN SA
VAWSNKSDFACANAFNNSIIPEDTFFFPSP ESSC D V K L V
EKS FETDTN LNFQNL SVIGFRILL LKVAGFNLLMTLR L
W S S (SEQ ID NO:3)

TCL1-TCR beta protein sequence

MGPGLLCWVLLCLLGAGPVDAGVTQSPTHLIKTRGQH V
TLRCSPIS SGHKSVSWYQQQVLGQQGPQFIFQ YYEKEEERGR
GNFPDRFSARQFPNYSSELNVNALLLGDSALYLC ASSF
TDGGTYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEA
EISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVC
TDPQPPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFR
CQVQFYGLSENDEWTQDRAKPV TQIVSAEAWGRADCGF
TSESYQQGVLSATILYEILLGKATLYAVLVSA L V
L M A M V K R K D S R G (SEQ ID NO:4)

Underlined: CDR1, CDR2, CDR3 sequence of TCL1-TCR alpha and beta sequence

FIG. 13 – cont'd

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - A61K 35/12, A61K 35/76, A61K 38/00 (2019.01)
 CPC - A61K 38/00, A61K 35/17, C12N 5/0636

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016/070119 A1 (BAYLOR COLLEGE OF MEDICINE) 6 May 2016 (06.05.2016); para [0007]-[0008], [0012], [0016], [0123]	1-7 and 10-17
A	WO 2013/075105 A2 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 23 May 2013 (23.05.2013); para [0002], [0006], [0115]	1-7 and 10-17
A	WO 2017/216324 A1 (MEDIGENE IMMUNOTHERAPIES GMBH) 21 December 2017 (21.12.2017); para [41]; SEQ ID NO: 34, 87 and 183	1-7 and 10-17
A	US 2015/0010631 A1 (COUR PHARMACEUTICALS DEVELOPMENT COMPANY) 8 January 2015 (08.01.2015); SEQ ID NO: 645	1-7 and 10-17
A	US 2015/0337369 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 26 November 2015 (26.11.2015); abstract; para [0151], SEQ ID NO: 1617	1-7 and 10-17
A	US 2014/0315735 A1 (INTERNATIONAL INSTITUTE OF CANCER IMMUNOLOGY, INC) 23 October 2014 (23.10.2014); abstract; SEQ ID NO: 2877	1-7 and 10-17

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 April 2019

Date of mailing of the international search report

13 MAY 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
 P.O. Box 1450, Alexandria, Virginia 22313-1450
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Lee W. Young

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 PCT OSP: 571-272-7774

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Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 18-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

*****Continued in Supplemental Box*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-7, 10 and 11-17(in part) limited to SEQ ID NOS: 1 and 2

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

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Continuation of Box No. III (Observations where unity of invention is lacking):

Group I+, claims 1-17, directed to an engineered T cell receptor (TCR) specific for a T-cell leukemia/lymphoma 1 (TCL1) or a TCL1 peptide, and a multivalent TCR complex comprising a plurality of said TCRs. The TCL1 specific TCR, and complexes thereof will be searched to the extent that the TCL1 TCR encompass an alpha chain variable region (VR) having the amino acid sequence of SEQ ID NO: 1 and a beta chain VR having the amino acid sequence of SEQ ID NO: 2 (note: SEQ ID NO:1 comprises SEQ ID Nos: 54, 55, 56, and SEQ ID NO: 2 comprises SEQ ID Nos: 57, 58, 59). It is believed that claims 1-7, 10 and 11-17(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that the TCL1 TCR alpha and beta chain VR sequences encompass SEQ ID Nos: 1 and 2, respectively. Additional TCL1 TCR alpha and beta chain VR sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected TCL1 TCR alpha and beta chain VR sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be TCL1 TCR alpha and beta chain VR sequences comprising SEQ ID Nos: 3 and 4, respectively, (claims 1, 8-10 and 11-17(in part)).

Group II, claims 69-71, directed to a method of treating a cancer in a mammalian subject.

The inventions listed as Groups I+ and II do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of an engineered TCR specific for a TCL1 or a TCL1 peptide, or a multivalent TCR complex comprising a plurality of TCRs, that is not required by Group II.

Group II has the special technical feature of administering a therapeutically effective amount of a TCL1-targeting immunotherapy to the subject, wherein the cancer is a solid tumor, that is not required by Group I+.

The inventions of Group I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

Common technical features

The inventions of Group I+ and Group II share the common technical feature of a TCL1-targeting immunotherapy.

No technical features are shared between the TCL1 TCR amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including: an engineered TCR having antigenic specificity for TCL1 or a TCL1 peptide, comprising an amino acid sequence, and a multivalent TCR complex comprising a plurality of TCRs, these shared technical features are previously made obvious by WO 2016/070119 A1 to Baylor College Of Medicine (hereinafter 'Baylor') in view of WO 2013/075105 A2 to Board Of Regents, The University Of Texas System (hereinafter 'Univ Texas').

Baylor teaches engineered TCRs having antigenic specificity for cancer antigens comprising an amino acid sequence (para [0007]-[0008] "there are compositions having cells with a modified T cell receptor comprising particular alpha and beta chains that provide selective antitumor activity"; [0012] "the cells express at least one non-endogenous molecule that targets a particular tumor antigen, and in at least some cases, the molecule comprises a TCR"), wherein the TCR comprises the amino acid sequences (para [0016]), however fails to teach wherein the antigens are T-cell leukemia/lymphoma 1 (TCL1) or a TCL1 peptide.

Univ Texas teaches TCL1 is a t-cell target, and use of TCL1 specific antibodies for treating cancer (para [0006] "The present invention is based, in part, on the discovery that peptides of the intracellular protein TCL1 are provided by MHC I (HLA-A2) on the surface of tumor cells that are recognized by T-cell receptors on T cells. In various aspects, TCL1 peptides are provided that can bind MHC I (HLA-A2) and can be recognized by T-cell receptors on T cells. The TCL1 peptides may be therapeutically used to treat a cancer"; para [0002] "epitopes of TCL1 that are recognized by T cells and may be used to treat a cancer."; para [0115] "The present invention further provides antibodies against a TCL1 peptide of the present invention or a TCL1 peptide-HLA-A2 complex"). Given that Univ Texas suggests that TCL1 can be recognized by T-cell receptors and wherein targeting TCL1 may be an effective method of treating cancer, it would have been obvious to an artisan of ordinary skill in the art to utilize the method taught by Baylor to engineer TCRs with specificity for TCL1.

Baylor further teaches use of a plurality of TCRs (para [0073]-[0074] "provided herein is a cell that has been genetically engineered to express one or more TCRs...RNAs or proteinaceous sequences may be co expressed with other selected RNAs or proteinaceous sequences in the same cell, such as the same CTL. Co expression may be achieved by co transfecting the CTL with two or more distinct recombinant vectors. Alternatively, a single recombinant vector may be constructed to include multiple distinct coding regions for RNAs, which could then be expressed in CTLs transfected with the single vector"; para [0081] "In the light of the present disclosure, the "proteinaceous compounds" providing an activation signal for immune effector cells may be, e.g. a further activation signal for T-cells (e.g. a further costimulatory molecule: molecules of the B7- family, OX40 L, 4-1BBL"; para [0084] "a TCR that has a high-avidity and reactivity toward target antigens that is selected, cloned"). It would have been obvious to one of ordinary skill in the art to have included said different TCRs in a multivalent TCR complex during the ordinary course of experimentation, in order to optimize targeting and binding efficacy.

*****Continued in Supplemental Box*****

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Continuation of Previous Page:

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 18-68 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).