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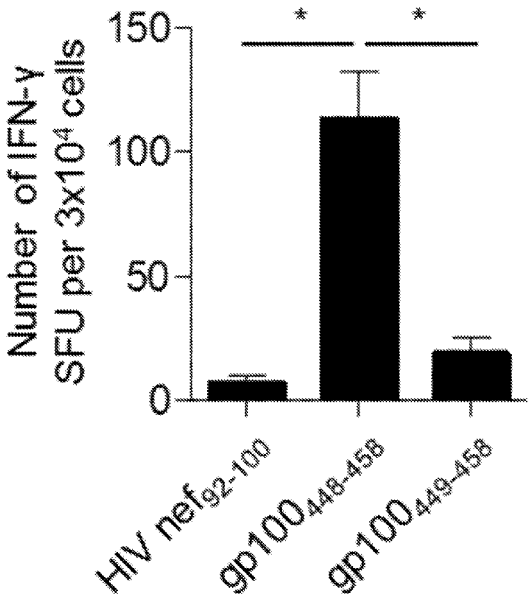
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(54) Title: T CELL RECEPTORS AND METHODS OF USE THEREOF

FIG. 3



(57) Abstract: The present disclosure is directed recombinant T cell receptors capable of binding a gp100 epitope and nucleic acid molecules encoding the same. In some embodiments, the nucleic acid molecules further comprise a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR. Other aspects of the disclosure are directed to vectors comprising the nucleic acid molecule and cells comprising the recombinant TCR, the nucleic acid molecule, or the vector. Still other aspects of the disclosure are directed to methods of using the same. In some embodiments, the methods comprise treating a cancer in a subject in need thereof.

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T CELL RECEPTORS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 62/813,645, filed March 4, 2019, which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0002] The content of the electronically submitted sequence listing (Name: 4285_004PC01_Seqlisting_ST25.txt, Size: 57,727 bytes; and Date of Creation: March 3, 2020) is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides recombinant T cell receptors ("TCRs") that specifically bind human gp100 and uses thereof.

BACKGROUND OF THE DISCLOSURE

[0004] Immunotherapy has immerged as a critical tool in the battle against a variety of diseases, including cancer. T cell therapies are at the forefront of immunotherapeutic development, and adoptive transfer of antitumor T cells has been shown induce clinical responses in cancer patients. Though many T cell therapies target mutated tumor antigens, the vast majority of neoantigens are not shared and are unique to each patient.

[0005] Potential non-mutated antigens out number mutated antigens by multiple orders of magnitude. The elucidation of T cell epitopes derived from shared antigens may facilitate the robust development of efficacious and safe adoptive T cell therapies that are readily available to a larger cohort of cancer patients. However, the sheer number of non-mutated antigens and the high polymorphism of HLA genes may have hampered comprehensive analyses of the specificity of antitumor T cell responses toward non-mutated antigens.

[0006] The present disclosure provides novel epitopes for the non-mutated antigen gp100 and TCRs capable of specifically binding the epitopes. These novel epitopes are associated with particular HLA alleles. The use of these tumor-reactive HLA-restricted gp100 TCRs stand to widen the applicability of anti-gp100 TCR gene therapy, particularly in immuno-oncology.

SUMMARY OF THE DISCLOSURE

[0007] Certain aspects of the present disclosure are directed to a nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR, wherein the anti-gp100 TCR cross competes for binding to human gp100 with a reference TCR, which comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

[0008] Certain aspects of the present disclosure are directed to a nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR, wherein the anti-gp100 TCR binds the same epitope or an overlapping epitope of human gp100 as a reference TCR, which comprises an alpha chain and a beta chain, wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

[0009] In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, the epitope is complexed with an HLA class I molecule.

[0010] In some embodiments, the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele. In some embodiments, the HLA class I molecule is an HLA-B*40 allele. In some embodiments, the HLA class I molecule is selected from an HLA-B*40:01 allele, HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele. In some embodiments, the HLA class I molecule is an HLA-B*40:01 allele.

[0011] In some embodiments, the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and wherein the beta chain

comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3; wherein the alpha chain CDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

[0012] In some embodiments, the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3; wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

[0013] In some embodiments, the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8. In some embodiments, the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6. In some embodiments, the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

[0014] In some embodiments, the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 1. In some embodiments, the beta chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 2.

[0015] In some embodiments, the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant region of the alpha chain. In some embodiments, the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 1. In some embodiments, the alpha chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence

set forth SEQ ID NO: 1. In some embodiments, the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant regions of the beta chain.

[0016] In some embodiments, the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the beta chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 2. In some embodiments, the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 2. In some embodiments, the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1.

[0017] In some embodiments, the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2. In some embodiments, the second nucleotide sequence is one or more siRNAs that reduce the expression of endogenous TCRs.

[0018] In some embodiments, the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the endogenous TCRs. In some embodiments, the one or more siRNAs comprise one or more nucleotide sequences selected from the group consisting of SEQ ID NOs: 53-56.

[0019] In some embodiments, the second nucleotide sequence encodes Cas9.

[0020] In some embodiments, the anti-gp100 TCR comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.

[0021] Certain aspects of the present disclosure are directed to a vector comprising a nucleic acid molecule disclosed herein. In some embodiments, the vector is a viral vector, a mammalian vector, or bacterial vector. In some embodiments, the vector is a retroviral vector. In some embodiments, the vector is selected from the group consisting of an adenoviral vector, a lentivirus, a Sendai virus vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, a

hybrid vector, and an adeno associated virus (AAV) vector. In some embodiments, the vector is a lentivirus.

[0022] Certain aspects of the present disclosure are directed to a T cell receptor (TCR) or an antigen binding portion thereof comprising an alpha chain variable domain of the anti-gp100 TCR disclosed herein and a beta chain variable domain of the anti-gp100 TCR disclosed herein. In some embodiments, the recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which cross competes for binding to human gp100 with a reference TCR; wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; and wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein (i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or (ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence of SEQ ID NO: 2.

[0023] Certain aspects of the present disclosure are directed to a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which binds the same epitope or an overlapping epitope of human gp100 as a reference TCR; wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; and wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein (i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or (ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO:

2. In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.

[0024] In some embodiments, the epitope is complexed with an HLA class I molecule. In some embodiments, the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele. In some embodiments, the HLA class I molecule is an HLA-B*40 allele. In some embodiments, the HLA class I molecule is selected from an HLA-B*40:01 allele, an HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele. In some embodiments, the HLA class I molecule is an HLA-B*40:01 allele.

[0025] In some embodiments, the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and wherein the beta chain of the anti-gp100 TCR comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3; wherein the alpha chain CDR3 of the anti-gp100 comprises an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

[0026] In some embodiments, the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chainCDR1, an alpha chain CDR2, and an alpha chain CDR3; and wherein the beta chain of the anti-gp100 TCR comprises a variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3; wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

[0027] In some embodiments, the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8. In some embodiments, the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6. In some embodiments, the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

[0028] In some embodiments, the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the beta chain variable

domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 2.

[0029] In some embodiments, the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 1.

[0030] In some embodiments, the beta chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 2.

[0031] In some embodiments, the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1. In some embodiments, the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

[0032] Certain aspects of the present disclosure are directed to a bispecific TCR comprising a first antigen-binding domain and a second antigen-binding domain, wherein the first antigen-binding domain comprises a TCR or an antigen-binding portion thereof disclosed herein or a TCR or an antigen-binding portion thereof disclosed herein. In some embodiments, the first antigen-binding domain comprises a single chain variable fragment ("scFv"). In some embodiments, the second antigen-binding domain binds specifically to a protein expressed on the surface of a T cell. In some embodiments, the second antigen-binding domain binds specifically to CD3. In some embodiments, the second antigen-binding domain comprises an scFv. In some embodiments, the first antigen-binding domain and the second antigen-binding domain are linked or associated by a covalent bond. In some embodiments, the first antigen-binding domain and the second antigen-binding domain are linked by a peptide bond.

[0033] Certain aspects of the present disclosure are directed to a cell comprising a nucleic acid molecule disclosed herein, a vector disclosed herein, a TCR disclosed herein, a recombinant TCR disclosed herein, or a bispecific TCR disclosed herein. In some embodiments, the cell further expresses CD3. In some embodiments, the cell is selected from the group consisting of a T cell, a natural killer (NK) cell, an natural killer T (NKT) cell, or an ILC cell.

[0034] Certain aspects of the present disclosure are directed to a method of treating a cancer in a subject in need thereof, comprising administering to the subject a cell disclosed herein. In some embodiments, the cancer is selected from the group consisting of melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers.

[0035] In some embodiments, the cancer is relapsed or refractory. In some embodiments, the cancer is locally advanced. In some embodiments, the cancer is advanced. In some embodiments, the cancer is metastatic.

[0036] In some embodiments, the cells are obtained from the subject. In some embodiments, the cells are obtained from a donor other than the subject. In some embodiments, the subject is preconditioned prior to the administering of the cells. In some embodiments, the preconditioning comprises administering to the subject a chemotherapy, a cytokine, a protein, a small molecule, or any combination thereof. In some embodiments, the preconditioning comprises administering an interleukin. In some embodiments, the preconditioning comprises administering IL-2, , IL-4, IL-7, IL-9, IL-15, IL-21, or any combination thereof. In some embodiments, the preconditioning comprises administering a preconditioning agent selected from the group consisting of cyclophosphamide, fludarabine, vitamin C, an AKT inhibitor, ATRA, Rapamycin, or any

combination thereof. In some embodiments, the preconditioning comprises administering cyclophosphamide, fludarabine, or both.

[0037] Certain aspects of the present disclosure are directed to a method of engineering an antigen-targeting cell, comprising transducing a cell collected from a subject in need of a T cell therapy with a nucleic acid disclosed herein or a vector disclosed herein. In some embodiments, the antigen-targeting cell further expresses CD3. In some embodiments, the cell is a T cell or a natural killer (NK) cell.

[0038] Certain aspects of the present disclosure are directed to an HLA class I molecule complexed to a peptide, wherein the HLA class I molecule comprises an α1 domain, an α2 domain, an α3 domain and a β2m, and wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 14.

[0039] In some embodiments, the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G. In some embodiments, the HLA class I molecule is an HLA-B. In some embodiments, the HLA class I molecule is an HLA-B*40 allele. In some embodiments, the HLA class I molecule is selected from an HLA-B*40:01 allele, HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele. In some embodiments, the HLA class I molecule is an HLA-B*40:01 allele. In some embodiments, the HLA class I molecule is an HLA-B*40:02 allele.

[0040] In some embodiments, the HLA class I molecule is a monomer. In some embodiments, the HLA class I molecule is a dimer. In some embodiments, the HLA class I molecule is a trimer. In some embodiments, the HLA class I molecule is a tetramer. In some embodiments, the HLA class I molecule is a pentamer.

[0041] Certain aspects of the present disclosure are directed to an antigen presenting cell (APC), comprising an HLA class I molecule disclosed herein. In some embodiments, the HLA class I molecule is expressed on the surface of the APC.

[0042] Certain aspects of the present disclosure are directed to a method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells with an HLA class I molecule disclosed herein or an APC disclosed herein, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class I molecule relative to the number of T cells capable of binding the HLA class I molecule prior to the contacting.

[0043] Certain aspects of the present disclosure are directed to a method of enriching a target population of T cells obtained from a human subject, comprising contacting the T

cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 13, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of targeting a tumor cell relative to the number of T cells capable of targeting a tumor cell prior to the contacting.

[0044] In some embodiments, the T cells obtained from the human subject are tumor infiltrating lymphocytes (TIL).

[0045] Certain aspects of the present disclosure are directed to a method of treating a tumor in a subject in need thereof, comprising administering to the subject an enriched population of T cells disclosed herein.

[0046] Certain aspects of the present disclosure are directed to a method of enhancing cytotoxic T cell-mediated targeting of cancer cells in a subject afflicted with a cancer, comprising administering to the subject a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.

[0047] Certain aspects of the present disclosure are directed to a cancer vaccine comprising a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.

[0048] Certain aspects of the present disclosure are directed to a method of selecting a T cell capable of targeting a tumor cell, comprising contacting a population of isolated T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 11. In some embodiments, the T cell is a tumor infiltrating lymphocytes (TIL).

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] FIG. 1 is a bar graph illustrating the number of B*40:01/gp100 T cells in melanoma TILs following stimulation with artificial APCs pulsed with overlapping peptides. The TILs stimulated once with B*40:01-artificial APCs pulsed with overlapping peptides to cover the whole protein of gp100 were employed as responder cells in IFN-γ ELISPOT analysis. B*40:01-artificial APCs pulsed with gp100-derived overlapping peptides were used as stimulator cells. Following one controlled peptide-specific stimulation, the TILs showed positive responses to two adjacent peptides with the shared sequence $_{446}$STESITGSLGPLLDG$_{460}$ (*see also* Table 5).

[0050] FIGs. 2A-2D are graphical representations of B*40:01/gp100$_{448\text{-}458}$ multimer staining of melanoma TILs. The TILs were stimulated once with B*40:01-artificial APCs pulsed with the gp100$_{448}$ESITGSLGPLL$_{458}$ peptide. Data on B*40:01/gp100$_{448\text{-}458}$ (FIGs. 2A-2B) or control B*40:01/HIV nef$_{92\text{-}100}$ (FIGs. 2C-2D) multimer staining before

stimulation (day 0; FIGs. 2A and 2C) and 14 days after stimulation (day 14; FIGs. 2B and 2D) are shown. The percentage of multimer$^+$ cells in CD8$^+$ T cells is shown.

[0051] FIG. 3 is a bar graph illustrating the functional assessment of B*40:01/gp100$_{448-458}$ multimer-positive melanoma TILs. IFN-γ production by the TILs in a B*40:01/gp100$_{448-458}$-specific manner following one peptide-specific stimulation. The TILs stimulated once with B*40:01-artificial APCs pulsed with the gp100$_{448-458}$ peptide were employed as responder cells in IFN-γ ELISPOT analysis. B*40:01-artificial APCs pulsed with the indicated peptides were used as stimulator cells. The HIV nef$_{92-100}$ and gp100$_{448-458}$ peptides were employed as controls. Experiments were carried out in triplicate, and error bars depict SD. $*P < 0.05$.

[0052] FIGs. 4A-4I are graphical representations of positive staining of Jurkat 76/CD8 cells transduced with B*40:01/gp100$_{448-458}$ TCR genes with a cognate multimer. Jurkat 76/CD8 cells transduced with the B*40:01/gp100$_{448-458}$ TCR (FIGs. 4B, 4E, and 4H) were stained with the B*40:01/gp100$_{448-458}$ multimer (FIG. 4B). The B*40:01/NY-ESO-1$_{125-133}$ multimer (FIGs. 4D, 4E, and 4F), B*40:01/unexchanged multimer (FIGs. 4G, 4H, and 4I), and Jurkat 76/CD8 cells transduced with B*40:01/NY-ESO-1$_{125-133}$ TCR, and untransduced (FIGs. 4A, 4D, and 4G) were employed as controls. The percentage of multimer$^+$ CD8$^+$ cells is shown.

[0053] FIGs. 5A-5D are graphical representations of positive staining of human primary T cells transduced with B*40:01/gp100$_{448-458}$ TCR genes (FIGs. 5B and 5D) with a cognate multimer. Primary T cells transduced with the B*40:01/gp100$_{448-458}$ TCR were stained with the B*40:01/gp100$_{448-458}$ (FIG. 5B) or B*40:01/HIV nef$_{92-100}$ control multimer (FIG. 5D). Untransduced primary T cells were employed as negative controls (FIGs. 5A and 5C). The percentage of multimer$^+$ CD8$^+$ T cells is shown.

[0054] FIG. 6 is a bar graph illustrating that human primary T cells transduced with B*40:01/gp100$_{448-458}$ TCR genes react strongly with the cognate peptide presented by the target class I molecule. Primary T cells transduced with B*40:01/gp100$_{448-458}$ TCR genes or untransduced primary T cells were used as responder cells in IFN-γ ELISPOT analysis. HLA-B*40:01-transduced T2 cells (T2-B*40:01) were generated. T2 or T2-B*40:01 cells pulsed with the gp100$_{448-458}$ or HIV nef$_{92-100}$ peptide (control) were used as stimulator cells. Experiments were carried out in triplicate, and error bars depict SD. $*P < 0.05$, $**P < 0.01$.

[0055] FIG. 7A is a graphical representation illustrating that primary T cells transduced with B*40:01/gp100$_{448-458}$ TCR genes recognize tumor cells. Primary T cells

transduced with B*40:01/gp100$_{448-458}$ TCR genes or untransduced primary T cells were employed as responder cells in IFN-γ ELISPOT analysis. SK-MEL-28 and SK-MEL-37 cells that were either untransduced or transduced with HLA-B*40:01 and/or gp100, as indicated in FIG. 7B (legend for FIG. 7A), were employed as stimulator cells following treatment with 100 ng/ml IFNγ for 48 hours. Experiments were carried out in triplicate, and error bars depict SD. $*P < 0.05$, $**P < 0.01$.

[0056] FIGs. 8A-8D are graphical representations of the expression of gp100 derived from endogenous or transduced full-length gene. The expression of gp100 derived from endogenous or transduced full-length gene in target cells was analyzed via intracellular flow cytometry following staining with anti-gp100 mAb (open curve) and an isotype control (filled curve).

[0057] FIGs. 9A-9B are graphical representations of the expression of ΔNGFR in target cells transduced with the full-length HLA-B*40:01 gene tagged with ΔNGFR (FIG. 9B). Surface expression of ΔNGFR in target cells transduced with the full-length HLA-B*40:01 gene tagged with ΔNGFR was analyzed by flow cytometry following staining with an anti-NGFR mAb (open curve) and an isotype control (filled curve). ΔNGFR alone was used as a control (FIG. 9A).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0058] The present disclosure is directed to TCRs or antigen binding portions thereof that specifically bind to an epitope on gp100, nucleic acid molecules that encode the same, and cells that comprise the TCR or the nucleic acid molecule. Some aspects of the present disclosure are directed to methods of treating a caner in a subject in need thereof, comprising administering to the subject the cell. Other aspects of the present disclosure are directed to HLA class I molecules complexed to a peptide comprising the epitope of gp100.

I. Terms

[0059] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0060] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more

nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0061] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0062] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

[0063] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0064] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0065] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0066] "Administering" refers to the physical introduction of an agent to a subject, using any of the various methods and delivery systems known to those skilled in the art.

Exemplary routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, *e.g.*, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

[0067] The term "T cell receptor" (TCR), as used herein, refers to a heteromeric cell-surface receptor capable of specifically interacting with a target antigen. As used herein, "TCR" includes but is not limited to naturally occurring and non-naturally occurring TCRs; full-length TCRs and antigen binding portions thereof; chimeric TCRs; TCR fusion constructs; and synthetic TCRs. In human, TCRs are expressed on the surface of T cells, and they are responsible for T cell recognition and targeting of antigen presenting cells. Antigen presenting cells (APCs) display fragments of foreign proteins (antigens) complexed with the major histocompatibility complex (MHC; also referred to herein as complexed with an HLA molecule, *e.g.*, an HLA class 1 molecule). A TCR recognizes and binds to the antigen:HLA complex and recruits CD3 (expressed by T cells), activating the TCR. The activated TCR initiates downstream signaling and an immune response, including the destruction of the EPC.

[0068] In general, a TCR can comprise two chains, an alpha chain and a beta chain (or less commonly a gamma chain and a delta chain), interconnected by disulfide bonds. Each chain comprises a variable domain (alpha chain variable domain and beta chain variable domain) and a constant region (alpha chain constant region and beta chain constant region). The variable domain is located distal to the cell membrane, and the variable domain interacts with an antigen. The constant region is located proximal to the cell membrane. A TCR can further comprises a transmembrane region and a short cytoplasmic tail. As used herein, the term "constant region" encompasses the

transmembrane region and the cytoplasmic tail, when present, as well as the traditional "constant region."

[0069] The variable domains can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each alpha chain variable domain and beta chain variable domain comprises three CDRs and four FRs: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Each variable domain contains a binding domain that interacts with an antigen. Though all three CDRs on each chain are involved in antigen binding, CDR3 is believed to be the primary antigen binding region. CDR1 is also interacts with the antigen, while CD2 is believed to primarily recognize the HLA complex.

[0070] Where not expressly stated, and unless the context indicates otherwise, the term "TCR" also includes an antigen-binding fragment or an antigen-binding portion of any TCR disclosed herein, and includes a monovalent and a divalent fragment or portion, and a single chain TCR. The term "TCR" is not limited to naturally occurring TCRs bound to the surface of a T cell. As used herein, the term "TCR" further refers to a TCR described herein that is expressed on the surface of a cell other than a T cell (*e.g.*, a cell that naturally expresses or that is modified to express CD3, as described herein), or a TCR described herein that is free from a cell membrane (*e.g.*, an isolated TCR or a soluble TCR).

[0071] An "antigen binding molecule," "portion of a TCR," or "TCR fragment" refers to any portion of an TCR less than the whole. An antigen binding molecule can include the antigenic complementarity determining regions (CDRs).

[0072] An "antigen" refers to any molecule, *e.g.*, a peptide, that provokes an immune response or is capable of being bound by a TCR. An "epitope," as used herein, refers to a portion of a polypeptide that provokes an immune response or is capable of being bound by a TCR. The immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. A person of skill in the art would readily understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. An antigen and/or an epitope can be endogenously expressed, *i.e.* expressed by genomic DNA, or can be recombinantly expressed. An antigen and/or an epitope can be specific to a certain tissue, such as a cancer cell, or it can be broadly expressed. In addition, fragments of larger molecules can act as antigens. In one embodiment, antigens are tumor antigens. An epitope can be present in a longer

polypeptide (*e.g.*, in a protein), or an epitope can be present as a fragment of a longer polypeptide. In some embodiments, an epitope is complexed with a major histocompatibility complex (MHC; also referred to herein as complexed with an HLA molecule, *e.g.*, an HLA class 1 molecule).

[0073] "gp100," "glycoprotein 100," "melanocyte protein PMEL," or "ME20M," as used herein, refers to a tumor antigen with expression in, e.g., melanoma. gp100 is a hydrophobic glycoprotein of 661 amino acids with a molecular mass of 70 kD (GenBank Acc No._NM_006928). *See, e.g.*, Eisenberg et al., *Cell Imunol. 266(1)*:98-103 (2010). *In vivo*, gp100 is involved in the maturation of melanosomes from stage I to stage II. As used herein, gp100 refers to not only the full-length canonical sequence, but also variants and fragments thereof. Known variants of gp100 are provided at www.uniprot.org (UniProtKB – P40967; last accessed March 1, 2019).

Table 1. gp100 Amino Acid Sequence

SEQ ID NO:	gp100 Amino Acid Sequence
52	MDLVLKRCLLHLAVIGALLAVGATKVPRNQDWLGVSRQLRTKAWNRQLYPEWTEAQRLDCWRGGQVS LKVSNDGPTLIGANASFSIALNFPGSQKVLPDGQVIWVNNTIINGSQVWGGQPVYPQETDDACIFPD GGPCPSGSWSQKRSFVYVWKTWGQYWQVLGGPVSGLSIGTGRAMLGTHTMEVTVYHRRGSRSYVPLA HSSSAFTITDQVPFSVSVSQLRALDGGNKHFLRNQPLTFALQLHDPSGYLAEADLSYTWDFGDSSGT LISRALVVTHTYLEPGPVTAQVVLQAAIPLTSCGSSPVPGTTDGHRPTAEAPNTTAGQVPTTEVVGT TPGQAPTAEPSGTTSVQVPTTEVISTAPVQMPTAESTGMTPEKVPVSEVMGTTLAEMSTPEATGMTP AEVSIVVLSGTTAAQVTTTEWVETTARELPIPEPEGPDASSIMSTESITGSLGPLLDGTATLRLVKR QVPLDCVLYRYGSFSVTLDIVQGIESAEILQAVPSGEGDAFELTVSCQGGLPKEACMEISSPGCQPP AQRLCQPVLPSPACQLVLHQILKGGSGTYCLNVSLADTNSLAVVSTQLIMPGQEAGLGQVPLIVGIL LVLMAVVLASLIYRRRLMKQDFSVPQLPHSSSHWLRLPRIFCSCPIGENSPLLSGQQV

[0074] The term "HLA," as used herein, refers to the human leukocyte antigen. HLA genes encode the major histocompatibility complex (MHC) proteins in humans. MHC proteins are expressed on the surface of cells, and are involved in activation of the immune response. HLA class I genes encode MHC class I molecules, which are expressed on the surface of cells in complex with peptide fragments (antigens) of self or non-self proteins. T cells expressing TCR and CD3 recognize the antigen:MHC class I complex and initiate an immune response to target and destroy antigen presenting cells displaying non-self proteins.

[0075] As used herein, an "HLA class I molecule" or "HLA class I molecule" refers to a protein product of a wild-type or variant HLA class I gene encoding an MHC class I molecule. Accordingly, "HLA class I molecule" and "MHC class I molecule" are used interchangeably herein.

[0076] The MHC Class I molecule comprises two protein chains: the alpha chain and the β2-microglobulin (β2m) chain. Human β2m is encoded by the B2M gene. The amino acid sequence of β2m is set forth in SEQ ID NO: 16 (Table 2). The alpha chain of the MHC Class I molecule is encoded by the HLA gene complex. The HLA complex is located within the 6p21.3 region on the short arm of human chromosome 6 and contains more than 220 genes of diverse function. The HLA gene are highly variant, with over 20,000 HLA alleles and related alleles, including over 15,000 HLA Class I alleles, known in the art, encoding thousands of HLA proteins, including over 10,000 HLA Class I proteins (*see, e.g.*, hla.alleles.org, last visited February 27, 2019). There are at least three genes in the HLA complex that encode an MHC Class I alpha chain protein: HLA-A, HLA-B, and HLA-C. In addition, HLA-E, HLA-F, and HLA-G encode proteins that associate with the MHC Class I molecule.

Table 2. Amino Acid Sequence of Human β2m

SEQ ID NO:	Sequence
16	MSRSVALAVLALLSLSGLEAIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLK NGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYACRVNHVTLSQPKIVKWDRDM

[0077] The term "autologous" refers to any material derived from the same individual to which it is later to be re-introduced. For example, an autologous T cell therapy comprises administering to a subject a T cell that was isolated from the same subject. The term "allogeneic" refers to any material derived from one individual which is then introduced to another individual of the same species. For example, an allogeneic T cell transplantation comprises administering to a subject a T cell that was obtained from a donor other than the subject.

[0078] A "cancer" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor. Examples of cancers that can be treated by the methods of the present invention include, but are not limited to, cancers of the immune system including lymphoma, leukemia, and other leukocyte malignancies. In some embodiments, the methods of the present invention can be used to reduce the tumor size of a tumor derived from, for example, bone cancer, renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, cutaneous or intraocular malignant melanoma, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular

malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers. The particular cancer can be responsive to chemo- or radiation therapy or the cancer can be refractory. A refractory cancer refers to a cancer that is not amendable to surgical intervention, and the cancer is either initially unresponsive to chemo- or radiation therapy or the cancer becomes unresponsive over time.

[0079] An "anti-tumor effect" as used herein, refers to a biological effect that can present as a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in tumor cell proliferation, a decrease in the number of metastases, an increase in overall or progression-free survival, an increase in life expectancy, or amelioration of various physiological symptoms associated with the tumor. An anti-tumor effect can also refer to the prevention of the occurrence of a tumor, *e.g.*, a vaccine.

[0080] The term "progression-free survival," which can be abbreviated as PFS, as used herein refers to the time from the treatment date to the date of disease progression per the revised IWG Response Criteria for Malignant Lymphoma or death from any cause.

[0081] "Disease progression" or "progressive disease," which can be abbreviated as PD, as used herein, refers to a worsening of one or more symptom associated with a particular disease. For example, disease progression for a subject afflicted with a cancer

can include an increase in the number or size of one or more malignant lesions, tumor metastasis, and death.

[0082] The "duration of response," which can be abbreviated as DOR, as used herein refers to the period of time between a subject's first objective response to the date of confirmed disease progression, per the revised IWG Response Criteria for Malignant Lymphoma, or death.

[0083] The term "overall survival," which can be abbreviated as OS, is defined as the time from the date of treatment to the date of death.

[0084] A "cytokine," as used herein, refers to a non-antibody protein that is released by one cell in response to contact with a specific antigen, wherein the cytokine interacts with a second cell to mediate a response in the second cell. A cytokine can be endogenously expressed by a cell or administered to a subject. Cytokines may be released by immune cells, including macrophages, B cells, T cells, and mast cells to propagate an immune response. Cytokines can induce various responses in the recipient cell. Cytokines can include homeostatic cytokines, chemokines, pro-inflammatory cytokines, effectors, and acute-phase proteins. For example, homeostatic cytokines, including interleukin (IL) 7 and IL-15, promote immune cell survival and proliferation, and pro-inflammatory cytokines can promote an inflammatory response. Examples of homeostatic cytokines include, but are not limited to, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p40, IL-12p70, IL-15, and interferon (IFN) gamma. Examples of pro-inflammatory cytokines include, but are not limited to, IL-1a, IL-1b, IL-6, IL-13, IL-17a, tumor necrosis factor (TNF)-alpha, TNF-beta, fibroblast growth factor (FGF) 2, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), vascular endothelial growth factor (VEGF), VEGF-C, VEGF-D, and placental growth factor (PLGF). Examples of effectors include, but are not limited to, granzyme A, granzyme B, soluble Fas ligand (sFasL), and perforin. Examples of acute phase-proteins include, but are not limited to, C-reactive protein (CRP) and serum amyloid A (SAA).

[0085] "Chemokines" are a type of cytokine that mediates cell chemotaxis, or directional movement. Examples of chemokines include, but are not limited to, IL-8, IL-16, eotaxin, eotaxin-3, macrophage-derived chemokine (MDC or CCL22), monocyte chemotactic protein 1 (MCP-1 or CCL2), MCP-4, macrophage inflammatory protein 1α (MIP-1α, MIP-1a), MIP-1β (MIP-1b), gamma-induced protein 10 (IP-10), and thymus and activation regulated chemokine (TARC or CCL17).

[0086] Other examples of analytes and cytokines of the present invention include, but are not limited to chemokine (C-C motif) ligand (CCL) 1, CCL5, monocyte-specific chemokine 3 (MCP3 or CCL7), monocyte chemoattractant protein 2 (MCP-2 or CCL8), CCL13, IL-1, IL-3, IL-9, IL-11, IL-12, IL-14, IL-17, IL-20, IL-21, granulocyte colony-stimulating factor (G-CSF), leukemia inhibitory factor (LIF), oncostatin M (OSM), CD154, lymphotoxin (LT) beta, 4-1BB ligand (4-1BBL), a proliferation-inducing ligand (APRIL), CD70, CD153, CD178, glucocorticoid-induced TNFR-related ligand (GITRL), tumor necrosis factor superfamily member 14 (TNFSF14), OX40L, TNF- and ApoL-related leukocyte-expressed ligand 1 (TALL-1), or TNF-related apoptosis-inducing ligand (TRAIL).

[0087] A "therapeutically effective amount," "effective dose," "effective amount," or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

[0088] The term "lymphocyte" as used herein includes natural killer (NK) cells, T cells, or B cells. NK cells are a type of cytotoxic (cell toxic) lymphocyte that represent a major component of the inherent immune system. NK cells reject tumors and cells infected by viruses. It works through the process of apoptosis or programmed cell death. They were termed “natural killers” because they do not require activation in order to kill cells. T-cells play a major role in cell-mediated-immunity (no antibody involvement). T-cell receptors (TCR) differentiate T cells from other lymphocyte types. The thymus, a specialized organ of the immune system, is primarily responsible for the T cell’s maturation. There are six types of T-cells, namely: Helper T-cells (*e.g.,* CD4+ cells), Cytotoxic T-cells (also known as TC, cytotoxic T lymphocyte, CTL, T-killer cell, cytolytic T cell, CD8+ T-cells or killer T cell), Memory T-cells ((i) stem memory T_{SCM} cells, like naive cells, are CD45RO−, CCR7+, CD45RA+, CD62L+ (L-selectin), CD27+, CD28+ and IL-7Rα+, but they also express large amounts of CD95, IL-2Rβ, CXCR3, and LFA-1, and show numerous functional attributes distinctive of memory cells); (ii) central

memory T_{CM} cells express L-selectin and the CCR7, they secrete IL-2, but not IFNγ or IL-4, and (iii) effector memory T_{EM} cells, however, do not express L-selectin or CCR7 but produce effector cytokines like IFNγ and IL-4), Regulatory T-cells (Tregs, suppressor T cells, or CD4+CD25+ regulatory T cells), Natural Killer T-cells (NKT) and Gamma Delta T-cells. B-cells, on the other hand, play a principal role in humoral immunity (with antibody involvement). A B cell makes antibodies and antigens and performs the role of antigen-presenting cells (APCs) and turns into memory B-cells after activation by antigen interaction. In mammals, immature B-cells are formed in the bone marrow, where its name is derived from.

[0089] The term "genetically engineered" or "engineered" refers to a method of modifying the genome of a cell, including, but not limited to, deleting a coding or non-coding region or a portion thereof or inserting a coding region or a portion thereof. In some embodiments, the cell that is modified is a lymphocyte, *e.g.*, a T cell or a modified cell that expresses CD3, which can either be obtained from a patient or a donor. The cell can be modified to express an exogenous construct, such as, *e.g.*, a T cell receptor (TCR) disclosed herein, which is incorporated into the cell's genome. In some embodiments, the cell is modified to express CD3.

[0090] An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0091] The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. Examples of immunotherapy include, but are not limited to, T cell therapies. T cell therapy can include adoptive T cell therapy, tumor-infiltrating lymphocyte (TIL) immunotherapy, autologous cell therapy, engineered autologous cell therapy (eACT), and allogeneic T cell transplantation.

[0092] Cells used in an immunotherapy described herein can come from any source known in the art. For example, T cells can be differentiated *in vitro* from a hematopoietic

stem cell population, or T cells can be obtained from a subject. T cells can be obtained from, *e.g.*, peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In addition, the T cells can be derived from one or more T cell lines available in the art. T cells can also be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation and/or apheresis. Additional methods of isolating T cells for a T cell therapy are disclosed in U.S. Patent Publication No. 2013/0287748, which is herein incorporated by references in its entirety. An immunotherapy can also comprise administering a modified cell to a subject, wherein the modified cell expresses CD3 and a TCR disclosed herein. In some embodiments, the modified cell is not a T cell.

[0093] A "patient" as used herein includes any human who is afflicted with a cancer (*e.g.*, a lymphoma or a leukemia). The terms "subject" and "patient" are used interchangeably herein.

[0094] The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0095] "Stimulation," as used herein, refers to a primary response induced by binding of a stimulatory molecule with its cognate ligand, wherein the binding mediates a signal transduction event. A "stimulatory molecule" is a molecule on a T cell, *e.g.*, the T cell receptor (TCR)/CD3 complex, that specifically binds with a cognate stimulatory ligand present on an antigen present cell. A "stimulatory ligand" is a ligand that when present on an antigen presenting cell (*e.g.*, an aAPC, a dendritic cell, a B-cell, and the like) can specifically bind with a stimulatory molecule on a T cell, thereby mediating a primary

response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands include, but are not limited to, an MHC Class I molecule loaded with a peptide, an anti-CD3 antibody, a superagonist anti-CD28 antibody, and a superagonist anti-CD2 antibody.

[0096] The terms "conditioning" and "pre-conditioning" are used interchangeably herein and indicate preparing a patient in need of a T cell therapy for a suitable condition. Conditioning as used herein includes, but is not limited to, reducing the number of endogenous lymphocytes, removing a cytokine sink, increasing a serum level of one or more homeostatic cytokines or pro-inflammatory factors, enhancing an effector function of T cells administered after the conditioning, enhancing antigen presenting cell activation and/or availability, or any combination thereof prior to a T cell therapy. In one embodiment, "conditioning" comprises increasing a serum level of one or more cytokines, *e.g.,* interleukin 7 (IL-7), interleukin 15 (IL-15), interleukin 10 (IL-10), interleukin 5 (IL-5), gamma-induced protein 10 (IP-10), interleukin 8 (IL-8), monocyte chemotactic protein 1 (MCP-1), placental growth factor (PLGF), C-reactive protein (CRP), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular adhesion molecule 1 (sVCAM-1), or any combination thereof. In another embodiment, "conditioning" comprises increasing a serum level of IL-7, IL-15, IP-10, MCP-1, PLGF, CRP, or any combination thereof.

[0097] "Treatment" or "treating" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease. In one embodiment, "treatment" or "treating" includes a partial remission. In another embodiment, "treatment" or "treating" includes a complete remission.

[0098] The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

[0099] The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the

measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% (*i.e.*, ±10%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

[0100] As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

[0101] Various aspects of the invention are described in further detail in the following subsections.

II. Compositions of the Disclosure

[0102] The present disclosure is directed to T Cell Receptors (TCRs) or antigen binding portions thereof that specifically bind to an epitope on gp100, nucleic acid molecules that encode the same, and cells that comprise the TCR or the nucleic acid molecule. Some aspects of the present disclosure are directed to methods of treating a caner in a subject in need thereof, comprising administering to the subject a cell comprising the TCRs described herein. Other aspects of the present disclosure are directed to an epitope of gp100 that the TCRs bind to and HLA class I molecules complexed to a peptide comprising the epitope of gp100.

[0103] The T-cell receptor, or TCR, is a molecule found on the surface of T cells, or T lymphocytes, that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR.

[0104] The TCR is composed of two different protein chains (that is, it is a heterodimer). In humans, in 95% of T cells the TCR consists of an alpha (α) chain and a beta (β) chain (encoded by TRA and TRB, respectively), whereas in 5% of T cells, the

TCR consists of gamma and delta (γ/δ) chains (encoded by TRG and TRD, respectively). This ratio changes during ontogeny and in diseased states (such as leukemia). It also differs between species. Orthologues of the 4 loci have been mapped in various species. Each locus can produce a variety of polypeptides with constant and variable regions.

[0105] When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction, that is, a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors.

II.A. Nucleic Acid Molecules

[0106] Certain aspects of the present disclosure are directed to nucleic acid molecules comprising (i) a first nucleotide sequence encoding a recombinant TCR or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR. In some embodiments, the second nucleotide sequence is a non-naturally occurring sequence. In other embodiments, the second nucleotide sequence is synthetic. In yet other embodiments, the second nucleotide sequence comprises a sequence that targets a nucleotide sequence encoding the endogenous TCR. In some embodiments, the anti-gp100 TCR cross competes for binding to human gp100 with a reference TCR. In some embodiments, the anti-gp100 TCR binds the same epitope or an overlapping epitope of human gp100 as a reference TCR.

[0107] In some embodiments, the reference TCR comprises an alpha chain and a beta chain; wherein the alpha chain comprises a complementarity determining region 1 (CDR1), a CDR2, and a CDR3; wherein the beta chain comprises a CDR1, a CDR2, and a CDR3; and wherein the reference TCR comprises the alpha chain CDR3 set forth in SEQ ID NO: 7 and the beta chain CDR3 set forth in SEQ ID NO: 10. In some embodiments, the alpha chain CDR1, CDR2, and CDR3 sequences present in the an amino acid sequence set forth in SEQ ID NO: 1, and reference TCR comprises the beta chain CDR1, CDR2, and CDR3 sequences present in the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the reference TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

Table 3. Alpha Chain and Beta Chain TCR Sequences

SEQ ID NO:	TCR Chain	Sequence
1	Alpha Chain (amino acid)	MKTFAGFSFLFLWLQLDCMSRGEDVEQSLFLSVREGDSSVINCTYTDSSSTYLYWYKQE PGAGLQLLTYIFSNMDMKQDQRLTVLLNKKDKHLSLRIADTQTGDSAIYFCAEGDTGRR ALTFGSGTRLQVQPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYIT DKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSF ETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSSZ
17	Alpha Chain (nucleotide)	ATGAAGACATTTGCTGGATTTTCGTTCCTGTTTTTGTGGCTGCAGCTGGACTGTATGAG TAGAGGAGAGGATGTGGAGCAGAGTCTTTTCCTGAGTGTCCGAGAGGGAGACAGCTCCG TTATAAACTGCACTTACACAGACAGCTCCTCCACCTACTTATACTGGTATAAGCAAGAA CCTGGAGCAGGTCTCCAGTTGCTGACGTATATTTTTTCAAATATGGACATGAAACAAGA CCAAAGACTCACTGTTCTATTGAATAAAAAGGATAAACATCTGTCTCTGCGCATTGCAG ACACCCAGACTGGGGACTCAGCTATCTACTTCTGTGCAGAGGGGGACACGGGCAGGAGA GCACTTACTTTTGGGAGTGGAACAAGACTCCAAGTGCAACCAAATATCCAGAACCCTGA CCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCA CCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACA GACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTG GAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAG ACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTT GAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCT CCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA
2	Beta Chain (amino acid)	MGCRLLCCAVLCLLGAVPIDTEVTQTPKHLVMGMTNKKSLKCEQHMGHRAMYWYKQKAK KPPELMFVYSYEKLSINESVPSRFSPECPNSSLLNLHLHALQPEDSALYLCASSPGASY EQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWW VNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSE NDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVS ALVLMAMVKRKDSRGZ
18	Beta Chain (nucleotide)	ATGGGCTGCAGGCTGCTCTGCTGTGCGGTTCTCTGTCTCCTGGGAGCAGTTCCCATAGA CACTGAAGTTACCCAGACACCAAAACACCTGGTCATGGGAATGACAAATAAGAAGTCTT TGAAATGTGAACAACATATGGGGCACAGGGCTATGTATTGGTACAAGCAGAAAGCTAAG AAGCCACCGGAGCTCATGTTTGTCTACAGCTATGAGAAACTCTCTATAAATGAAAGTGT GCCAAGTCGCTTCTCACCTGAATGCCCCAACAGCTCTCTCTTAAACCTTCACCTACACG CCCTGCAGCCAGAAGACTCAGCCCTGTATCTCTGCGCCAGCAGCCCAGGGGCATCCTAC GAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTT CCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGG CCACACTGGTATGCCTGGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGGTGG GTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCA GCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCT TCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAG AATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGCGCCGAGGC CTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCAGCAAGGGGTCCTGTCTG CCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGTGCTGGTCAGT GCCCTCGTGCTGATGGCCATGGTCAAGAGAAAGGATTCCAGAGGCTAG

II.A.1. TCR Encoded by the First Nucleotide Sequence

[0108] The present disclosure is directed to a TCR encoded by the first nucleotide sequence described herein. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable domain comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3. In some

embodiments, the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7 (CAEGDTGRRALTF). In some embodiments, the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10 (CASSPGASYEQYF). In some embodiments, the non-CDR regions in the alpha chain and/or the beta chain are further modified, *e.g.*, substitution or mutation of one amino acid, two amino acids, three amino acids, four amino acids, five amino acids, or six amino acids, thereby the alpha chain and/or the beta chain are not naturally occurring. In some embodiments, the substitutions or mutations can improve the TCRs described herein in various ways, *e.g.*, binding affinity, binding specificity, stability, viscosity, or any combination thereof.

[0109] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain CDR1, wherein the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5 (DSSSTY). In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain CDR1, wherein the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8 (IFSNMDM).

[0110] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain CDR2, wherein the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6 (MGHRA). In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain CDR2, wherein the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9 (YSYEKL).

[0111] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a variable domain of the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a variable domain of the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an

alpha chain variable domain present in the alpha chain amino acid sequence set forth in SEQ ID NO: 1.

[0112] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a variable domain of the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a variable domain of the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain variable domain present in the amino acid sequence set forth in SEQ ID NO: 2.

[0113] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises an alpha chain constant region, a beta chain constant region, or both an alpha chain constant region and a beta chain constant region. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain constant region present in the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises an alpha constant region that is different from endogenous, *e.g.*, naturally occurring, constant regions of the alpha chain. In some embodiments, the alpha chain constant region comprises an amino

acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the amino acid sequence of the constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1.

[0114] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain constant region present in the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises a beta constant region that is different from endogenous, *e.g.*, naturally occurring, constant regions of the beta chain. In some embodiments, the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the amino acid sequence of the constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2.

[0115] In certain embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide

sequence comprises an alpha chain comprising the amino acid sequence set forth in SEQ ID NO: 1.

[0116] In certain embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises a beta chain comprising the amino acid sequence set forth in SEQ ID NO: 2.

[0117] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.

II.A.2. Epitopes

[0118] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide sequence binds the same epitope as a reference TCR. In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 comprising the amino acid sequence set forth in SEQ ID NO: 13 (ESITGSLGPLL). In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, the epitope consists of amino acid residues 448-458 of gp100 (SEQ ID NO: 52), *e.g.*, "$gp100_{448-458}$."

[0119] In certain embodiments, the epitope is complexed with an HLA class I molecule. The human leukocyte antigen (HLA) system (the major histocompatibility complex [MHC] in humans) is an important part of the immune system and is controlled by genes located on chromosome 6. It encodes cell surface molecules specialized to

present antigenic peptides to the T-cell receptor (TCR) on T cells. (See also Overview of the Immune System.) MHC molecules that present antigen (Ag) are divided into 2 main classes: Class I MHC molecules and Class II MHC molecules.

[0120] Class I MHC molecules are present as transmembrane glycoproteins on the surface of all nucleated cells. Intact class I molecules consist of an alpha heavy chain bound to a beta-2 microglobulin molecule. The heavy chain consists of 2 peptide-binding domains, an Ig-like domain, and a transmembrane region with a cytoplasmic tail. The heavy chain of the class I molecule is encoded by genes at HLA-A, HLA-B, and HLA-C loci. T cells that express CD8 molecules react with class I MHC molecules. These lymphocytes often have a cytotoxic function, requiring them to be capable of recognizing any infected cell. Because every nucleated cell expresses class I MHC molecules, all infected cells can act as antigen-presenting cells for CD8 T cells (CD8 binds to the nonpolymorphic part of the class I heavy chain). Some class I MHC genes encode nonclassical MHC molecules, such as HLA-G (which may play a role in protecting the fetus from the maternal immune response) and HLA-E (which presents peptides to certain receptors on natural killer [NK] cells).

[0121] In some embodiments, the HLA class 1 molecule is selected from an HLA-A, HLA-B, and HLA-C allele. In some embodiments, the HLA class 1 molecule is selected from an HLA-E, HLA-F, and HLA-G allele. In certain embodiments, the HLA class 1 molecule is an HLA-A allele. In certain embodiments, the HLA class 1 molecule is an HLA-B allele. In certain embodiments, the HLA class 1 molecule is an HLA-C allele.

[0122] Many HLA-A, HLA-B, and HLA-C alleles are known in the art, and any of the known alleles can be used in the present disclosure. An updated list of HLA alleles is available at hla.alleles.org/ (last visited on February 27, 2019). In some embodiments, the HLA class 1 molecule is an HLA-B allele selected from an HLA-B*07, an HLA-B*08, an HLA-B*13, an HLA-B*14, an HLA-B*15, an HLA-B*18, an HLA-B*27, an HLA-B*35, an HLA-B*37, an HLA-B*38, an HLA-B*39, an HLA-B*40, an HLA-B*41, an HLA-B*42, an HLA-B*44, an HLA-B*45, an HLA-B*46, an HLA-B*47, an HLA-B*48, an HLA-B*49, an HLA-B*50, an HLA-B*51, an HLA-B*52, an HLA-B*53, an HLA-B*54, an HLA-B*55, an HLA-B*56, an HLA-B*57, an HLA-B*58, an HLA-B*59, an HLA-B*67, an HLA-B*73, an HLA-B*78, an HLA-B*79, an HLA-B*81, an HLA-B*82, and an HLA-B*83. In certain embodiments, the HLA-B allele is an HLA-B*40:01 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:02 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:03 allele. In certain embodiments, the

HLA-B allele is an HLA-B*40:04 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:05 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:06 allele.

[0123] In certain embodiments, the HLA class 1 molecule is an HLA-B allele selected from the group consisting of HLA-B*40:01:01, HLA-B*40:01:02:01, HLA-B*40:01:02:02, HLA-B*40:01:02:03, HLA-B*40:01:02:04, HLA-B*40:01:02:05, HLA-B*40:01:02:06, HLA-B*40:01:02:07, HLA-B*40:01:02:08, HLA-B*40:01:02:09, HLA-B*40:01:03, HLA-B*40:01:04, HLA-B*40:01:05, HLA-B*40:01:06, HLA-B*40:01:07, HLA-B*40:01:08, HLA-B*40:01:09, HLA-B*40:01:10, HLA-B*40:01:11, HLA-B*40:01:12, HLA-B*40:01:13, HLA-B*40:01:14, HLA-B*40:01:15, HLA-B*40:01:16, HLA-B*40:01:17, HLA-B*40:01:18, HLA-B*40:01:19, HLA-B*40:01:20, HLA-B*40:01:21, HLA-B*40:01:22, HLA-B*40:01:23, HLA-B*40:01:24, HLA-B*40:01:25, HLA-B*40:01:26, HLA-B*40:01:27, HLA-B*40:01:28, HLA-B*40:01:29, HLA-B*40:01:30, HLA-B*40:01:31, HLA-B*40:01:32, HLA-B*40:01:33, HLA-B*40:01:34, HLA-B*40:01:35, HLA-B*40:01:36, HLA-B*40:01:37, HLA-B*40:01:38, HLA-B*40:01:39, HLA-B*40:01:40, HLA-B*40:01:41, HLA-B*40:01:42, HLA-B*40:01:43, HLA-B*40:01:44, HLA-B*40:01:45, HLA-B*40:01:46, HLA-B*40:01:47, HLA-B*40:01:48, HLA-B*40:01:49, HLA-B*40:01:50, HLA-B*40:01:51, HLA-B*40:01:52, HLA-B*40:01:53, HLA-B*40:01:54, HLA-B*40:01:55, HLA-B*40:01:56, HLA-B*40:01:57, HLA-B*40:01:58, HLA-B*40:01:59, and HLA-B*40:01:60. In some embodiments, the HLA class 1 molecule is an HLA-B allele selected from the group consisting of HLA-B*40:02:01:01, HLA-B*40:02:01:02, HLA-B*40:02:01:03, HLA-B*40:02:01:04, HLA-B*40:02:01:05, HLA-B*40:02:01:06, HLA-B*40:02:01:07, HLA-B*40:02:01:08, HLA-B*40:02:02, HLA-B*40:02:03, HLA-B*40:02:04, HLA-B*40:02:05, HLA-B*40:02:06, HLA-B*40:02:07, HLA-B*40:02:08, HLA-B*40:02:09, HLA-B*40:02:10, HLA-B*40:02:11, HLA-B*40:02:12, HLA-B*40:02:13, HLA-B*40:02:14, HLA-B*40:02:15, HLA-B*40:02:16, HLA-B*40:02:17, HLA-B*40:02:18, HLA-B*40:02:19, HLA-B*40:02:20, HLA-B*40:02:21, HLA-B*40:02:22, HLA-B*40:02:23, HLA-B*40:02:24, HLA-B*40:02:25, HLA-B*40:02:26, HLA-B*40:03:01:01, HLA-B*40:03:01:02, HLA-B*40:04:01, HLA-B*40:04:02, HLA-B*40:05:01:01, HLA-B*40:05:01:02, HLA-B*40:06:01:01, HLA-B*40:06:01:02, HLA-B*40:06:02, HLA-B*40:06:03, HLA-B*40:06:04:01, HLA-B*40:06:04:02, HLA-B*40:06:05, HLA-B*40:06:06, HLA-B*40:06:07, HLA-B*40:06:08, HLA-B*40:06:09, HLA-B*40:06:10, HLA-B*40:06:11, HLA-B*40:06:12, HLA-B*40:06:13, HLA-

B*40:06:14, HLA-B*40:06:15, HLA-B*40:06:16, HLA-B*40:06:17, HLA-B*40:06:18, HLA-B*40:07, HLA-B*40:08, HLA-B*40:09, HLA-B*40:100, HLA-B*40:101, HLA-B*40:102, HLA-B*40:103, HLA-B*40:104, HLA-B*40:105, HLA-B*40:106, HLA-B*40:107, HLA-B*40:108, HLA-B*40:109, HLA-B*40:10:01:01, HLA-B*40:10:01:02, HLA-B*40:10:02, HLA-B*40:110, HLA-B*40:111, HLA-B*40:112, HLA-B*40:113, HLA-B*40:114:01, HLA-B*40:114:02, HLA-B*40:115, HLA-B*40:116, HLA-B*40:117, HLA-B*40:118, HLA-B*40:119, HLA-B*40:11:01, HLA-B*40:11:02, HLA-B*40:12, HLA-B*40:120, HLA-B*40:121, HLA-B*40:122, HLA-B*40:123, HLA-B*40:124:01, HLA-B*40:124:02, HLA-B*40:125:01, HLA-B*40:125:02, HLA-B*40:126, HLA-B*40:127, HLA-B*40:128, HLA-B*40:129, HLA-B*40:13, HLA-B*40:130:01, HLA-B*40:130:02, HLA-B*40:131, HLA-B*40:132, HLA-B*40:133, HLA-B*40:134, HLA-B*40:135, HLA-B*40:136, HLA-B*40:137, HLA-B*40:138, HLA-B*40:139, HLA-B*40:140, HLA-B*40:141, HLA-B*40:142, HLA-B*40:143, HLA-B*40:144, HLA-B*40:145, HLA-B*40:146, HLA-B*40:147, HLA-B*40:148, HLA-B*40:149, HLA-B*40:14:01, HLA-B*40:14:02, HLA-B*40:14:03, HLA-B*40:15, HLA-B*40:150, HLA-B*40:151, HLA-B*40:152, HLA-B*40:153, HLA-B*40:154, HLA-B*40:155:01, HLA-B*40:155:02, HLA-B*40:156, HLA-B*40:157, HLA-B*40:158, HLA-B*40:159, HLA-B*40:16, HLA-B*40:160:01, HLA-B*40:160:02, HLA-B*40:161, HLA-B*40:162, HLA-B*40:163, HLA-B*40:164, HLA-B*40:165, HLA-B*40:166, HLA-B*40:167, HLA-B*40:168, HLA-B*40:169, HLA-B*40:170, HLA-B*40:171, HLA-B*40:172, HLA-B*40:173, HLA-B*40:174, HLA-B*40:175, HLA-B*40:176, HLA-B*40:177, HLA-B*40:178, HLA-B*40:179, HLA-B*40:18, HLA-B*40:180, HLA-B*40:181, HLA-B*40:182, HLA-B*40:183, HLA-B*40:184, HLA-B*40:185, HLA-B*40:186:01, HLA-B*40:186:02, HLA-B*40:187, HLA-B*40:188, HLA-B*40:189, HLA-B*40:19, HLA-B*40:190, HLA-B*40:191, HLA-B*40:192, HLA-B*40:193, HLA-B*40:194, HLA-B*40:195, HLA-B*40:196, HLA-B*40:197, HLA-B*40:198, HLA-B*40:199, HLA-B*40:200, HLA-B*40:201, HLA-B*40:202, HLA-B*40:203, HLA-B*40:204, HLA-B*40:205, HLA-B*40:206, HLA-B*40:207, HLA-B*40:208, HLA-B*40:209, HLA-B*40:20:01:01, HLA-B*40:20:01:02, HLA-B*40:21, HLA-B*40:210, HLA-B*40:211, HLA-B*40:212, HLA-B*40:213, HLA-B*40:214, HLA-B*40:215, HLA-B*40:216, HLA-B*40:217, HLA-B*40:218, HLA-B*40:219, HLA-B*40:220, HLA-B*40:221, HLA-B*40:222, HLA-B*40:223, HLA-B*40:224, HLA-B*40:225, HLA-B*40:226, HLA-B*40:227, HLA-B*40:228, HLA-B*40:229, HLA-B*40:22 57, HLA-B*40:23, HLA-B*40:230, HLA-B*40:231, HLA-B*40:232,

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II.A.3 The Second Nucleotide Sequence

[0124] The second nucleotide sequence of the nucleic acid molecule disclosed herein can be any sequence or can encode for any polypeptide that is capable of inhibiting the expression of an endogenous TCR. In some embodiments, the second nucleotide sequence is one or more siRNAs. In some embodiments, the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of an endogenous TCR. In certain embodiments, the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of wild-type, human TCR. In some embodiments, the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the alpha chain of wild-type TCR. In some embodiments, the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the beta chain of wild-type TCR. In some embodiments, the one or more siRNAs comprise (i) one or more siRNA's that are complementary to a target sequence within a nucleotide sequence encoding a constant region of the alpha chain of

wild-type TCR and (ii) one or more siRNA's that are complementary to a target sequence within a nucleotide sequence encoding a constant region of the beta chain of wild-type TCR.

[0125] In some embodiments, the one or more siRNAs comprise a nucleotide sequence selected from the group consisting of SEQ ID NOs: 53-56 (Table 4). In some embodiments, the second nucleotide sequence of the nucleic acid molecule encodes one or more siRNAs, wherein the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the alpha chain of wild-type TCR, and wherein the one or more siRNAs comprise the nucleic acid sequences set forth in SEQ ID NOs: 53 and 54.

Table 4. siRNA Sequences

SEQ ID NO:	siRNA	Sequence (Nucleotides 1-19 are ribonucleotides; nucleotides 20-21 are deoxyribonucleotides)
53	siRNA-TCRa-1	GUAAGGAUUCUGAUGUGUATT
54	siRNA-TCRa-2	UACACAUCAGAAUCCUUACTT
55	siRNA-TCRb-1	CCACCAUCCUCUAUGAGAUTT
56	siRNA-TCRb-2	AUCUCAUAGAGGAUGGUGGTT

[0126] In some embodiments, the second nucleotide sequence of the nucleic acid molecule encodes one or more siRNAs, wherein the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the beta chain of wild-type TCR, and wherein the one or more siRNAs comprise the nucleic acid sequences set forth in SEQ ID NOs: 55 and 56. In some embodiments, the second nucleotide sequence of the nucleic acid molecule encodes one or more siRNAs, wherein the one or more siRNAs comprise (i) one or more siRNAs that are complementary to a target sequence within a nucleotide sequence encoding a constant region of the alpha chain of wild-type TCR, wherein the one or more siRNAs comprise the nucleic acid sequences set forth in SEQ ID NOs: 53 and 54; and (ii) one or more siRNAs that are complementary to a target sequence within a nucleotide sequence encoding a constant region of the beta chain of wild-type TCR, wherein the one or more siRNAs comprise the nucleic acid sequences set forth in SEQ ID NOs: 55 and 56.

[0127] In some embodiments, the second nucleotide sequence of the nucleic acid molecule comprises SEQ ID NOs: 53-56. In some embodiments, the second nucleotide sequence comprises SEQ ID NOs: 53-56, wherein one or more of SEQ ID NOs: 53-56 is separated by one or more nucleic acids that do not encode an siRNA. In certain embodiments, the one or more siRNAs are selected from the siRNAs disclosed in U.S.

Publication No. 2010/0273213 A1, which is incorporated by reference herein in its entirety.

[0128] In some embodiments, the second nucleotide sequence of the nucleic acid molecule encodes a protein, wherein the protein is capable of inhibiting the expression of an endogenous, *e.g.*, wild-type, TCR. In some embodiments, the second nucleotide sequence encodes Cas9.

II.A.3 Vectors

[0129] Certain aspects of the present disclosure are directed to vectors comprising a nucleic acid molecule disclosed herein. In some embodiments, the vector is a viral vector. In some embodiments, the vector is a viral particle or a virus. In some embodiments, the vector is a mammalian vector. In some embodiments, the vector is a bacterial vector.

[0130] In certain embodiments, the vector is a retroviral vector. In some embodiments, the vector is selected from the group consisting of an adenoviral vector, a lentivirus, a Sendai virus, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, and an adeno associated virus (AAV) vector. In particular embodiments, the vector is an AAV vector. In some embodiments, the vector is a lentivirus. In particular embodiments, the vector is an AAV vector. In some embodiments, the vector is a Sendai virus. In some embodiments, the vector is a hybrid vector. Examples of hybrid vectors that can be used in the present disclosure can be found in Huang and Kamihira, *Biotechnol. Adv. 31(2)*:208-23 (2103), which is incorporated by reference herein in its entirety.

II.B. Recombinant T Cell Receptors (TCRs)

[0131] Certain aspects of the present disclosure are directed to recombinant T cell receptors (TCRs) or an antigen binding portion thereof that specifically bind human gp100 ("an anti-gp100 TCR"). In some embodiments, the anti-gp100 TCR is encoded by the a nucleic acid molecule disclosed herein.

[0132] In some embodiments, the anti-gp100 TCR cross competes for binding to human gp100 with a reference TCR. In some embodiments, the anti-gp100 TCR binds the same epitope or an overlapping epitope of human gp100 as a reference TCR. In some embodiments, the reference TCR comprises an alpha chain and a beta chain, and the alpha chain comprises of the reference TCR comprises an amino acid sequence as set

forth in SEQ ID NO: 1. In some embodiments, the beta chain of the reference TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

[0133] In some embodiments, the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the constant region of an alpha chain comprising the amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the constant region of a beta chain comprising the amino acid sequence set forth in SEQ ID NO: 2.

[0134] In some embodiments, the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein (i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the constant region of an alpha chain comprising the amino acid sequence set forth in SEQ ID NO: 1; and (ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the constant region of a beta chain comprising the amino acid sequence set forth in SEQ ID NO: 2.

[0135] In some embodiments, the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and the beta chain of the anti-gp100 TCR comprises a variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3. In some embodiments, the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10.

[0136] In some embodiments, the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8.

[0137] In some embodiments, the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6. In some embodiments, the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

[0138] In some embodiments, the anti-gp100 TCR comprises an alpha chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a variable domain of the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR comprises an alpha chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a variable domain of the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR comprises an alpha chain variable domain present in the alpha chain amino acid sequence set forth in SEQ ID NO: 1.

[0139] In some embodiments, the anti-gp100 TCR comprises a beta chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a variable domain of the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR comprises a beta chain variable domain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a variable domain of the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR comprises a beta chain variable domain present in the beta chain amino acid sequence set forth in SEQ ID NO: 2.

[0140] In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises an alpha chain constant region, a beta chain constant region, or both an alpha chain constant region and a beta chain constant region. In some embodiments, the anti-gp100 TCR comprises an alpha chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a

constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR comprises an alpha chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR comprises an alpha chain constant region present in the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises an alpha constant region that is different from endogenous, *e.g.*, naturally occurring, constant regions of the alpha chain. In some embodiments, the alpha chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the amino acid sequence of the constant region of the alpha chain amino acid sequence set forth in SEQ ID NO: 1.

[0141] In some embodiments, the anti-gp100 TCR comprises a beta chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with a constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR comprises a beta chain constant region having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, or at least about 99% sequence identity with a constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR comprises a beta chain constant region present in the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR encoded by the first nucleotide further comprises a beta constant region that is different from endogenous, *e.g.*, naturally occurring, constant regions of the beta chain. In some embodiments, the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to the amino acid sequence of the constant region of the beta chain amino acid sequence set forth in SEQ ID NO: 2.

[0142] In certain embodiments, the anti-gp100 TCR comprises an alpha chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the alpha chain amino acid sequence set forth in SEQ ID NO: 1. In some embodiments, the anti-gp100 TCR comprises an alpha chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the alpha chain amino acid sequence set forth in SEQ ID NO: 1, wherein the anti-gp100 TCR comprises an alpha chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 7. In some embodiments, the anti-gp100 TCR comprises an alpha chain comprising the amino acid sequence set forth in SEQ ID NO: 1.

[0143] In certain embodiments, the anti-gp100 TCR comprises a beta chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the beta chain amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the anti-gp100 TCR comprises a beta chain having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the beta chain amino acid sequence set forth in SEQ ID NO: 2, wherein the anti-gp100 TCR comprises a beta chain CDR3 comprising an amino acid sequence as set forth in SEQ ID NO: 10. In some embodiments, the anti-gp100 TCR comprises a beta chain comprising the amino acid sequence set forth in SEQ ID NO: 2.

[0144] In some embodiments, the anti-gp100 TCR comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.

II.B.2. Epitopes

[0145] In some embodiments, the anti-gp100 TCR binds the same epitope as a reference TCR. In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 comprising the amino acid sequence set forth in SEQ ID NO: 13. In some embodiments, the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as

set forth in SEQ ID NO: 13. In some embodiments, the epitope consists of amino acid residues 448-458 of gp100 (SEQ ID NO: 52), *e.g.*, "gp100$_{448-458}$."

[0146] In certain embodiments, the epitope is complexed with an HLA class I molecule. In some embodiments, the HLA class 1 molecule is selected from an HLA-A, HLA-B, and HLA-C allele. In some embodiments, the HLA class 1 molecule is selected from an HLA-E, HLA-F, and HLA-G allele. In certain embodiments, the HLA class 1 molecule is an HLA-A allele. In certain embodiments, the HLA class 1 molecule is an HLA-B allele. In certain embodiments, the HLA class 1 molecule is an HLA-C allele.

[0147] Many HLA-A, HLA-B, and HLA-C alleles are known in the art, and any of the known alleles can be used in the present disclosure. An updated list of HLA alleles is available at hla.alleles.org/ (last visited on February 27, 2019). In some embodiments, the HLA class 1 molecule is an HLA-B allele selected from an HLA-B*07, an HLA-B*08, an HLA-B*13, an HLA-B*14, an HLA-B*15, an HLA-B*18, an HLA-B*27, an HLA-B*35, an HLA-B*37, an HLA-B*38, an HLA-B*39, an HLA-B*40, an HLA-B*41, an HLA-B*42, an HLA-B*44, an HLA-B*45, an HLA-B*46, an HLA-B*47, an HLA-B*48, an HLA-B*49, an HLA-B*50, an HLA-B*51, an HLA-B*52, an HLA-B*53, an HLA-B*54, an HLA-B*55, an HLA-B*56, an HLA-B*57, an HLA-B*58, an HLA-B*59, an HLA-B*67, an HLA-B*73, an HLA-B*78, an HLA-B*79, an HLA-B*81, an HLA-B*82, and an HLA-B*83. In certain embodiments, the HLA-B allele is an HLA-B*40:01 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:02 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:03 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:04 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:05 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:06 allele.

[0148] In certain embodiments, the HLA class 1 molecule is an HLA-B allele selected from the group consisting of HLA-B*40:01:01, HLA-B*40:01:02:01, HLA-B*40:01:02:02, HLA-B*40:01:02:03, HLA-B*40:01:02:04, HLA-B*40:01:02:05, HLA-B*40:01:02:06, HLA-B*40:01:02:07, HLA-B*40:01:02:08, HLA-B*40:01:02:09, HLA-B*40:01:03, HLA-B*40:01:04, HLA-B*40:01:05, HLA-B*40:01:06, HLA-B*40:01:07, HLA-B*40:01:08, HLA-B*40:01:09, HLA-B*40:01:10, HLA-B*40:01:11, HLA-B*40:01:12, HLA-B*40:01:13, HLA-B*40:01:14, HLA-B*40:01:15, HLA-B*40:01:16, HLA-B*40:01:17, HLA-B*40:01:18, HLA-B*40:01:19, HLA-B*40:01:20, HLA-B*40:01:21, HLA-B*40:01:22, HLA-B*40:01:23, HLA-B*40:01:24, HLA-B*40:01:25, HLA-B*40:01:26, HLA-B*40:01:27, HLA-B*40:01:28, HLA-B*40:01:29, HLA-

B*40:01:30, HLA-B*40:01:31, HLA-B*40:01:32, HLA-B*40:01:33, HLA-B*40:01:34, HLA-B*40:01:35, HLA-B*40:01:36, HLA-B*40:01:37, HLA-B*40:01:38, HLA-B*40:01:39, HLA-B*40:01:40, HLA-B*40:01:41, HLA-B*40:01:42, HLA-B*40:01:43, HLA-B*40:01:44, HLA-B*40:01:45, HLA-B*40:01:46, HLA-B*40:01:47, HLA-B*40:01:48, HLA-B*40:01:49, HLA-B*40:01:50, HLA-B*40:01:51, HLA-B*40:01:52, HLA-B*40:01:53, HLA-B*40:01:54, HLA-B*40:01:55, HLA-B*40:01:56, HLA-B*40:01:57, HLA-B*40:01:58, HLA-B*40:01:59, and HLA-B*40:01:60. In some embodiments, the HLA class 1 molecule is an HLA-B allele selected from the group consisting of HLA-B*40:02:01:01, HLA-B*40:02:01:02, HLA-B*40:02:01:03, HLA-B*40:02:01:04, HLA-B*40:02:01:05, HLA-B*40:02:01:06, HLA-B*40:02:01:07, HLA-B*40:02:01:08, HLA-B*40:02:02, HLA-B*40:02:03, HLA-B*40:02:04, HLA-B*40:02:05, HLA-B*40:02:06, HLA-B*40:02:07, HLA-B*40:02:08, HLA-B*40:02:09, HLA-B*40:02:10, HLA-B*40:02:11, HLA-B*40:02:12, HLA-B*40:02:13, HLA-B*40:02:14, HLA-B*40:02:15, HLA-B*40:02:16, HLA-B*40:02:17, HLA-B*40:02:18, HLA-B*40:02:19, HLA-B*40:02:20, HLA-B*40:02:21, HLA-B*40:02:22, HLA-B*40:02:23, HLA-B*40:02:24, HLA-B*40:02:25, HLA-B*40:02:26, HLA-B*40:03:01:01, HLA-B*40:03:01:02, HLA-B*40:04:01, HLA-B*40:04:02, HLA-B*40:05:01:01, HLA-B*40:05:01:02, HLA-B*40:06:01:01, HLA-B*40:06:01:02, HLA-B*40:06:02, HLA-B*40:06:03, HLA-B*40:06:04:01, HLA-B*40:06:04:02, HLA-B*40:06:05, HLA-B*40:06:06, HLA-B*40:06:07, HLA-B*40:06:08, HLA-B*40:06:09, HLA-B*40:06:10, HLA-B*40:06:11, HLA-B*40:06:12, HLA-B*40:06:13, HLA-B*40:06:14, HLA-B*40:06:15, HLA-B*40:06:16, HLA-B*40:06:17, HLA-B*40:06:18, HLA-B*40:07, HLA-B*40:08, HLA-B*40:09, HLA-B*40:100, HLA-B*40:101, HLA-B*40:102, HLA-B*40:103, HLA-B*40:104, HLA-B*40:105, HLA-B*40:106, HLA-B*40:107, HLA-B*40:108, HLA-B*40:109, HLA-B*40:10:01:01, HLA-B*40:10:01:02, HLA-B*40:10:02, HLA-B*40:110, HLA-B*40:111, HLA-B*40:112, HLA-B*40:113, HLA-B*40:114:01, HLA-B*40:114:02, HLA-B*40:115, HLA-B*40:116, HLA-B*40:117, HLA-B*40:118, HLA-B*40:119, HLA-B*40:11:01, HLA-B*40:11:02, HLA-B*40:12, HLA-B*40:120, HLA-B*40:121, HLA-B*40:122, HLA-B*40:123, HLA-B*40:124:01, HLA-B*40:124:02, HLA-B*40:125:01, HLA-B*40:125:02, HLA-B*40:126, HLA-B*40:127, HLA-B*40:128, HLA-B*40:129, HLA-B*40:13, HLA-B*40:130:01, HLA-B*40:130:02, HLA-B*40:131, HLA-B*40:132, HLA-B*40:133, HLA-B*40:134, HLA-B*40:135, HLA-B*40:136, HLA-B*40:137, HLA-B*40:138, HLA-B*40:139, HLA-B*40:140, HLA-B*40:141, HLA-B*40:142, HLA-B*40:143,

HLA-B*40:144, HLA-B*40:145, HLA-B*40:146, HLA-B*40:147, HLA-B*40:148, HLA-B*40:149, HLA-B*40:14:01, HLA-B*40:14:02, HLA-B*40:14:03, HLA-B*40:15, HLA-B*40:150, HLA-B*40:151, HLA-B*40:152, HLA-B*40:153, HLA-B*40:154, HLA-B*40:155:01, HLA-B*40:155:02, HLA-B*40:156, HLA-B*40:157, HLA-B*40:158, HLA-B*40:159, HLA-B*40:16, HLA-B*40:160:01, HLA-B*40:160:02, HLA-B*40:161, HLA-B*40:162, HLA-B*40:163, HLA-B*40:164, HLA-B*40:165, HLA-B*40:166, HLA-B*40:167, HLA-B*40:168, HLA-B*40:169, HLA-B*40:170, HLA-B*40:171, HLA-B*40:172, HLA-B*40:173, HLA-B*40:174, HLA-B*40:175, HLA-B*40:176, HLA-B*40:177, HLA-B*40:178, HLA-B*40:179, HLA-B*40:18, HLA-B*40:180, HLA-B*40:181, HLA-B*40:182, HLA-B*40:183, HLA-B*40:184, HLA-B*40:185, HLA-B*40:186:01, HLA-B*40:186:02, HLA-B*40:187, HLA-B*40:188, HLA-B*40:189, HLA-B*40:19, HLA-B*40:190, HLA-B*40:191, HLA-B*40:192, HLA-B*40:193, HLA-B*40:194, HLA-B*40:195, HLA-B*40:196, HLA-B*40:197, HLA-B*40:198, HLA-B*40:199, HLA-B*40:200, HLA-B*40:201, HLA-B*40:202, HLA-B*40:203, HLA-B*40:204, HLA-B*40:205, HLA-B*40:206, HLA-B*40:207, HLA-B*40:208, HLA-B*40:209, HLA-B*40:20:01:01, HLA-B*40:20:01:02, HLA-B*40:21, HLA-B*40:210, HLA-B*40:211, HLA-B*40:212, HLA-B*40:213, HLA-B*40:214, HLA-B*40:215, HLA-B*40:216, HLA-B*40:217, HLA-B*40:218, HLA-B*40:219, HLA-B*40:220, HLA-B*40:221, HLA-B*40:222, HLA-B*40:223, HLA-B*40:224, HLA-B*40:225, HLA-B*40:226, HLA-B*40:227, HLA-B*40:228, HLA-B*40:229, HLA-B*40:22 57, HLA-B*40:23, HLA-B*40:230, HLA-B*40:231, HLA-B*40:232, HLA-B*40:233, HLA-B*40:234, HLA-B*40:235, HLA-B*40:236, HLA-B*40:237, HLA-B*40:238, HLA-B*40:239, HLA-B*40:24, HLA-B*40:240, HLA-B*40:241, HLA-B*40:242, HLA-B*40:243, HLA-B*40:244, HLA-B*40:245, HLA-B*40:246, HLA-B*40:247, HLA-B*40:248, HLA-B*40:249, HLA-B*40:25, HLA-B*40:250, HLA-B*40:251, HLA-B*40:252, HLA-B*40:253, HLA-B*40:254, HLA-B*40:255, HLA-B*40:256, HLA-B*40:257, HLA-B*40:258, HLA-B*40:259, HLA-B*40:26, HLA-B*40:260, HLA-B*40:261, HLA-B*40:262, HLA-B*40:263, HLA-B*40:264, HLA-B*40:265, HLA-B*40:266, HLA-B*40:267, HLA-B*40:268, HLA-B*40:269, HLA-B*40:270, HLA-B*40:271, HLA-B*40:272, HLA-B*40:273, HLA-B*40:274, HLA-B*40:275, HLA-B*40:276, HLA-B*40:277, HLA-B*40:278, HLA-B*40:279, HLA-B*40:27:01, HLA-B*40:27:02, HLA-B*40:28, HLA-B*40:280, HLA-B*40:281, HLA-B*40:282, HLA-B*40:283, HLA-B*40:284, HLA-B*40:285, HLA-B*40:286, HLA-B*40:287, HLA-B*40:288, HLA-B*40:289, HLA-B*40:29, HLA-B*40:290, HLA-

B*40:291, HLA-B*40:292, HLA-B*40:293, HLA-B*40:294, HLA-B*40:295, HLA-B*40:296, HLA-B*40:297, HLA-B*40:298:01, HLA-B*40:298:02, HLA-B*40:299, HLA-B*40:30, HLA-B*40:300, HLA-B*40:301, HLA-B*40:302, HLA-B*40:303, HLA-B*40:304, HLA-B*40:305, HLA-B*40:306, HLA-B*40:307, HLA-B*40:308, HLA-B*40:309, HLA-B*40:31, HLA-B*40:310, HLA-B*40:311, HLA-B*40:312, HLA-B*40:313, HLA-B*40:314, HLA-B*40:315, HLA-B*40:316, HLA-B*40:317, HLA-B*40:318, HLA-B*40:319, HLA-B*40:32, HLA-B*40:320, HLA-B*40:321, HLA-B*40:322, HLA-B*40:323, HLA-B*40:324, HLA-B*40:325, HLA-B*40:326, HLA-B*40:327, HLA-B*40:328, HLA-B*40:329, HLA-B*40:33, HLA-B*40:330, HLA-B*40:331, HLA-B*40:332, HLA-B*40:333, HLA-B*40:334, HLA-B*40:335, HLA-B*40:336, HLA-B*40:337 98, HLA-B*40:338, HLA-B*40:339, HLA-B*40:34, HLA-B*40:340, HLA-B*40:341, HLA-B*40:342, HLA-B*40:343, HLA-B*40:344, HLA-B*40:345, HLA-B*40:346, HLA-B*40:347, HLA-B*40:348, HLA-B*40:349, HLA-B*40:350, HLA-B*40:351, HLA-B*40:352, HLA-B*40:353, HLA-B*40:354, HLA-B*40:355, HLA-B*40:356, HLA-B*40:357, HLA-B*40:358, HLA-B*40:359, HLA-B*40:35:01, HLA-B*40:35:02, HLA-B*40:36, HLA-B*40:360, HLA-B*40:361 86, HLA-B*40:362, HLA-B*40:363, HLA-B*40:364, HLA-B*40:365, HLA-B*40:366, HLA-B*40:367, HLA-B*40:368, HLA-B*40:369, HLA-B*40:37, HLA-B*40:370, HLA-B*40:371, HLA-B*40:372, HLA-B*40:373, HLA-B*40:374, HLA-B*40:375, HLA-B*40:376, HLA-B*40:377, HLA-B*40:378, HLA-B*40:379, HLA-B*40:38, HLA-B*40:380, HLA-B*40:381, HLA-B*40:382, HLA-B*40:383, HLA-B*40:384, HLA-B*40:385, HLA-B*40:386, HLA-B*40:387, HLA-B*40:388, HLA-B*40:389, HLA-B*40:39, HLA-B*40:390, HLA-B*40:391, HLA-B*40:392, HLA-B*40:393, HLA-B*40:394, HLA-B*40:395, HLA-B*40:396, HLA-B*40:397, HLA-B*40:398, HLA-B*40:399 57, HLA-B*40:40, HLA-B*40:400, HLA-B*40:401, HLA-B*40:402, HLA-B*40:403, HLA-B*40:404, HLA-B*40:406, HLA-B*40:407, HLA-B*40:408, HLA-B*40:409, HLA-B*40:410, HLA-B*40:411, HLA-B*40:412, HLA-B*40:413, HLA-B*40:414, HLA-B*40:42, HLA-B*40:43, HLA-B*40:44, HLA-B*40:45, HLA-B*40:46, HLA-B*40:47, HLA-B*40:48, HLA-B*40:49, HLA-B*40:50, HLA-B*40:51, HLA-B*40:52, HLA-B*40:53, HLA-B*40:54, HLA-B*40:55, HLA-B*40:56, HLA-B*40:57, HLA-B*40:58, HLA-B*40:59, HLA-B*40:60, HLA-B*40:61, HLA-B*40:62, HLA-B*40:63, HLA-B*40:64:01:01, HLA-B*40:64:01:02, HLA-B*40:65, HLA-B*40:66, HLA-B*40:67, HLA-B*40:68, HLA-B*40:69, HLA-B*40:70:01, HLA-B*40:70:02, HLA-B*40:71, HLA-B*40:72:01, HLA-B*40:72:02, HLA-B*40:73, HLA-B*40:74,

HLA-B*40:75, HLA-B*40:76, HLA-B*40:77, HLA-B*40:78, HLA-B*40:79, HLA-B*40:80, HLA-B*40:81, HLA-B*40:82, HLA-B*40:83, HLA-B*40:84, HLA-B*40:85, HLA-B*40:86, HLA-B*40:87:01, HLA-B*40:87:02, HLA-B*40:88, HLA-B*40:89, HLA-B*40:90, HLA-B*40:91, HLA-B*40:92, HLA-B*40:93, HLA-B*40:94, HLA-B*40:95, HLA-B*40:96, HLA-B*40:97, HLA-B*40:98, and HLA-B*40:99.

II.B.3. Bispecific T Cell Receptors (TCRs)

[0149] Certain aspects of the present disclosure are directed to a bispecific TCR comprising a first antigen-binding domain and a second antigen-binding domain, wherein the first antigen-binding domain comprises a TCR or an antigen-binding portion thereof disclosed herein. In some embodiments, the first antigen-binding domain comprises a single chain variable fragment ("scFv").

[0150] In some embodiments, the second antigen-binding domain binds specifically to a protein expressed on the surface of a T cell. Any protein expressed on the surface of a T cell can be targeted by the bispecific antibody disclosed herein. In certain embodiments, the protein expressed on the surface of a T cell is not expressed by other cells. In some embodiments, the protein expressed on the surface of a T cell is expressed on the surface of one or more other human immune cells. In some embodiments, the protein expressed on the surface of a T cell is expressed on the surface of one or more other human immune cells, but it is not expressed on the surface of a human non-immune cell. In some embodiments, the second antigen-binding domain binds specifically to a protein expressed on the surface of a T cell selected from CD3, CD2, CD5, CD6, CD8, CD11a (LFA-1α), CD43, CD45, and CD53. In certain embodiments, the second antigen-binding domain binds specifically to CD3. In some embodiments, the second antigen-binding domain comprises an scFv.

[0151] In some embodiments, the first antigen-binding domain and the second antigen-binding domain are linked or associated by a covalent bond. In some embodiments, the first antigen-binding domain and the second antigen-binding domain are linked by a peptide bond.

II.C. Cells Expressing TCRs

[0152] Certain aspects of the present disclosure are directed to cells comprising a nucleic acid molecule disclosed herein, a vector disclosed herein, a recombinant TCR

disclosed herein, a bispecific TCR disclosed herein, or any combination thereof. Any cell can be used in the present disclosure.

[0153] In certain embodiments, the cell expresses CD3. CD3 expression can be naturally occurring, *e.g.*, the CD3 is expressed from a nucleic acid sequence that is endogenously expressed by the cell. For example, T cells and natural killer (NK) cells naturally express CD3. Thus, in some embodiments, the cell is a T cell or a natural killer cell. In certain embodiments, the cell is a T cell selected from a natural killer T (NKT) cell and an innate lymphoid cell (ILC).

[0154] In some embodiments, the T cell is isolated from a human subject. In some embodiments, the human subject is the same subject that will ultimately receive the T cell therapy. In other embodiments, the subject is a donor subject, wherein the donor subject is not the same subject that will receive the T cell therapy.

[0155] In some embodiments, the cell is a cell that does not naturally express CD3, wherein the cell has been modified to express CD3. In some embodiments, the cell comprises a transgene encoding CD3, wherein the transgene is expressed by the cell. In some embodiments, the cell comprises a transgene encoding a protein that activates expression of endogenous CD3 by the cell. In some embodiments, the cell comprises a transgene encoding a protein or siRNA that inhibits an inhibitor of CD3 expression in the cell. In some embodiments, the transgene is incorporated into the genome of the cell. In some embodiments, the transgene is not incorporated into the genome of the cell.

[0156] In some embodiments, the cell that is modified to express CD3 is isolated from a human subject. In some embodiments, the human subject is the same subject that will ultimately receive the cell therapy. In other embodiments, the subject is a donor subject, wherein the donor subject is not the same subject that will receive the cell therapy.

II.D. HLA Class I Molecules

[0157] Certain aspects of the present disclosure are directed to a HLA class I molecule complexed to a peptide, wherein the peptide comprises the amino acid sequence set forth in SEQ ID NO: 13. In some embodiments, he peptide consists of the amino acid sequence set forth in SEQ ID NO: 13.

[0158] In some embodiments, the HLA Class I molecule is an HLA-A, HLA-B, or an HLA-C. In some embodiments, the HLA Class I molecule is an HLA-E, HLA-F, or HLA-G. In some embodiments, the HLA class 1 molecule is an HLA-B allele selected from an

HLA-B*07, an HLA-B*08, an HLA-B*13, an HLA-B*14, an HLA-B*15, an HLA-B*18, an HLA-B*27, an HLA-B*35, an HLA-B*37, an HLA-B*38, an HLA-B*39, an HLA-B*40, an HLA-B*41, an HLA-B*42, an HLA-B*44, an HLA-B*45, an HLA-B*46, an HLA-B*47, an HLA-B*48, an HLA-B*49, an HLA-B*50, an HLA-B*51, an HLA-B*52, an HLA-B*53, an HLA-B*54, an HLA-B*55, an HLA-B*56, an HLA-B*57, an HLA-B*58, an HLA-B*59, an HLA-B*67, an HLA-B*73, an HLA-B*78, an HLA-B*79, an HLA-B*81, an HLA-B*82, and an HLA-B*83. In certain embodiments, the HLA-B allele is an HLA-B*40:01 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:02 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:03 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:04 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:05 allele. In certain embodiments, the HLA-B allele is an HLA-B*40:06 allele. In some embodiments, the HLA allele is any HLA allele disclosed herein, *e.g.*, *supra*.

[0159] In some embodiments, the HLA Class I molecule comprises an alpha chain and a β2m. In some embodiments, the alpha chain comprises an α1 domain, an α2 domain, an α3 domain. In some embodiments, the β2m comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97% at least about 98%, at least about 99%, or about 100% sequence identity with the amino acid sequence set forth in SEQ ID NO: 16. In some embodiments, the sequence of the alpha chain is selected from any of the HLA protein sequences available at hla.alleles.org (last visited February 27, 2019).

[0160] In some embodiments, the HLA class I molecule is a monomer. In some embodiments, the HLA class I molecule is a dimer. In some embodiments, the HLA class I molecule is a multimer. In some embodiments, the HLA class I molecule is a trimer. In some embodiments, the HLA class I molecule is a tetramer. In some embodiments, the HLA class I molecule is a pentamer.

[0161] Certain aspects of the present disclosure are directed to antigen presenting cells (APCs) comprising any HLA class I molecule disclosed herein. In certain embodiments, the APC expressed the HLA class I molecule on the surface of the APC. In certain embodiments, the APC comprises more than one HLA class I molecule disclosed herein.

II.D. Vaccines

[0162] Certain aspects of the present disclosure a cancer vaccine comprising a peptide comprising an amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, the cancer vaccine comprises a peptide that consists of the amino acid sequence set forth in SEQ ID NO: 13. In some embodiments, the vaccine further comprises one or more excipient. In some embodiments, the vaccine further comprises one or more additional peptides. In some embodiments, the one or more additional peptides comprise one or more additional epitopes.

III. Methods of the Disclosure

[0163] Certain aspects of the present disclosure are directed to methods of treating a cancer in a subject in need thereof. Other aspects of the present disclosure are directed to methods of engineering an antigen-targeting cell. Other aspects of the present disclosure are directed to methods of enriching a target population of T cells obtained from a human subject.

III.A. Methods of Treating Cancer

[0164] Certain aspects of the present disclosure are directed to methods of treating a cancer in a subject in need thereof, comprising administering to the subject a nucleic acid molecule disclosed herein, a recombinant TCR disclosed herein, a bispecific TCR disclosed herein, an epitope disclosed herein, or an HLA class I molecule disclosed herein, or a vector or cell comprising any of the above.

[0165] In some embodiments, the cancer is selected from melanoma, bone cancer, renal cancer, prostate cancer, breast cancer, colon cancer, lung cancer, cutaneous or intraocular malignant melanoma, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute

myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers. In some embodiments, the cancer melanoma.

[0166] In some embodiments, the cancer is relapsed. In some embodiments, the cancer is refractory. In some embodiments, the cancer is advanced. In some embodiments, the cancer is metastatic.

[0167] In some embodiments, the methods disclosed herein treat a cancer in a subject. In some embodiments, the methods disclosed herein reduce the severity of one or more symptom of the cancer. In some embodiments, the methods disclosed herein reduce the size or number of a tumor derived from the cancer. In some embodiments, the methods disclosed herein increase the overall survival of the subject, relative to a subject not provided the methods disclosed herein. In some embodiments, the methods disclosed herein increase the progressive-free survival of the subject, relative to a subject not provided the methods disclosed herein. In some embodiments, the methods disclosed herein lead to a partial response in the subject. In some embodiments, the methods disclosed herein lead to a complete response in the subject.

[0168] In some embodiments, the methods disclosed herein comprise treating a cancer in a subject in need thereof, comprising administering to the subject a cell described herein, wherein the cell comprises a nucleic acid molecule disclosed herein, a vector disclosed herein, a recombinant TCR disclosed herein, and/or a bispecific antibody disclosed herein. In some embodiments, the cell is a T cell. In some embodiments, the cell is a cell that is modified to express CD3.

[0169] In some embodiments, the cell, *e.g.*, a T cell, is obtained from the subject. In some embodiments, the cell, *e.g.*, a T cell, is obtained from a donor other than the subject.

[0170] In some embodiments, the subject is preconditioned prior to administering the cells. The preconditioning can comprise any substance that promotes T cell function and/or survival. In some embodiments, the preconditioning comprises administering to the subject a chemotherapy, a cytokine, a protein, a small molecule, or any combination

thereof. In some embodiments, the preconditioning comprises administering an interleukin. In some embodiments, the preconditioning comprises administering IL-2, , IL-4, IL-7, IL-9, IL-15, IL-21, or any combination thereof. In some embodiments, the preconditioning comprises administering cyclophosphamide, fludarabine, or both. In some embodiments, the preconditioning comprises administering vitamin C, an AKT inhibitor, ATRA (vesanoid, tretinoin), rapamycin, or any combination thereof.

III.B. Methods of Engineering an Antigen-Targeting Cell

[0171] Certain aspects of the present disclosure are directed to methods of engineering an antigen-targeting cell. In some embodiments, the antigen is a gp100 antigen. In some embodiments, the method comprises transducing a cell with a nucleic acid molecule disclosed herein or a vector disclosed herein. The cell can be any cell described herein. In some embodiments, the cell is a T cell described herein. In some embodiments, the cell is a cell that is modified to express CD3, as described herein. In some embodiments, the cell, *e.g.*, the T cell, is obtained from a subject in need of a T cell therapy. In some embodiments, the cell is obtained from a donor other than the subject in need of the T cell therapy. In some embodiments, the cell is a T cell or a natural killer cell.

III.C. Methods of Enriching a Target Population of T Cells

[0172] Certain aspects of the present disclosure are directed to methods of enriching a target population of T cells obtained from a human subject. In some embodiments, the method comprises contacting the T cells with an HLA class I molecule disclosed herein. In some embodiments, the method comprises contacting the T cells with an APC disclosed herein. In some embodiments, following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class I molecule relative to the number of T cells capable of binding the HLA class I molecule prior to the contacting.

[0173] In some embodiments, the method comprises contacting the T cells *in vitro* with a peptide, wherein the peptide comprises the amino acid sequence set forth in SEQ ID NO: 13. In some embodiments, the method comprises contacting the T cells *in vitro* with a peptide, wherein the peptide consists of the amino acid sequence set forth in SEQ ID NO: 13. In some embodiments, following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class I molecule

relative to the number of T cells capable of binding the HLA class I molecule prior to the contacting.

[0174] Some aspects of the present disclosure are directed to a method of selecting a T cell capable of targeting a tumor cell. In some embodiments, the method comprises contacting a population of isolated T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 13. In some embodiments, the T cells are obtained from a human subject.

[0175] The T cells obtained from the human subject can be any T cells disclosed herein. In some embodiments, the T cells obtained from the human subject are tumor infiltrating lymphocytes (TIL).

[0176] In some embodiments, the method further comprises administering to the human subject the enriched T cells. In some embodiments, the subject is preconditioned prior to receiving the T cells, as described herein.

[0177] All of the various aspects, embodiments, and options described herein can be combined in any and all variations.

[0178] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0179] Having generally described this disclosure, a further understanding can be obtained by reference to the examples provided herein. These examples are for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1

[0180] TILs were isolated from a metastatic melanoma patient, then polyclonally expanded *in vitro*, and their gp100 antigen specificity for HLA-B*40:01 allele was examined. The combination of structure-based analysis using peptide/HLA (pHLA) multimers and functional analysis has been used to measure Ag-specific T cell responses.

[0181] Since pHLA multimer production requires the use of a peptide with a known exact sequence, it is not straightforward or practical to conduct high-throughput screening for new epitope peptides using a pHLA multimer-based strategy. In addition to structure-based analysis using pHLA multimers, functional analysis can be applied to determine the antigen specificity of T cells. We conducted functional assays using artificial antigen-

presenting cells (APCs), which can take up and process longer peptides and present epitope peptides via class I molecules, as stimulator cells. B*40:01-artificial APCs were pulsed with overlapping peptides to cover the whole protein of gp100 (Table 5) and used as stimulators in cytokine ELISPOT assays. Following one controlled stimulation with B*40:01-artificial APCs pulsed with the gp100-derived overlapping peptides, B*40:01^{+} melanoma TILs showed positive responses to two adjacent peptides with the shared sequence $_{446}$STESITGSLGPLLDG$_{460}$ in the IFN-γ ELISPOT analysis (FIG. 1). Using a series of mutant deletion peptides, we determined the minimally required peptide epitope, $_{448}$ESITGSLGPLL$_{458}$ presented by B*40:01 molecules. We identified HLA-B*40:01/gp100$_{448-458}$ T cells, which accounted for 0.11% of CD8^{+} T cells among the polyclonally expanded TILs (FIG. 2). Following one controlled peptide-specific stimulation with B*40:01-artificial APCs, the frequency of B*40:01/gp100$_{448-458}$ T cells increased to 1.8%, excluding the possibility that the low percentage of staining represented false positivity (FIG. 2). The multimer-positive T cells secreted detectable IFN-γ in an HLA-restricted peptide-specific manner according to ELISPOT analysis (FIG. 3).

Table 5. gp100 Overlapping Peptides.

Position	Peptide sequence	SEQ ID NO	Position	Peptide sequence	SEQ ID NO
1	MDLVLKRCLLHLAVIGALLA	64	326	QVPTTEVVGTTPGQAPTAEP	129
6	KRCLLHLAVIGALLAVGATK	65	331	EVVGTTPGQAPTAEPSGTTS	130
11	HLAVIGALLAVGATKVPRNQ	66	336	TPGQAPTAEPSGTTSVQVPT	131
16	GALLAVGATKVPRNQDWLGV	67	341	PTAEPSGTTSVQVPTTEVIS	132
21	VGATKVPRNQDWLGVSRQLR	68	346	SGTTSVQVPTTEVISTAPVQ	133
26	VPRNQDWLGVSRQLRTKAWN	69	351	VQVPTTEVISTAPVQMPTAE	134
31	DWLGVSRQLRTKAWNRQLYP	70	356	TEVISTAPVQMPTAESTGMT	135
36	SRQLRTKAWNRQLYPEWTEA	71	361	TAPVQMPTAESTGMTPEKVP	136
41	TKAWNRQLYPEWTEAQRLDC	72	366	MPTAESTGMTPEKVPVSEVM	137
46	RQLYPEWTEAQRLDCWRGGQ	73	371	STGMTPEKVPVSEVMGTTLA	138
51	EWTEAQRLDCWRGGQVSLKV	74	376	PEKVPVSEVMGTTLAEMSTP	139
56	QRLDCWRGGQVSLKVSNDGP	75	381	VSEVMGTTLAEMSTPEATGM	140
61	WRGGQVSLKVSNDGPTLIGA	76	386	GTTLAEMSTPEATGMTPAEV	141
66	VSLKVSNDGPTLIGANASFS	77	391	EMSTPEATGMTPAEVSIVVL	142
71	SNDGPTLIGANASFSIALNF	78	396	EATGMTPAEVSIVVLSGTTA	143
76	TLIGANASFSIALNFPGSQK	79	401	TPAEVSIVVLSGTTAAQVTT	144
81	NASFSIALNFPGSQKVLPDG	80	406	SIVVLSGTTAAQVTTTEWVE	145
86	IALNFPGSQKVLPDGQVIWV	81	411	SGTTAAQVTTTEWVETTARE	146
91	PGSQKVLPDGQVIWVNNTII	82	416	AQVTTTEWVETTARELPIPE	147
96	VLPDGQVIWVNNTIINGSQV	83	421	TEWVETTARELPIPEPEGPD	148
101	QVIWVNNTIINGSQVWGGQP	84	426	TTARELPIPEPEGPDASSIM	149
106	NNTIINGSQVWGGQPVYPQE	85	431	LPIPEPEGPDASSIMSTESI	150
111	NGSQVWGGQPVYPQETDDAC	86	436	PEGPDASSIMSTESITGSLG	151
116	WGGQPVYPQETDDACIFPDG	87	441	ASSIMSTESITGSLGPLLDG	152
121	VYPQETDDACIFPDGGPCPS	88	446	STESITGSLGPLLDGTATLR	153

126	TDDACIFPDGGPCPSGSWSQ	89	451	TGSLGPLLDGTATLRLVKRQ	154
131	IFPDGGPCPSGSWSQKRSFV	90	456	PLLDGTATLRLVKRQVPLDC	155
136	GPCPSGSWSQKRSFVYVWKT	91	461	TATLRLVKRQVPLDCVLYRY	156
141	GSWSQKRSFVYVWKTWGQYW	92	466	LVKRQVPLDCVLYRYGSFSV	157
146	KRSFVYVWKTWGQYWQVLGG	93	471	VPLDCVLYRYGSFSVTLDIV	158
151	YVWKTWGQYWQVLGGPVSGL	94	476	VLYRYGSFSVTLDIVQGIES	159
156	WGQYWQVLGGPVSGLSIGTG	95	481	GSFSVTLDIVQGIESAEILQ	160
161	QVLGGPVSGLSIGTGRAMLG	96	486	TLDIVQGIESAEILQAVPSG	161
166	PVSGLSIGTGRAMLGTHTME	97	491	QGIESAEILQAVPSGEGDAF	162
171	SIGTGRAMLGTHTMEVTVYH	98	496	AEILQAVPSGEGDAFELTVS	163
176	RAMLGTHTMEVTVYHRRGSR	99	501	AVPSGEGDAFELTVSCQGGL	164
181	THTMEVTVYHRRGSRSYVPL	100	506	EGDAFELTVSCQGGLPKEAC	165
186	VTVYHRRGSRSYVPLAHSSS	101	511	ELTVSCQGGLPKEACMEISS	166
191	RRGSRSYVPLAHSSSAFTIT	102	516	CQGGLPKEACMEISSPGCQP	167
196	SYVPLAHSSSAFTITDQVPF	103	521	PKEACMEISSPGCQPPAQRL	168
201	AHSSSAFTITDQVPFSVSVS	104	526	MEISSPGCQPPAQRLCQPVL	169
206	AFTITDQVPFSVSVSQLRAL	105	531	PGCQPPAQRLCQPVLPSPAC	170
211	DQVPFSVSVSQLRALDGGNK	106	536	PAQRLCQPVLPSPACQLVLH	171
216	SVSVSQLRALDGGNKHFLRN	107	541	CQPVLPSPACQLVLHQILKG	172
221	QLRALDGGNKHFLRNQPLTF	108	546	PSPACQLVLHQILKGGSGTY	173
226	DGGNKHFLRNQPLTFALQLH	109	551	QLVLHQILKGGSGTYCLNVS	174
231	HFLRNQPLTFALQLHDPSGY	110	556	QILKGGSGTYCLNVSLADTN	175
236	QPLTFALQLHDPSGYLAEAD	111	561	GSGTYCLNVSLADTNSLAVV	176
241	ALQLHDPSGYLAEADLSYTW	112	566	CLNVSLADTNSLAVVSTQLI	177
246	DPSGYLAEADLSYTWDFGDS	113	571	LADTNSLAVVSTQLIMPGQE	178
251	LAEADLSYTWDFGDSSGTLI	114	576	SLAVVSTQLIMPGQEAGLGQ	179
256	LSYTWDFGDSSGTLISRALV	115	581	STQLIMPGQEAGLGQVPLIV	180
261	DFGDSSGTLISRALVVTHTY	116	586	MPGQEAGLGQVPLIVGILLV	181
266	SGTLISRALVVTHTYLEPGP	117	591	AGLGQVPLIVGILLVLMAVV	182
271	SRALVVTHTYLEPGPVTAQV	118	596	VPLIVGILLVLMAVVLASLI	183
276	VTHTYLEPGPVTAQVVLQAA	119	601	GILLVLMAVVLASLIYRRRL	184
281	LEPGPVTAQVVLQAAIPLTS	120	606	LMAVVLASLIYRRRLMKQDF	185
286	VTAQVVLQAAIPLTSCGSSP	121	611	LASLIYRRRLMKQDFSVPQL	186
291	VLQAAIPLTSCGSSPVPGTT	122	616	YRRRLMKQDFSVPQLPHSSS	187
296	IPLTSCGSSPVPGTTDGHRP	123	621	MKQDFSVPQLPHSSSHWLRL	188
301	CGSSPVPGTTDGHRPTAEAP	124	626	SVPQLPHSSSHWLRLPRIFC	189
306	VPGTTDGHRPTAEAPNTTAG	125	631	PHSSSHWLRLPRIFCSCPIG	190
311	DGHRPTAEAPNTTAGQVPTT	126	636	HWLRLPRIFCSCPIGENSPL	191
316	TAEAPNTTAGQVPTTEVVGT	127	641	PRIFCSCPIGENSPLLSGQQ	192
321	NTTAGQVPTTEVVGTTPGQA	128	642	RIFCSCPIGENSPLLSGQQV	193

[0182] The multimer-positive antitumor T cells were collected and their TCR genes were molecularly cloned (Fig. 4, SEQ ID NOs: 1 and 2). The antigen specificity and functional reactivity of the cloned TCR were verified by multimer staining and ELISPOT assay of TCR-reconstituted T cells. When reconstituted on primary T cells, B*40:01/gp100$_{448-458}$ TCR-transduced T cells were successfully stained with the cognate multimer (FIG. 5) and strongly reacted with the gp100$_{448-458}$ peptide presented by surface B*40:01 molecules (FIG. 6). Importantly, these cells were able to recognize B*40:01-matched and peptide-unpulsed tumor cells naturally expressing the gp100 gene (FIG. 7).

SK-MEL-37 melanoma cells, which lack endogenous expression of both B*40:01 and gp100, became reactive to B*40:01/gp100$_{448-458}$ TCR-transduced T cells only when both the B*40:01 and gp100 genes (but not either of the single genes) were transduced (FIGs. 7-9). These results clearly demonstrate that the B*40:01/gp100$_{448-458}$ TCR-transduced T cells were sufficiently avid to recognize tumor cells and that the cloned B*40:01/gp100$_{448-458}$ TCR was tumor-reactive.

[0183] Gp100 is one of the shared antigens that have been promising and extensively studied in bispecific T cell engager (BiTE) therapy, and clinical trials targeting gp100 are ongoing in patients with metastatic uveal melanoma, using IMCgp100 which is a bispecific biologic comprised of a soluble TCR recognizing the gp100 antigen fused to a scFV anti-CD3 that redirects T cell lysis of melanoma cells expressing gp100 in the context of HLA-A*02:01 molecules. The use of the newly cloned tumor-reactive B*40:01-restricted gp100 TCR genes may widen the applicability of BiTE therapy targeting gp100 beyond HLA-A*02:01-positive cancer patients.

[0184] **Methods**

[0185] *Cell samples*

[0186] Peripheral blood samples were obtained from healthy donors after Institutional Review Board approval. Mononuclear cells were obtained via density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare). K562 is an erythroleukemic cell line with defective HLA expression. T2 is an HLA-A*02:01^{+} T cell leukemia/B-LCL hybrid cell line. Jurkat 76 is a T cell leukemic cell line lacking TCR and CD8 expression. SK-MEL-28 and A375 cell lines were grown in DMEM supplemented with 10% FBS and 50 μg/ml gentamicin (Invitrogen). The K562, T2, and Jurkat 76 cell lines were cultured in RPMI 1640 supplemented with 10% FBS and 50 μg/ml gentamicin. TILs isolated from a metastatic melanoma patient were grown *in vitro*.

[0187] *Peptides*

[0188] Synthetic peptides were dissolved to 50 μg/ml in DMSO. Peptides used were 20-mer overlapping peptides to cover the whole protein of gp100 (Table 1) and B*40:01-restricted gp100$_{448-458}$ (ESITGSLGPLL; SEQ ID NO: 13), gp100$_{449-458}$ (SITGSLGPLL; SEQ ID NO: 194), NY-ESO-1$_{125-133}$ (EFTVSGNIL; SEQ ID NO: 195), and HIV nef$_{92-100}$ (KEKGGLEGL; SEQ ID NO: 63) peptides. The gp100$_{449-458}$, NY-ESO-1$_{125-133}$, and HIV nef$_{92-100}$ peptides were utilized as negative controls.

[0189] *Genes*

[0190] HLA-B*40:01 gene was fused with a truncated version of the human nerve growth factor receptor (ΔNGFR) via the internal ribosome entry site. ΔNGFR-transduced cells were isolated using anti-NGFR monoclonal antibody (mAb). TCR genes were cloned by 5'-rapid amplification of cDNA ends (RACE) PCR using a SMARTer RACE cDNA amplification kit (Takara Bio). The 5'-RACE PCR products were cloned into a retrovirus vector and sequenced. All genes were cloned into the pMX retrovirus vector and transduced using the 293GPG cell-based retrovirus system.

[0191] *Transfectants*

[0192] Jurkat 76/CD8 cells were transduced with individual TCRα and TCRβ genes as reported previously[42-44]. The Jurkat 76/CD8-derived TCR transfectants were purified (>95% purity) using CD3 Microbeads (Miltenyi Biotec). The K562-based artificial APCs individually expressing various HLA class I genes as a single HLA allele in conjunction with CD80 and CD83 have been reported previously (Butler and Hirano, *Immunol. Rev. 257*:191-209 (2014); Hirano et al., *Clin. Cancer Res. 12*:2967-75 (2006)). PG13-derived retrovirus supernatants were used to transduce TCR genes into human primary T cells. TransIT293 (Mirus Bio) was used to transfect TCR genes into the 293GPG cell line. $Gp100^-$ SK-MEL-37 cells were retrovirally transduced with the full-length gp100 gene to generate SK-MEL-37/gp100. The expression of transduced gp100 was evaluated by flow cytometry after staining with an anti-gp100 mAb (clone 7E3; LifeSpan Biosciences). HLA-B*40:01$^-$ SK-MEL-37 cells were retrovirally transduced with HLA-B*40:01 to generate SK-MEL-37/B*40:01 cells. HLA-B*40:01 gene was tagged with the ΔNGFR gene as described above, and the $\Delta NGFR^+$ cells were purified (>95% purity) and used in subsequent experiments. The ΔNGFR gene alone was retrovirally transduced as a control.

[0193] *Flow cytometry and cell sorting*

[0194] Cell surface molecules were stained with a PC5-conjugated anti-CD8 mAb (clone B9.11; Beckman Coulter), FITC-conjugated anti-NGFR (clone ME20.4; Biolegend), and APC/Cy7-conjugated anti-CD3 (clone UCHT1; Biolegend). Dead cells were discriminated with the LIVE/DEAD Fixable Aqua Dead Cell Stain kit (Life Technologies). For intracellular staining, cells were fixed and permeabilized by using a Cytofix/Cytoperm kit (BD Biosciences). Stained cells were analyzed with flow cytometry (BD Biosciences), and data analysis was performed using FlowJo (Tree Star). Cell sorting was conducted using a FACS Aria II (BD Bioscience).

[0195] *Cytokine ELISPOT analysis*

[0196] IFN-γ ELISPOT assays were conducted. PVDF plates (Millipore, Bedford, MA) were coated with the capture mAb (1-D1K; MABTECH, Mariemont, OH), and T cells were incubated with 2 x 10^4 target cells per well in the presence or absence of a peptide for 20–24 hours at 37°C. The plates were subsequently washed and incubated with a biotin-conjugated detection mAb (7-B6-1; MABTECH). HRP-conjugated SA (Jackson ImmunoResearch) was then added, and IFN-γ spots were developed. The reaction was stopped by rinsing thoroughly with cold tap water. ELISPOT plates were scanned and counted using an ImmunoSpot plate reader and ImmunoSpot version 5.0 software (Cellular Technology Limited, Shaker Heights, OH).

[0197] *Expansion of $CD8^+$ TILs in an HLA-restricted peptide-specific manner*

[0198] $CD8^+$ TILs were purified through negative magnetic selection using the $CD8^+$ T Cell Isolation Kit (Miltenyi Biotec). B*40:01-artificial APCs were pulsed with 10 μg/mL gp100 peptides for 6 hours. The artificial APCs were then irradiated at 200 Gy, washed, and added to the TILs at an effector to target (E:T) ratio of 20:1. Starting on the next day, 10 IU/ml IL-2 (Novartis), 10 ng/ml IL-15 (Peprotech), and 30 ng/ml IL-21 (Peprotech) were added to the cultures every three days.

[0199] *Expansion of primary $CD8^+$ T cells transduced with the cloned TCR*

[0200] $CD3^+$ T cells were purified through negative magnetic selection using a Pan T Cell Isolation Kit (Miltenyi Biotec). Purified T cells were stimulated with artificial APC/mOKT3 irradiated with 200 Gy at an E:T ratio of 20:1. Starting on the next day, activated T cells were retrovirally transduced with the cloned TCR genes via centrifugation for 1 hour at 1,000 g at 32°C for 3 consecutive days. On the following day, 100 IU/ml IL-2 and 10 ng/ml IL-15 were added to the TCR-transduced T cells. The culture medium was replenished every 2-3 days.

[0201] *Production of human cell-based pHLA multimers*

[0202] The affinity-matured HLA class I gene was engineered to carry a Glu (E) residue in lieu of the Gln (Q) residue at position 115 of the α2 domain and a mouse K^b gene-derived α3 domain instead of the HLA class I α3 domain. By fusing the extracellular domain of the affinity-matured HLA class I gene with a Gly-Ser (GS) flexible linker followed by a 6x His tag, we generated the soluble HLA class I^{Q115E}-K^b gene. HEK293T cells were individually transduced with various soluble HLA class I^{Q115E}-K^b genes along with the β2m gene using the 293GPG cell-based retrovirus system. Stable HEK293T cells ectopically expressing soluble affinity-matured class I^{Q115E}-K^b were grown until confluent, and the medium was then changed. Forty-eight hours later,

the conditioned medium was harvested and immediately used or frozen until use. The soluble HLA class I^{Q115E}-K^b-containing supernatant produced by the HEK293T transfectants was mixed with 100-1000 μg/ml of class I-restricted peptide of interest overnight at 37°C for *in vitro* peptide exchange. Soluble monomeric class I^{Q115E}-K^b loaded with the peptide was dimerized using an anti-His mAb (clone AD1.1.10; Abcam) conjugated to a fluorochrome such as phycoerythrin (PE) at a 2:1 molar ratio for 2 hours at room temperature or overnight at 4°C. The concentration of functional soluble HLA class I^{Q115E}-K^b molecules was measured by specific ELISA using an anti-pan class I mAb (clone W6/32, in-house) and an anti-His tag biotinylated mAb (clone AD1.1.10, R&D systems) as capture and detection Abs, respectively.

[0203] *pHLA multimer staining*

[0204] T cells (1×10^5) were incubated for 30 minutes at 37°C in the presence of 50 nM dasatinib (LC laboratories). The cells were then washed and incubated with 5-10 μg/ml of multimer for 30 minutes at room temperature, and R-phycoerythrin-conjugated AffiniPure Fab fragment goat anti-mouse IgG1 (Jackson ImmunoResearch Laboratories) was added for 15 minutes at 4°C. Next, the cells were washed three times and costained with an anti-CD8 mAb for 15 minutes at 4°C. Dead cells were finally discriminated using the LIVE/DEAD Fixable Dead Cell Stain kit.

[0205] *Statistical analysis*

[0206] Statistical analysis was performed using GraphPad Prism 5.0e. To determine whether two groups were significantly different for a given variable, we conducted an analysis using Welch's t test (two-sided). P values < 0.05 were considered significant.

Claims

1. A nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR,

   wherein the anti-gp100 TCR cross competes for binding to human gp100 with a reference TCR, which comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

2. A nucleic acid molecule comprising (i) a first nucleotide sequence encoding a recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("anti-gp100 TCR"); and (ii) a second nucleotide sequence, wherein the second nucleotide sequence or the polypeptide encoded by the second nucleotide sequence inhibits the expression of an endogenous TCR,

   wherein the anti-gp100 TCR binds the same epitope or an overlapping epitope of human gp100 as a reference TCR, which comprises an alpha chain and a beta chain, wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2.

3. The nucleic acid molecule of claim 1 or 2, wherein the anti-gp100 TCR binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.

4. The nucleic acid molecule of claim 2 or 3, wherein the epitope is complexed with an HLA class I molecule.

5. The nucleic acid molecule of claim 4, wherein the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele.

6. The nucleic acid molecule of claim 4, wherein the HLA class I molecule is an HLA-B*40 allele.

7. The nucleic acid molecule of any one of claims 4 to 6, wherein the HLA class I molecule is selected from an HLA-B*40:01 allele, HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele.

8. The nucleic acid molecule of any one of claims 4 to 7, wherein the HLA class I molecule is an HLA-B*40:01 allele.

9. The nucleic acid molecule of any one of claims 1 to 8, wherein the anti-gp100 TCR comprises an alpha chain and a beta chain,

wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and

wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein the alpha chain CDR3 comprises an amino acid sequence as set forth in SEQ ID NO: 7.

10. The nucleic acid molecule of claim 9, wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

11. The nucleic acid molecule of any one of claims 1 to 8, wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a variable region comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and

wherein the beta chain comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

12. The nucleic acid molecule of claim 11, wherein the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

13. The nucleic acid molecule of any one of claims 9 to 12, wherein the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5.

14. The nucleic acid molecule of any one of claims 9 to 13, wherein the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8.

15. The nucleic acid molecule of any one of claims 9 to 14, wherein the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6.

16. The nucleic acid molecule of any one of claims 9 to 15, wherein the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

17. The nucleic acid molecule of any one of claims 9 to 16, wherein the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 1.

18. The nucleic acid molecule of any one of claims 9 to 17, wherein the beta chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth SEQ ID NO: 2.

19. The nucleic acid molecule of any one of claims 9 to 18, wherein the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant region of the alpha chain.

20. The nucleic acid molecule of any one of claims 9 to 19, wherein the alpha chain of the anti-gp100 TCR further comprises a constant region, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 1.

21. The nucleic acid molecule of claim 19 or 20, wherein the alpha chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least

4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 1.

22. The nucleic acid molecule of any one of claims 9 to 21, wherein the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the constant region is different from endogenous constant regions of the beta chain.

23. The nucleic acid molecule of any one of claims 9 to 22, wherein the beta chain of the anti-gp100 TCR further comprises a constant region, wherein the beta chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to a constant region present in the amino acid sequence set forth SEQ ID NO: 2.

24. The nucleic acid molecule of claim 22 or 23, wherein the beta chain constant region comprises an amino acid sequence comprising at least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth SEQ ID NO: 2.

25. The nucleic acid molecule of any one of claims 9 to 24, wherein the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1.

26. The nucleic acid molecule of any one of claims 9 to 25, wherein the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

27. The nucleic acid molecule of any one of claims 1 to 26, wherein the second nucleotide sequence is one or more siRNAs that reduce the expression of endogenous TCRs.

28. The nucleic acid molecule of claim 27, wherein the one or more siRNAs are complementary to a target sequence within a nucleotide sequence encoding a constant region of the endogenous TCRs.

29. The nucleic acid molecule of claim 27 or 28, wherein the one or more siRNAs comprise one or more nucleotide sequences selected from the group consisting of SEQ ID NOs: 53-56.

30. The nucleic acid molecule of any one of claims 1 to 29, wherein the second nucleotide sequence encodes Cas9.

31. The nucleic acid molecule of any one of claims 1 to 30, wherein the anti-gp100 TCR comprises an alpha chain constant region, a beta chain constant region, or both; and wherein the alpha chain constant region, the beta chain constant region, or both comprises an amino acid sequence having at least 1, at least 2, at least 3, at least 4, or at least 5 substitutions within the target sequence relative to the corresponding amino acid sequence of an endogenous TCR.

32. A vector comprising the nucleic acid molecule of any one of claims 1 to 31.

33. The vector of claim 32, which is a viral vector, a mammalian vector, or bacterial vector.

34. The vector of claim 32 or 33, which is a retroviral vector.

35. The vector of any one of claims 32 to 34, which is selected from the group consisting of an adenoviral vector, a lentivirus, a Sendai virus vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, a hybrid vector, and an adeno associated virus (AAV) vector.

36. The vector of any one of claims 32 to 35, which is a lentivirus.

37. A T cell receptor (TCR) or an antigen binding portion thereof comprising the alpha chain variable domain of the anti-gp100 TCR of any one of claims 9 to 31 and the beta chain variable domain of the anti-gp100 TCR of any one of claims 9 to 31.

38. A recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which cross competes for binding to human gp100 with a reference TCR;

wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; and

wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein

(i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or

(ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence of SEQ ID NO: 2.

39. A recombinant T cell receptor (TCR) or an antigen binding portion thereof that specifically binds human gp100 ("an anti-gp100 TCR"), which binds the same epitope or an overlapping epitope of human gp100 as a reference TCR;

wherein the reference TCR comprises an alpha chain and a beta chain, and wherein the alpha chain comprises an amino acid sequence as set forth in SEQ ID NO: 1 and the beta chain comprises an amino acid sequence as set forth in SEQ ID NO: 2; an

wherein the anti-gp100 TCR comprises an alpha chain and a beta chain, wherein the alpha chain comprises a constant region, and wherein the beta chain comprises a constant region; wherein

(i) the alpha chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 1 or

(ii) the beta chain constant region comprises an amino acid sequence having a least 1, at least 2, at least 3, at least 4, or at least 5 amino acid substitutions relative to a constant region present in the amino acid sequence set forth in SEQ ID NO: 2.

40. The anti-gp100 TCR of claim 38 or 39, which binds to an epitope of gp100 consisting of an amino acid sequence as set forth in SEQ ID NO: 13.

41. The anti-gp100 TCR of claim 39 or 40, wherein the epitope is complexed with an HLA class I molecule.

42. The anti-gp100 TCR of claim 41, wherein the HLA class I molecule is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G allele.

43. The anti-gp100 TCR of claim 41 or 42, wherein the HLA class I molecule is an HLA-B*40 allele.

44. The anti-gp100 TCR of any one of claims 41 to 43, wherein the HLA class I molecule is selected from an HLA-B*40:01 allele, an HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele.

45. The anti-gp100 TCR of any one of claims 41 to 44, wherein the HLA class I molecule is an HLA-B*40:01 allele.

46. The anti-gp100 TCR of any one of claims 38 to 45, wherein the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chain CDR1, an alpha chain CDR2, and an alpha chain CDR3; and

wherein the beta chain of the anti-gp100 TCR comprises variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein the alpha chain CDR3 of the anti-gp100 comprises an amino acid sequence as set forth in SEQ ID NO: 7.

47. The anti-gp100 TCR of claim 46, wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

48. The anti-gp100 TCR of any one of claims 38 to 45, wherein the alpha chain of the anti-gp100 TCR comprises a variable domain comprising an alpha chainCDR1, an alpha chain CDR2, and an alpha chain CDR3; and

wherein the beta chain of the anti-gp100 TCR comprises a variable domain comprising a beta chain CDR1, a beta chain CDR2, and a beta chain CDR3;

wherein the beta chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 10.

49. The anti-gp100 TCR of claim 48, wherein the alpha chain CDR3 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 7.

50. The anti-gp100 TCR of claim 49, wherein the alpha chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 5.

51. The anti-gp100 TCR of any one of claims 46 to 50, wherein the beta chain CDR1 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 8.

52. The anti-gp100 TCR of any one of claims 46 to 51, wherein the alpha chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 6.

53. The anti-gp100 TCR of any one of claims 46 to 52, wherein the beta chain CDR2 of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 9.

54. The anti-gp100 TCR of any one of claims 46 to 53, wherein the alpha chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 1.

55. The anti-gp100 TCR of any one of claims 46 to 54, wherein the beta chain variable domain of the anti-gp100 TCR comprises an amino acid sequence of a variable domain present in the amino acid sequence set forth in SEQ ID NO: 2.

56. The anti-gp100 TCR of any one of claims 38 to 55, wherein the alpha chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 1.

57. The anti-gp100 TCR of any one of claims 38 to 56, wherein the beta chain constant region comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence of a constant region present in the amino acid sequence set forth in SEQ ID NO: 2.

58. The anti-gp100 TCR of any one of claims 38 to 57, wherein the alpha chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 1.

59. The anti-gp100 TCR of any one of claims 38 to 58, wherein the beta chain of the anti-gp100 TCR comprises an amino acid sequence as set forth in SEQ ID NO: 2.

60. A bispecific TCR comprising a first antigen-binding domain and a second antigen-binding domain, wherein the first antigen-binding domain comprises the TCR or an antigen-binding portion thereof of claim 37 or the TCR or an antigen-binding portion thereof of any one of claims 38 to 59.

61. The bispecific TCR of claim 60, wherein the first antigen-binding domain comprises a single chain variable fragment ("scFv").

62. The bispecific TCR of claim 60 or 61, wherein the second antigen-binding domain binds specifically to a protein expressed on the surface of a T cell.

63. The bispecific TCR of any one of claims 60 to 62, wherein the second antigen-binding domain binds specifically to CD3.

64. The bispecific TCR of any one of claims 60 to 63, wherein the second antigen-binding domain comprises an scFv.

65. The bispecific TCR of any one of claims 60 to 64, wherein the first antigen-binding domain and the second antigen-binding domain are linked or associated by a covalent bond.

66. The bispecific TCR of any one of claims 60 to 65, wherein the first antigen-binding domain and the second antigen-binding domain are linked by a peptide bond.

67. A cell comprising the nucleic acid molecule of any one of claims 1 to 31, the vector of any one of claims 32 to 36, the TCR of claim 37, the recombinant TCR of any one of claims 38 to 59, or the bispecific TCR of any one of claims 60 to 66.

68. The cell of claim 67, which further expresses CD3.

69. The cell of claim 67 or 68, which is selected from the group consisting of a T cell, a natural killer (NK) cell, an natural killer T (NKT) cell, or an ILC cell.

70. A method of treating a cancer in a subject in need thereof, comprising administering to the subject the cell of any one of claims 67 to 69.

71. The method of claim 70, wherein the cancer is selected from the group consisting of melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma (NHL), primary mediastinal large B cell lymphoma (PMBC), diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), transformed follicular lymphoma, splenic marginal zone lymphoma (SMZL), cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia (ALL) (including non T cell ALL), chronic lymphocytic leukemia (CLL), solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, other B cell malignancies, and combinations of said cancers.

72. The method of claim 70 or 71, wherein the cancer is relapsed or refractory.

73. The method of any one of claims 70 to 72, wherein the cancer is locally advanced.

74. The method of any one of claims 70 to 73, wherein the cancer is advanced.

75. The method of any one of claims 70 to 74, wherein the cancer is metastatic.

76. The method of any one of claims 70 to 75, wherein the cells are obtained from the subject.

77. The method of any one of claims 70 to 76, wherein the cells are obtained from a donor other than the subject.

78. The method of any one of claims 70 to 77, wherein the subject is preconditioned prior to the administering of the cells.

79. The method of any one of claims 68 to 78, wherein the preconditioning comprises administering to the subject a chemotherapy, a cytokine, a protein, a small molecule, or any combination thereof.

80. The method of claim 78 or 79, wherein the preconditioning comprises administering an interleukin.

81. The method of any one of claims 78 to 80, wherein the preconditioning comprises administering IL-2, , IL-4, IL-7, IL-9, IL-15, IL-21, or any combination thereof.

82. The method of any one of claims 78 to 81, wherein the preconditioning comprises administering a preconditioning agent selected from the group consisting of cyclophosphamide, fludarabine, vitamin C, an AKT inhibitor, ATRA, Rapamycin, or any combination thereof.

83. The method of any one of claims 78 to 82, wherein the preconditioning comprises administering cyclophosphamide, fludarabine, or both.

84. A method of engineering an antigen-targeting cell, comprising transducing a cell collected from a subject in need of a T cell therapy with the nucleic acid molecule of any one of claims 1 to 31 or the vector of any one of claims 32 to 36.

85. The method of claim 84, wherein the antigen-targeting cell further expresses CD3.

86. The method of claim 84 or 85, wherein the cell is a T cell or a natural killer (NK) cell.

87. An HLA class I molecule complexed to a peptide, wherein the HLA class I molecule comprises an α1 domain, an α2 domain, an α3 domain and a β2m, and wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 14.

88. The HLA class I molecule of claim 87, which is an HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G.

89. The HLA class I molecule of claim 87 or 88, which is an HLA-B.

90. The HLA class I molecule of any one of claims 87 to 89, which is an HLA-B*40 allele.

91. The HLA class I molecule of any one of claims 87 to 90, wherein the HLA class I molecule is selected from an HLA-B*40:01 allele, HLA-B*40:02 allele, an HLA-B*40:03 allele, an HLA-B*40:04 allele, an HLA-B*40:05 allele, and an HLA-B*40:06 allele.

92. The HLA class I molecule of any one of claims 87 to 91, wherein the HLA class I molecule is an HLA-B*40:01 allele.

93. The HLA class I molecule of any one of claims 87 to 92, wherein the HLA class I molecule is an HLA-B*40:02 allele.

94. The HLA class I molecule of any one of claims 87 to 93, which is a monomer.

95. The HLA class I molecule of any one of claims 87 to 93, which is a dimer.

96. The HLA class I molecule of any one of claims 87 to 93, which is a trimer.

97. The HLA class I molecule of any one of claims 87 to 93, which is a tetramer.

98. The HLA class I molecule of any one of claims 87 to 93, which is a pentamer.

99. An antigen presenting cell (APC), comprising the HLA class I molecule of any one of claims 87 to 98.

100. The APC of claims 99, wherein the HLA class I molecule is expressed on the surface of the APC.

101. A method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells with the HLA class I molecule of any one of claims 87 to 98 or the APC of claim 99 or 100, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of binding the HLA class I molecule relative to the number of T cells capable of binding the HLA class I molecule prior to the contacting.

102. A method of enriching a target population of T cells obtained from a human subject, comprising contacting the T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 13, wherein following the contacting, the enriched population of T cells comprises a higher number of T cells capable of targeting a tumor cell relative to the number of T cells capable of targeting a tumor cell prior to the contacting.

103. The method of claim 101 or 102, wherein the T cells obtained from the human subject are tumor infiltrating lymphocytes (TIL).

104. A method of treating a tumor in a subject in need thereof, comprising administering to the subject the enriched T cells of claim 101 or 102.

105. A method of enhancing cytotoxic T cell-mediated targeting of cancer cells in a subject afflicted with a cancer, comprising administering to the subject a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.

106. A cancer vaccine comprising a peptide having an amino acid sequence as set forth in SEQ ID NO: 13.

107. A method of selecting a T cell capable of targeting a tumor cell, comprising contacting a population of isolated T cells *in vitro* with a peptide, wherein the peptide consists of an amino acid sequence as set forth in SEQ ID NO: 11.

108. The method of claim 107, wherein the T cell is a tumor infiltrating lymphocytes (TIL).

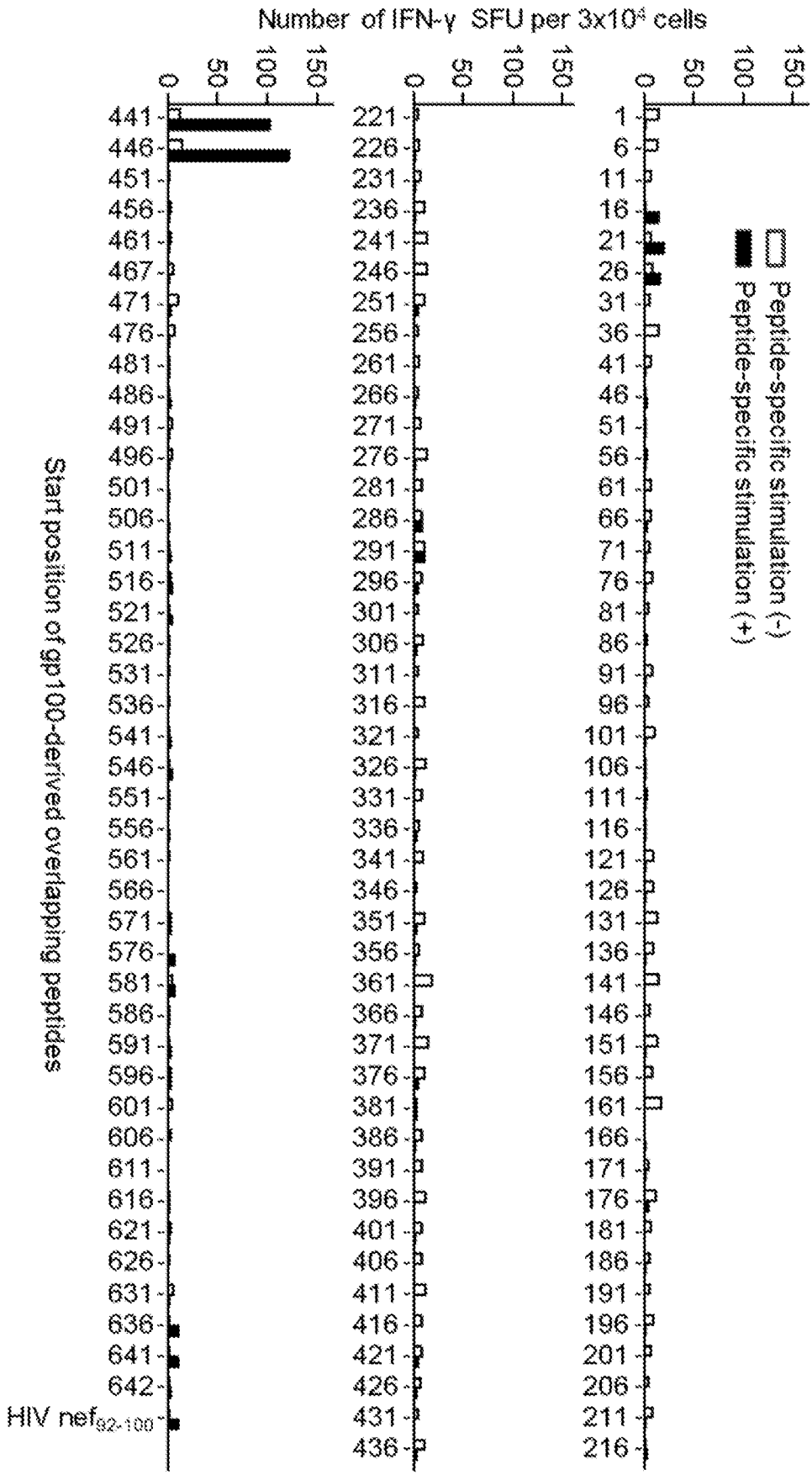

Number of IFN-γ SFU per 3x10⁵ cells
0
50
100
150
Peptide-specific stimulation (-)
Peptide-specific stimulation (+)
Start position of gp100-derived overlapping peptides
HIV nef92-100

FIG. 1

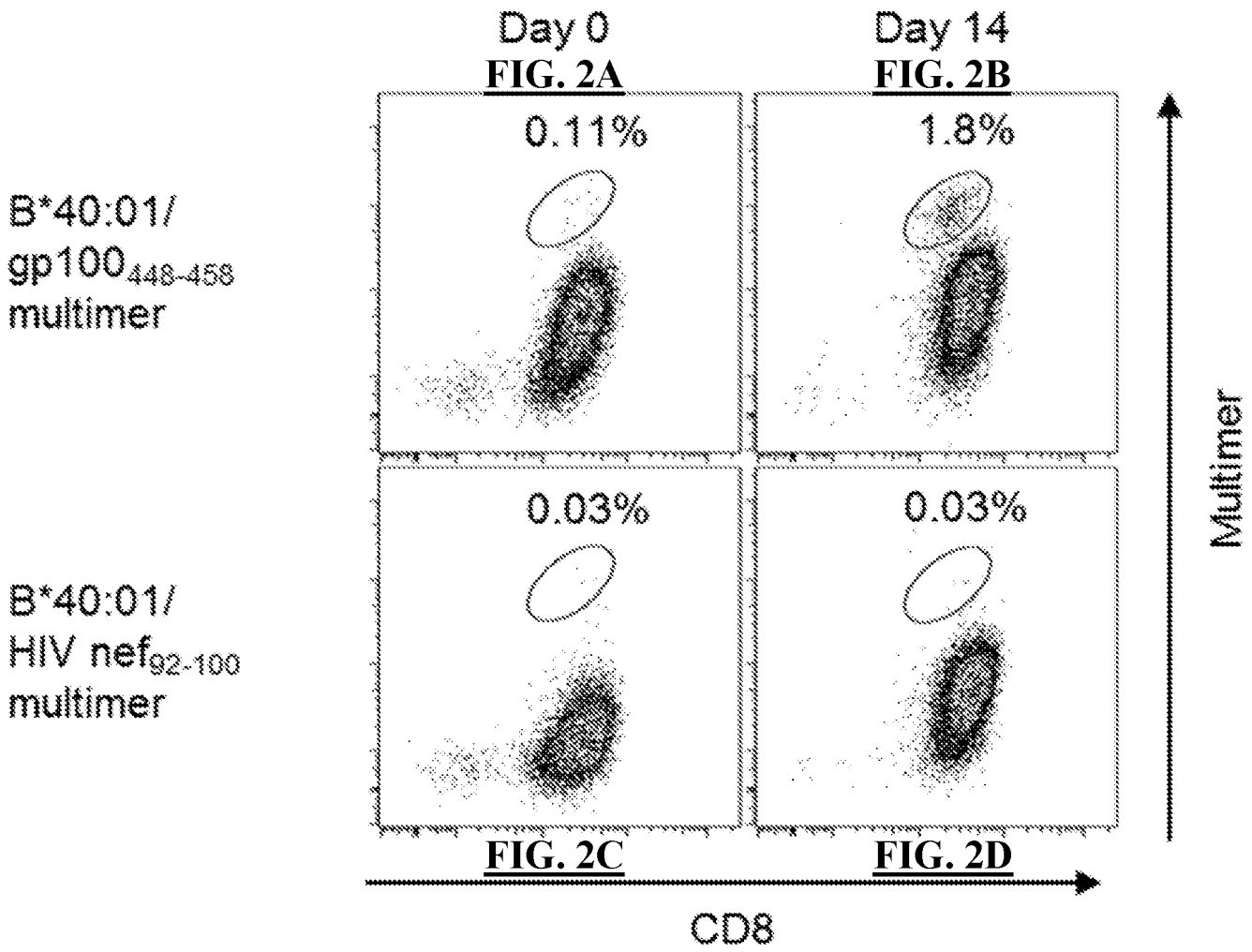
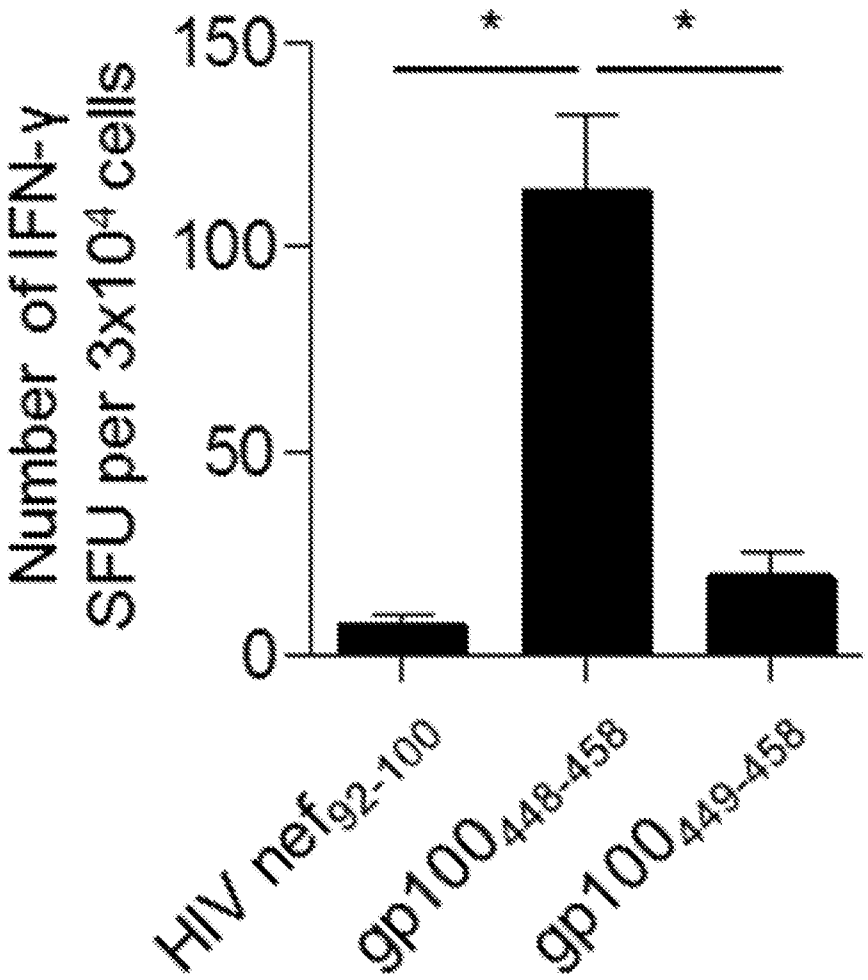
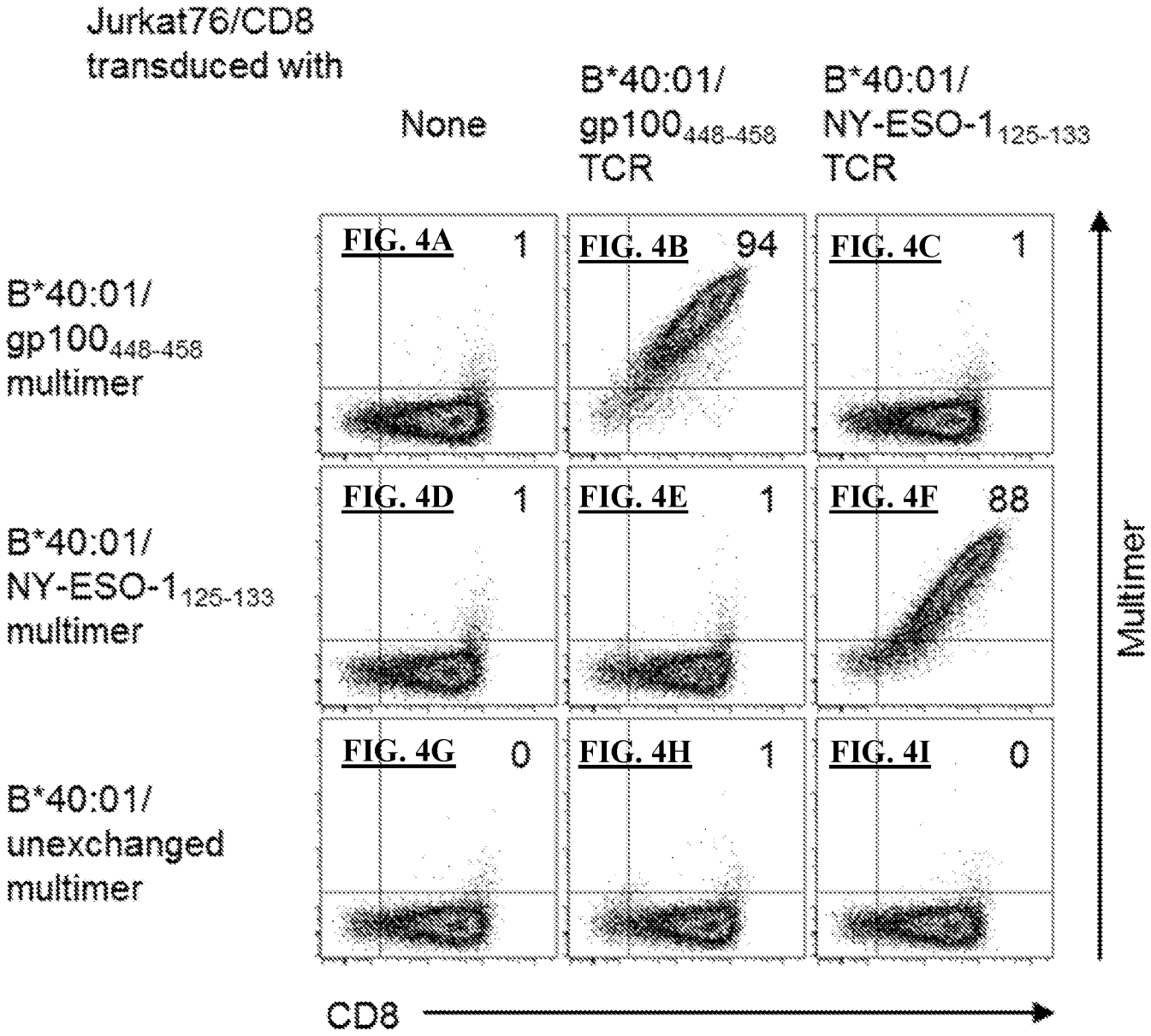

Day 0
Day 14
FIG. 2A
FIG. 2B
0.11%
1.8%
B*40:01/
gp100448-458
multimer
0.03%
0.03%
B*40:01/
HIV nef92-100
multimer
FIG. 2C
FIG. 2D
Multimer
CD8

FIG. 3


Number of IFN-γ SFU per 3x10^4 cells
150
100
50
0
*
*
HIV nef92-100
gp100448-458
gp100449-458


Jurkat76/CD8
transduced with
None
B*40:01/
gp100448-458
TCR
B*40:01/
NY-ESO-1125-133
TCR
B*40:01/
gp100448-458
multimer
B*40:01/
NY-ESO-1125-133
multimer
B*40:01/
unexchanged
multimer
FIG. 4A 1
FIG. 4B 94
FIG. 4C 1
FIG. 4D 1
FIG. 4E 1
FIG. 4F 88
FIG. 4G 0
FIG. 4H 1
FIG. 4I 0
Multimer
CD8

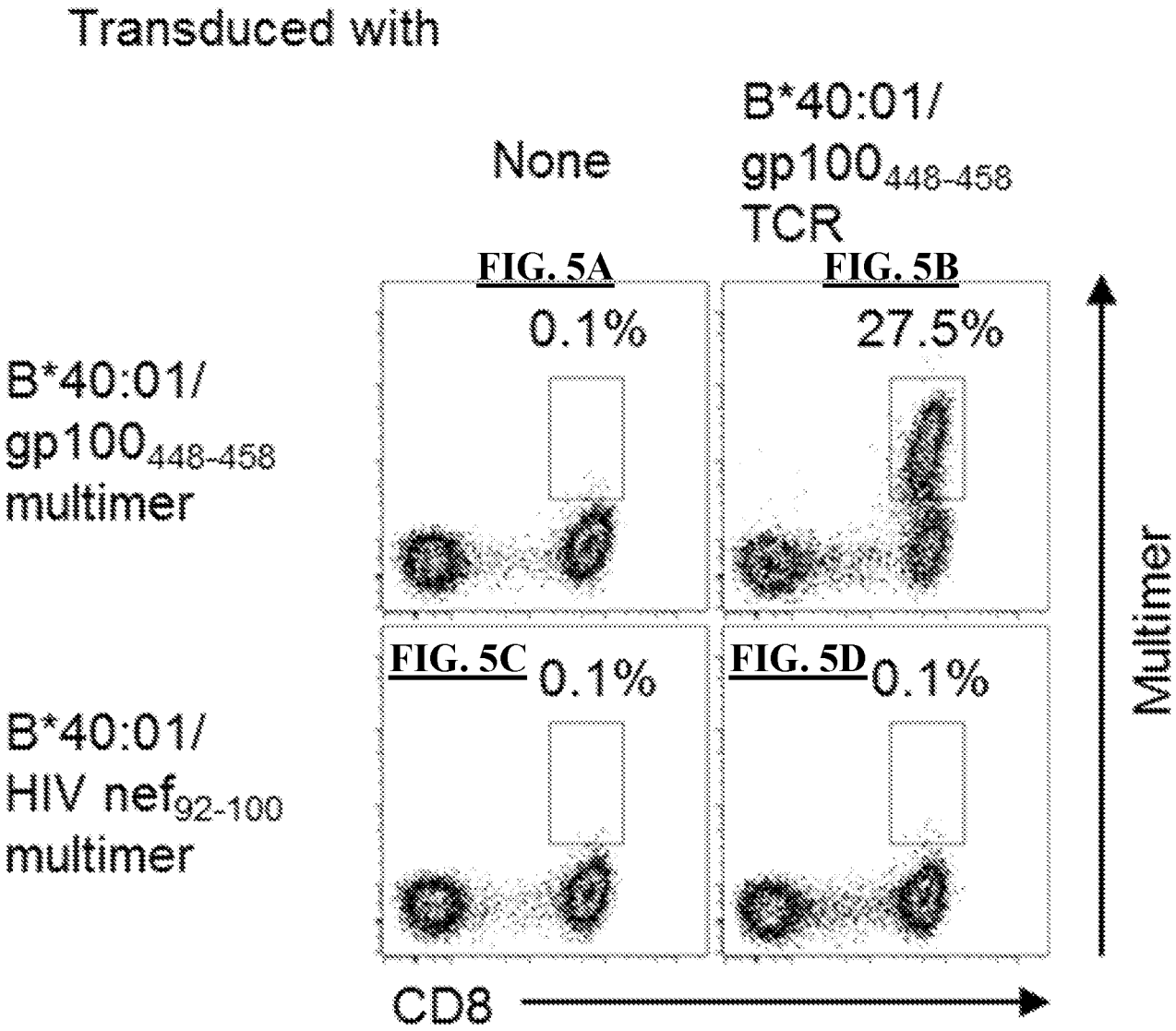

Transduced with
None
B*40:01/ gp100448-458 TCR
FIG. 5A
FIG. 5B
0.1%
27.5%
B*40:01/ gp100448-458 multimer
FIG. 5C
0.1%
FIG. 5D
0.1%
B*40:01/ HIV nef92-100 multimer
Multimer
CD8

FIG. 6

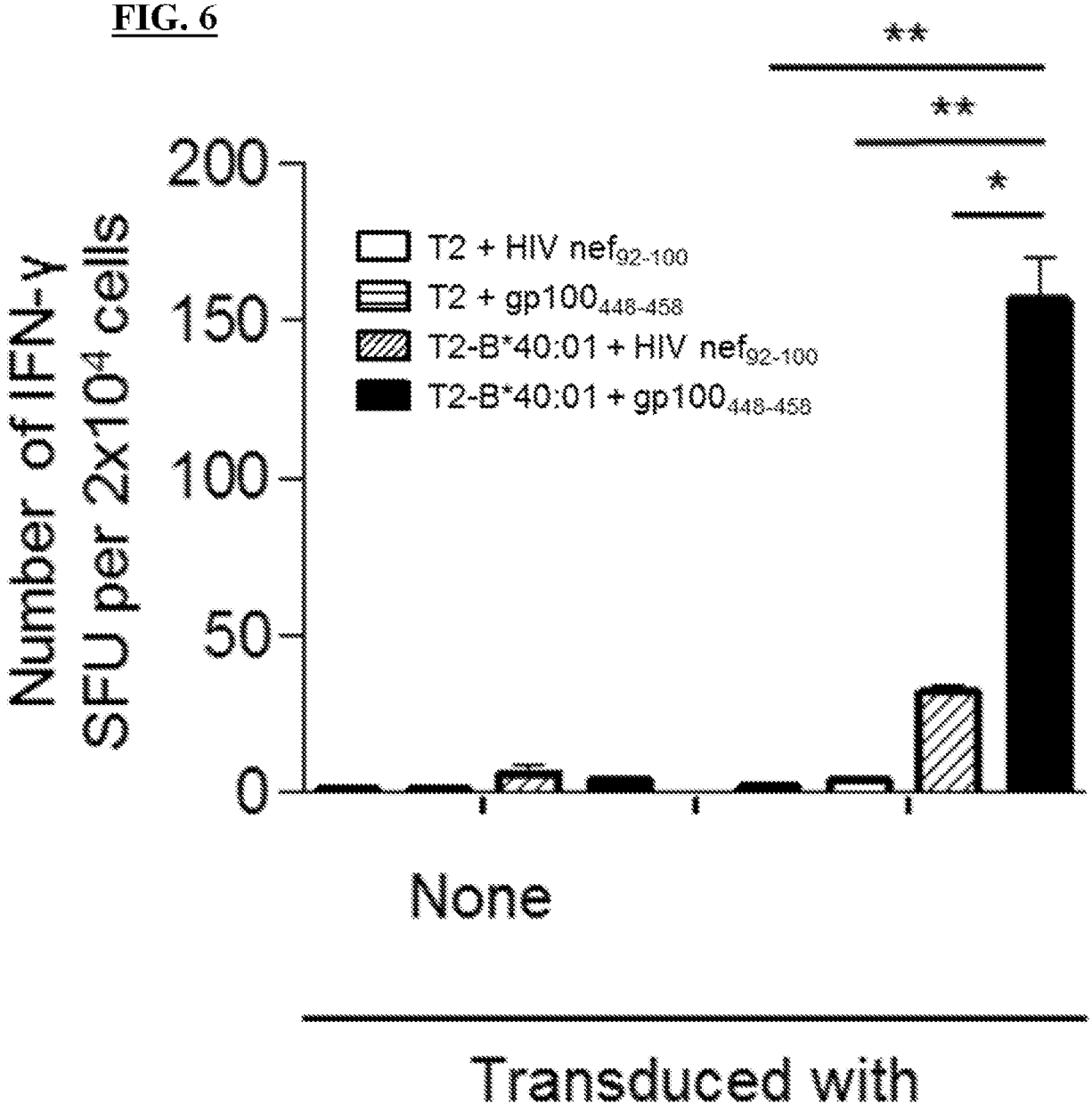

**
**
*
200
150
100
50
0
Number of IFN-γ SFU per 2x10^4 cells
T2 + HIV nef92-100
T2 + gp100448-458
T2-B*40:01 + HIV nef92-100
T2-B*40:01 + gp100448-458
None
Transduced with

FIG. 7A

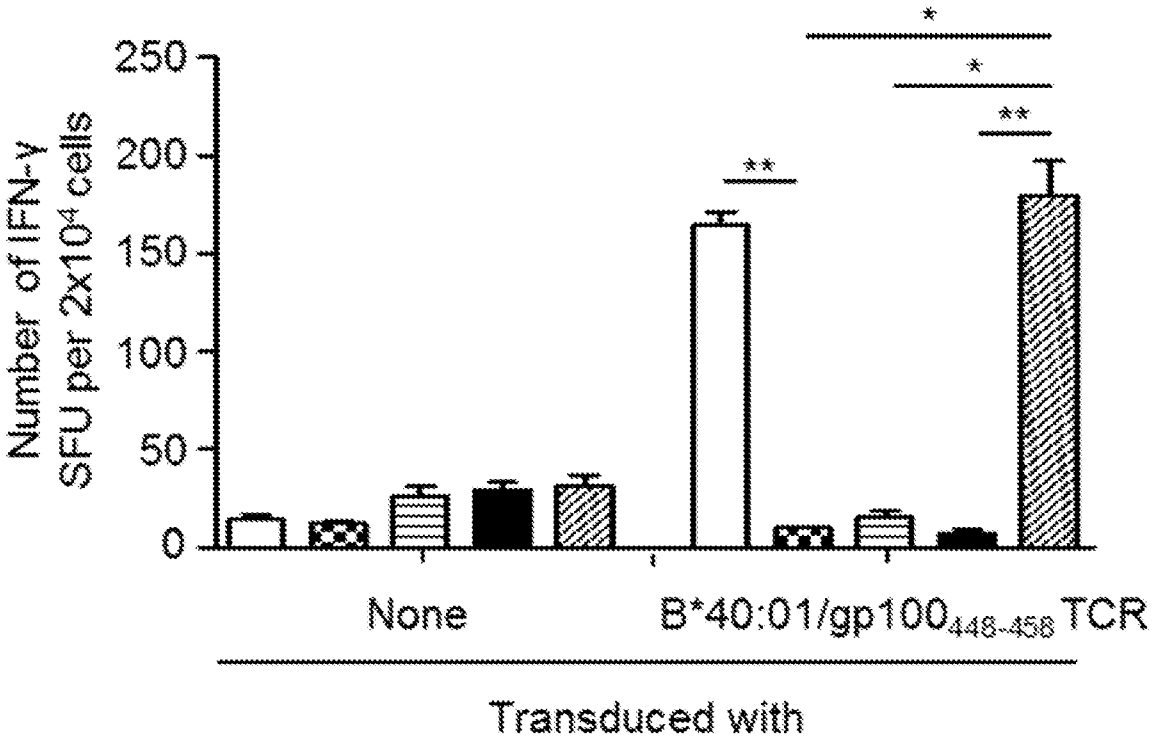


FIG. 7B

	B*40:01	gp100
SK-MEL-28	+	+
SK-MEL-37	-	-
SK-MEL-37/B*40:01	+	-
SK-MEL-37/gp100	-	+
SK-MEL-37/B*40:01/gp100	+	+

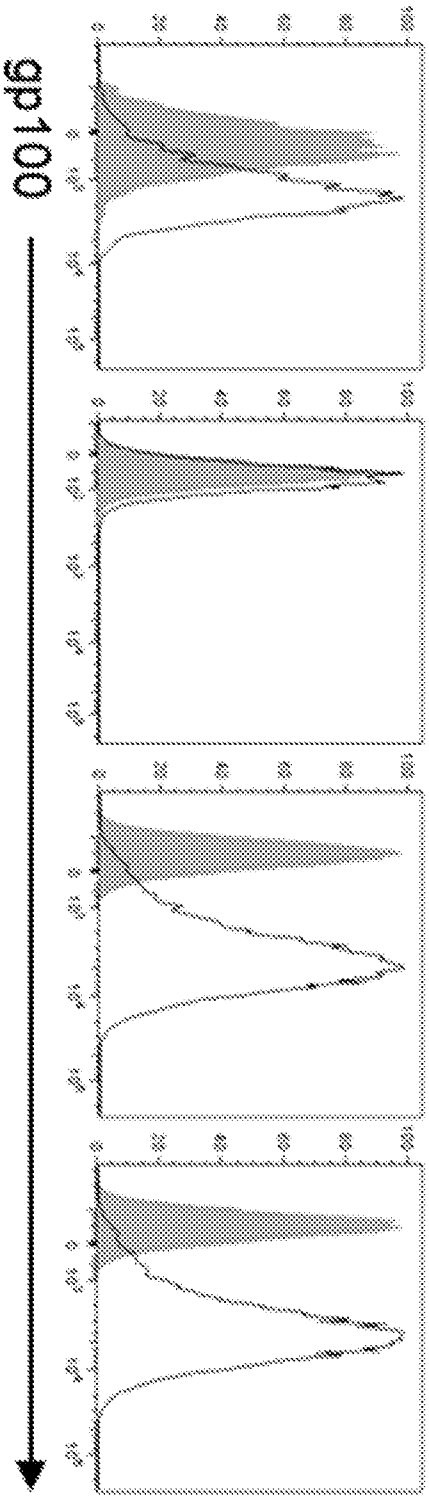


FIG. 8A FIG. 8B FIG. 8C FIG. 8D

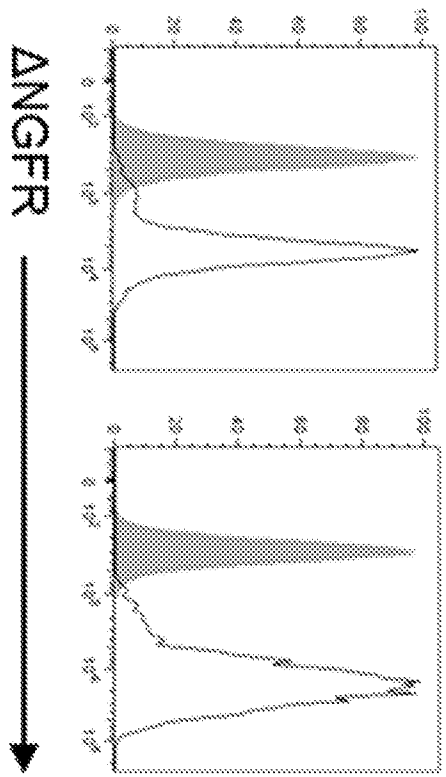


FIG. 9A

SK-MEL-37

FIG. 9B

SK-MEL-37/B*40:01

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<151> 2019-03-04

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Thr Asp Lys Thr Val Leu Asp Met Arg Ser Met Asp Phe Lys Ser Asn
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Gly Phe His Pro Ser Asp Ile Glu Val Asp Leu Leu Lys Asn Gly Glu
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Arg Ile Glu Lys Val Glu His Ser Asp Leu Ser Phe Ser Lys Asp Trp
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Ser Phe Tyr Leu Leu Tyr Tyr Thr Glu Phe Thr Pro Thr Glu Lys Asp
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Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
50 55 60

Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65 70 75 80

Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
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Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
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Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser

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Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
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Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
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Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
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Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
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Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
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Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
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Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
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Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
260 265 270

Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
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Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
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Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
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Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
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Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr
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Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
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Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
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Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
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Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
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Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
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Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
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Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
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Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
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Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
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Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
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Gly Ser Trp Ser Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp
1 5 10 15

Gly Gln Tyr Trp
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<210> 93
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 93

Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp Gln
1 5 10 15

Val Leu Gly Gly
20

<210> 94
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 94

Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp Gln Val Leu Gly Gly Pro
1 5 10 15

Val Ser Gly Leu
20

<210> 95
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 95

Trp Gly Gln Tyr Trp Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser
1 5 10 15

Ile Gly Thr Gly
20

<210> 96
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 96

Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
1 5 10 15

Ala Met Leu Gly
20

<210> 97
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 97

Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg Ala Met Leu Gly Thr
1 5 10 15

His Thr Met Glu
20

<210> 98
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 98

Ser Ile Gly Thr Gly Arg Ala Met Leu Gly Thr His Thr Met Glu Val
1 5 10 15

Thr Val Tyr His
20

<210> 99
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 99

Arg Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg
1 5 10 15

Arg Gly Ser Arg
20

<210> 100
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 100

Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg Gly Ser Arg Ser
1 5 10 15

Tyr Val Pro Leu
20

<210> 101
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 101

Val Thr Val Tyr His Arg Arg Gly Ser Arg Ser Tyr Val Pro Leu Ala
1 5 10 15

His Ser Ser Ser
20

<210> 102
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 102

Arg Arg Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala
1 5 10 15

Phe Thr Ile Thr
20

<210> 103
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 103

Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr Ile Thr Asp
1 5 10 15

Gln Val Pro Phe
20

<210> 104
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 104

Ala His Ser Ser Ser Ala Phe Thr Ile Thr Asp Gln Val Pro Phe Ser
1 5 10 15

Val Ser Val Ser
20

<210> 105
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 105

Ala Phe Thr Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln
1 5 10 15

Leu Arg Ala Leu
20

<210> 106
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 106

Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala Leu Asp
1 5 10 15

Gly Gly Asn Lys
20

<210> 107
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 107

Ser Val Ser Val Ser Gln Leu Arg Ala Leu Asp Gly Gly Asn Lys His
1 5 10 15

Phe Leu Arg Asn
20

<210> 108
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 108

Gln Leu Arg Ala Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln
1 5 10 15

Pro Leu Thr Phe
20

<210> 109
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 109

Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe Ala
1 5 10 15

Leu Gln Leu His
20

<210> 110
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 110

His Phe Leu Arg Asn Gln Pro Leu Thr Phe Ala Leu Gln Leu His Asp
1 5 10 15

Pro Ser Gly Tyr
20

<210> 111
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 111

Gln Pro Leu Thr Phe Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu
1 5 10 15

Ala Glu Ala Asp
20

<210> 112
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 112

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
1 5 10 15

Ser Tyr Thr Trp
20

<210> 113
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 113

Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu Ser Tyr Thr Trp Asp
1 5 10 15

Phe Gly Asp Ser
20

<210> 114
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 114

Leu Ala Glu Ala Asp Leu Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser
1 5 10 15

Gly Thr Leu Ile
20

<210> 115
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 115

Leu Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser
1 5 10 15

Arg Ala Leu Val
20

<210> 116
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 116

Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg Ala Leu Val Val
1 5 10 15

Thr His Thr Tyr
20

<210> 117
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 117

Ser Gly Thr Leu Ile Ser Arg Ala Leu Val Val Thr His Thr Tyr Leu
1 5 10 15

Glu Pro Gly Pro
20

<210> 118
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 118

Ser Arg Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val
1 5 10 15

Thr Ala Gln Val
20

<210> 119
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 119

Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala Gln Val Val
1 5 10 15

Leu Gln Ala Ala
20

<210> 120
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 120

Leu Glu Pro Gly Pro Val Thr Ala Gln Val Val Leu Gln Ala Ala Ile
1 5 10 15

Pro Leu Thr Ser
20

<210> 121
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 121

Val Thr Ala Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys
1 5 10 15

Gly Ser Ser Pro
20

<210> 122
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 122

Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser Pro Val
1 5 10 15

Pro Gly Thr Thr
20

<210> 123
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 123

Ile Pro Leu Thr Ser Cys Gly Ser Ser Pro Val Pro Gly Thr Thr Asp
1 5 10 15

Gly His Arg Pro
20

<210> 124
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 124

Cys Gly Ser Ser Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr
1 5 10 15

Ala Glu Ala Pro
20

<210> 125
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 125

Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro Asn
1 5 10 15

Thr Thr Ala Gly
20

<210> 126
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 126

Asp Gly His Arg Pro Thr Ala Glu Ala Pro Asn Thr Thr Ala Gly Gln
1 5 10 15

Val Pro Thr Thr
20

<210> 127
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 127

Thr Ala Glu Ala Pro Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu
1 5 10 15

Val Val Gly Thr
20

<210> 128
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 128

Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
1 5 10 15

Pro Gly Gln Ala
20

<210> 129
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 129

Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr Pro Gly Gln Ala Pro
1 5 10 15

Thr Ala Glu Pro
20

<210> 130
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 130

Glu Val Val Gly Thr Thr Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser
1 5 10 15

Gly Thr Thr Ser
20

<210> 131
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 131

Thr Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val
1 5 10 15

Gln Val Pro Thr
20

<210> 132
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 132

Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln Val Pro Thr Thr
1 5 10 15

Glu Val Ile Ser
20

<210> 133
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 133

Ser Gly Thr Thr Ser Val Gln Val Pro Thr Thr Glu Val Ile Ser Thr
1 5 10 15

Ala Pro Val Gln
20

<210> 134
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 134

Val Gln Val Pro Thr Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met
1 5 10 15

Pro Thr Ala Glu
20

<210> 135
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 135

Thr Glu Val Ile Ser Thr Ala Pro Val Gln Met Pro Thr Ala Glu Ser
1 5 10 15

Thr Gly Met Thr
20

<210> 136
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 136

Thr Ala Pro Val Gln Met Pro Thr Ala Glu Ser Thr Gly Met Thr Pro
1 5 10 15

Glu Lys Val Pro
20

<210> 137
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 137

Met Pro Thr Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val
1 5 10 15

Ser Glu Val Met
20

<210> 138
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 138

Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val Met Gly
1 5 10 15

Thr Thr Leu Ala
20

<210> 139
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 139

Pro Glu Lys Val Pro Val Ser Glu Val Met Gly Thr Thr Leu Ala Glu
1 5 10 15

Met Ser Thr Pro
20

<210> 140
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 140

Val Ser Glu Val Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu
1 5 10 15

Ala Thr Gly Met
20

<210> 141
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 141

Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met Thr
1 5 10 15

Pro Ala Glu Val
20

<210> 142
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 142

Glu Met Ser Thr Pro Glu Ala Thr Gly Met Thr Pro Ala Glu Val Ser
1 5 10 15

Ile Val Val Leu
20

<210> 143
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 143

Glu Ala Thr Gly Met Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser
1 5 10 15

Gly Thr Thr Ala
20

<210> 144
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 144

Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
1 5 10 15

Gln Val Thr Thr
20

<210> 145
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 145

Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala Gln Val Thr Thr Thr
1 5 10 15

Glu Trp Val Glu
20

<210> 146
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 146

Ser Gly Thr Thr Ala Ala Gln Val Thr Thr Thr Glu Trp Val Glu Thr
1 5 10 15

Thr Ala Arg Glu
20

<210> 147
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 147

Ala Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu
1 5 10 15

Pro Ile Pro Glu
20

<210> 148
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 148

Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro Ile Pro Glu Pro
1 5 10 15

Glu Gly Pro Asp
            20

<210> 149
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 149

Thr Thr Ala Arg Glu Leu Pro Ile Pro Glu Pro Glu Gly Pro Asp Ala
1   5   10   15

Ser Ser Ile Met
            20

<210> 150
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 150

Leu Pro Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser
1   5   10   15

Thr Glu Ser Ile
            20

<210> 151
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 151

Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu Ser Ile Thr
1 5 10 15

Gly Ser Leu Gly
20

<210> 152
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 152

Ala Ser Ser Ile Met Ser Thr Glu Ser Ile Thr Gly Ser Leu Gly Pro
1 5 10 15

Leu Leu Asp Gly
20

<210> 153
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 153

Ser Thr Glu Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr
1 5 10 15

Ala Thr Leu Arg
20

<210> 154
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 154

Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu Arg Leu
1 5 10 15

Val Lys Arg Gln
20

<210> 155
<211> 20
<212> PRT
<213> Artificial sequence

<220>
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<400> 155

Pro Leu Leu Asp Gly Thr Ala Thr Leu Arg Leu Val Lys Arg Gln Val
1 5 10 15

Pro Leu Asp Cys
20

<210> 156
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 156

Thr Ala Thr Leu Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val
1 5 10 15

Leu Tyr Arg Tyr
20

<210> 157
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 157

Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr Gly
1 5 10 15

Ser Phe Ser Val
20

<210> 158
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 158

Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr Gly Ser Phe Ser Val Thr
1 5 10 15

Leu Asp Ile Val
20

<210> 159
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 159

Val Leu Tyr Arg Tyr Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln
1 5 10 15

Gly Ile Glu Ser
20

<210> 160
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 160

Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
1 5 10 15

Glu Ile Leu Gln
20

<210> 161
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 161

Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala Glu Ile Leu Gln Ala
1 5 10 15

Val Pro Ser Gly
20

<210> 162
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 162

Gln Gly Ile Glu Ser Ala Glu Ile Leu Gln Ala Val Pro Ser Gly Glu
1 5 10 15

Gly Asp Ala Phe
20

<210> 163
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 163

Ala Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu
1 5 10 15

Leu Thr Val Ser
20

<210> 164
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 164

Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu Thr Val Ser Cys
1 5 10 15

Gln Gly Gly Leu
20

<210> 165
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 165

Glu Gly Asp Ala Phe Glu Leu Thr Val Ser Cys Gln Gly Gly Leu Pro
1 5 10 15

Lys Glu Ala Cys
20

<210> 166
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 166

Glu Leu Thr Val Ser Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met
1 5 10 15

Glu Ile Ser Ser
20

<210> 167
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 167

Cys Gln Gly Gly Leu Pro Lys Glu Ala Cys Met Glu Ile Ser Ser Pro
1 5 10 15

Gly Cys Gln Pro
20

<210> 168
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 168

Pro Lys Glu Ala Cys Met Glu Ile Ser Ser Pro Gly Cys Gln Pro Pro
1 5 10 15

Ala Gln Arg Leu
20

<210> 169
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 169

Met Glu Ile Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys
1 5 10 15

Gln Pro Val Leu
20

<210> 170
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 170

Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val Leu Pro
1 5 10 15

Ser Pro Ala Cys
20

<210> 171
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 171

Pro Ala Gln Arg Leu Cys Gln Pro Val Leu Pro Ser Pro Ala Cys Gln
1 5 10 15

Leu Val Leu His
20

<210> 172
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 172

Cys Gln Pro Val Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln
1 5 10 15

Ile Leu Lys Gly
20

<210> 173
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 173

Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly Gly
1 5 10 15

Ser Gly Thr Tyr
20

<210> 174
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 174

Gln Leu Val Leu His Gln Ile Leu Lys Gly Gly Ser Gly Thr Tyr Cys
1 5 10 15

Leu Asn Val Ser
20

<210> 175
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 175

Gln Ile Leu Lys Gly Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu
1 5 10 15

Ala Asp Thr Asn
20

<210> 176
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 176

Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
1 5 10 15

Leu Ala Val Val
20

<210> 177
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 177

Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser Leu Ala Val Val Ser
1 5 10 15

Thr Gln Leu Ile
20

<210> 178
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 178

Leu Ala Asp Thr Asn Ser Leu Ala Val Val Ser Thr Gln Leu Ile Met
1 5 10 15

Pro Gly Gln Glu
20

<210> 179
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 179

Ser Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala
1 5 10 15

Gly Leu Gly Gln
20

<210> 180
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 180

Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly Leu Gly Gln Val
1 5 10 15

Pro Leu Ile Val
20

<210> 181
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 181

Met Pro Gly Gln Glu Ala Gly Leu Gly Gln Val Pro Leu Ile Val Gly
1 5 10 15

Ile Leu Leu Val
20

<210> 182
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 182

Ala Gly Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu
1 5 10 15

Met Ala Val Val
20

<210> 183
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 183

Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala Val Val Leu
1 5 10 15

Ala Ser Leu Ile
20

<210> 184
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 184

Gly Ile Leu Leu Val Leu Met Ala Val Val Leu Ala Ser Leu Ile Tyr
1 5 10 15

Arg Arg Arg Leu
20

<210> 185
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 185

Leu Met Ala Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met
1 5 10 15

Lys Gln Asp Phe
20

<210> 186
<211> 20
<212> PRT

<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 186

Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp Phe Ser
1 5 10 15

Val Pro Gln Leu
20

<210> 187
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> Synthetic Peptide fragment

<400> 187

Tyr Arg Arg Arg Leu Met Lys Gln Asp Phe Ser Val Pro Gln Leu Pro
1 5 10 15

His Ser Ser Ser
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Met Lys Gln Asp Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His
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Trp Leu Arg Leu
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Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu Pro
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Arg Ile Phe Cys
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Cys Pro Ile Gly
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His Trp Leu Arg Leu Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu
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Asn Ser Pro Leu
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Ser Gly Gln Gln
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Gly Gln Gln Val
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Glu Phe Thr Val Ser Gly Asn Ile Leu
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