

T CELL RECEPTORS RECOGNIZING R175H OR Y220C MUTATION IN P53

CROSS REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/867,619, filed June 27, 2019, which is incorporated by reference in its entirety herein.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support under project number BC010985 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 357,775 Byte ASCII (Text) file named "749338_ST25.txt," dated June 23, 2020.

BACKGROUND OF THE INVENTION

[0004] Some cancers may have very limited treatment options, particularly when the cancer becomes metastatic and unresectable. Despite advances in treatments such as, for example, surgery, chemotherapy, and radiation therapy, the prognosis for many cancers, such as, for example, pancreatic, colorectal, lung, endometrial, ovarian, and prostate cancers, may be poor. Accordingly, there exists an unmet need for additional treatments for cancer.

BRIEF SUMMARY OF THE INVENTION

[0005] An embodiment of the invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for a human p53^{R175H} or human p53^{Y220C} amino acid sequence, wherein the TCR comprises the amino acid sequences of: (1) all of SEQ ID NOs: 3-8; (2) all of SEQ ID NOs: 14-19; (3) all of SEQ ID NOs: 25-30; (4) all of SEQ ID NOs: 36-41; (5) all of SEQ ID NOs: 47-52; (6) all of SEQ ID NOs: 58-63; (7) all of SEQ ID NOs: 69-74; (8) all of SEQ ID NOs: 80-85; or (9) all of SEQ ID NOs: 131-136.

[0006] In an embodiment of the invention, the TCR has antigenic specificity for a human p53^{R175H} or human p53^{Y220C} amino acid sequence, wherein the human p53^{R175H} amino acid sequence is SEQ ID NO: 2 or SEQ ID NO: 96.

[0007] In an embodiment of the invention, the TCR has antigenic specificity for a human p53^{R175H} or human p53^{Y220C} amino acid sequence, wherein the human p53^{Y220C} amino acid sequence is SEQ ID NO: 113.

[0008] In an embodiment of the invention, the TCR does not have antigenic specificity for the wild-type human p53 amino acid sequence of SEQ ID NO: 95.

[0009] In an embodiment of the invention, the TCR does not have antigenic specificity for the wild-type human p53 amino acid sequence of SEQ ID NO: 112.

[0010] Further embodiments of the invention provide related polypeptides and proteins, as well as related nucleic acids, recombinant expression vectors, host cells, populations of cells, and pharmaceutical compositions relating to the TCRs of the invention.

[0011] An embodiment of the invention provides an isolated or purified nucleic acid comprising, from 5' to 3', a first nucleic acid sequence and a second nucleotide sequence, wherein the first and second nucleotide sequence, respectively, encode the amino sequences of SEQ ID NOs: 9 and 10; 10 and 9; 20 and 21; 21 and 20; 31 and 32; 32 and 31; 42 and 43; 43 and 42; 53 and 54; 54 and 53; 64 and 65; 65 and 64; 75 and 76; 76 and 75; 86 and 87; 87 and 86; 137 and 138; 138 and 137; 142 and 143; 143 and 142; 144 and 145; 145 and 144; 146 and 147; 147 and 146; 148 and 149; 149 and 148; 150 and 151; 151 and 150; 152 and 153; 153 and 152; 154 and 155; 155 and 154; 156 and 157; 157 and 156; 159 and 158; 158 and 159; 178 and 10; 10 and 178; 181 and 21; 21 and 181; 184 and 32; 32 and 184; 187 and 43; 43 and 187; 190 and 54; 54 and 190; 193 and 65; 65 and 193; 196 and 76; 76 and 196; 199 and 87; 87 and 199; 137 and 202; 202 and 137; 9 and 205; 205 and 9; 20 and 207; 207 and 20; 31 and 209; 209 and 31; 42 and 211; 211 and 42; 53 and 213; 213 and 53; 64 and 215; 215 and 64; 75 and 217; 217 and 75; 86 and 219; 219 and 86; 137 and 221; 221 and 137; 223 and 202; 202 and 223; 223 and 221; 221 and 223; 20 and 226; 226 and 20; 181 and 226; or 226 and 181.

[0012] An embodiment of the invention provides an isolated or purified nucleic acid comprising, from 5' to 3', a first nucleic acid sequence and a second nucleotide sequence, wherein the first and second nucleotide sequence, respectively, encode the amino sequences of SEQ ID NOs: 11 and 12; 12 and 11; 22 and 23; 23 and 22; 33 and 34; 34 and 33; 44 and 45; 45 and 44; 55 and 56; 56 and 55; 66 and 67; 67 and 66; 77 and 78; 78 and 77; 88 and 89;

89 and 88; 139 and 140; 140 and 139; 160 and 161; 161 and 160; 162 and 163; 163 and 162; 164 and 165; 165 and 164; 166 and 167; 167 and 166; 168 and 169; 169 and 168; 170 and 171; 171 and 170; 172 and 173; 173 and 172; 174 and 175; 175 and 174; 176 and 177; 177 and 176; 179 and 12; 12 and 179; 182 and 23; 23 and 182; 185 and 34; 34 and 185; 188 and 45; 45 and 188; 191 and 56; 56 and 191; 194 and 67; 67 and 194; 197 and 78; 78 and 197; 200 and 89; 89 and 200; 139 and 203; 203 and 139; 11 and 206; 206 and 11; 22 and 208; 208 and 22; 33 and 210; 210 and 33; 44 and 212; 212 and 44; 55 and 214; 214 and 55; 66 and 216; 216 and 66; 77 and 218; 218 and 77; 88 and 220; 220 and 88; 139 and 222; 222 and 139; 224 and 203; 203 and 224; 224 and 222; 222 and 224; 22 and 227; 227 and 22; 182 and 227; or 227 and 182.

[0013] In an embodiment of the invention, the isolated or purified nucleic acid further comprises a third nucleotide acid sequence interposed between the first and second nucleotide sequence, wherein the third nucleotide sequence encodes a cleavable linker peptide.

[0014] In an embodiment of the invention, the cleavable linker peptide comprises the amino acid sequence of SEQ ID NO: 94.

[0015] In an embodiment of the invention, the isolated or purified nucleic acid encodes an amino acid sequence selected from the group consisting of: SEQ ID NO: 13, 24, 35, 46, 57, 68, 79, 90, 141, 180, 183, 186, 189, 192, 195, 198, 201, 204, 225, 228, and 229.

[0016] In an embodiment of the invention, the recombinant expression vector is a transposon or a lentiviral vector.

[0017] Another embodiment of the invention provides an isolated or purified TCR, polypeptide, or protein encoded by any of the nucleic acids or vectors described herein.

[0018] Another embodiment of the invention provides an isolated or purified TCR, polypeptide, or protein that results from expression of any of the nucleic acids or vectors described herein in a cell.

[0019] Another embodiment of the invention provides a method of producing a host cell expressing a TCR that has antigenic specificity for the peptide of SEQ ID NO: 2, 96 or 113, the method comprising contacting a cell with any of the vectors described herein under conditions that allow introduction of the vector into the cell.

[0020] Another embodiment of the invention provides an isolated or purified host cell comprising any of the nucleic acids or recombinant expression vectors described herein.

[0021] In an embodiment of the invention, the host cell is a human lymphocyte.

[0022] In an embodiment of the invention, the host cell is selected from the group consisting of a T cell, a natural killer T (NKT) cell, an invariant natural killer T (iNKT) cell, and a natural killer (NK) cell.

[0023] Another embodiment of the invention provides a method of producing any of the TCRs, polypeptides, or proteins described herein, the method comprising culturing any of the host cells or populations of host cells described herein, so that the TCR, polypeptide, or protein is produced.

[0024] Another embodiment of the invention provides any of the TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or pharmaceutical compositions described herein, for use in inducing an immune response against a cancer in a mammal. In one embodiment, the invention provides a method of inducing an immune response against a cancer in a mammal comprising administering any of the TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or pharmaceutical compositions described herein.

[0025] Still further embodiments of the invention provide methods of detecting the presence of cancer in a mammal and methods of treating or preventing cancer in a mammal. In one embodiment, the invention provides a method of treating or preventing cancer in a mammal comprising administering any of the TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or pharmaceutical compositions described herein. In an embodiment, the cancer is cholangiocarcinoma, melanoma, colon cancer, rectal cancer, ovarian cancer, endometrial cancer, non-small cell lung cancer (NSCLC), glioblastoma, uterine cervical cancer, head and neck cancer, breast cancer, pancreatic cancer, or bladder cancer.

[0026] In an embodiment of the invention, the cancer is known to comprise an R175H or a Y220C mutation in human p53.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0027] Figure 1A is a schematic showing an experimental design wherein the parent gate was lymphocytes → single cells → live (PI negative) → CD3⁺ (T cells).

[0028] Figures 1B-1C are graphs showing the percentage of 4-1BB positive cells detected after IVS (*TP53*-TMG-IVS (B) or p53-LP-IVS (C)) and 4-1BB/OX40 enrichment. Cultures deemed positive are bolded. Responses to mutated *TP53* (TMG (closed circles); LP (closed

squares)) and the wild type (WT) counterparts (TMG (open circles); LP (open squares)) are shown.

[0029] Figure 1D shows representative flow cytometry plots from the 4141-CD8 *TP53*-TMG-IVS culture following co-culture with autologous antigen presenting cells electroporated with WT or mutated (MUT) *TP53* tandem minigenes (TMG).

[0030] Figure 1E shows the interferon-gamma secretion (left axis) or upregulation of 4-1BB (right axis) measured after antigen experienced CD4 T cells were sorted and *in vitro* stimulated with immature dendritic cells electroporated with mutated *TP53*-TMG. The culture was then co-cultured with immature dendritic cells electroporated with mutated *TP53*-TMG and the following day 4-1BB⁺ and/or OX40⁺ cells were sorted and expanded by rapid expansion protocol. After 12-14 days of expansion the culture (4285-CD4 *TP53*-TMG-IVS) was tested for specificity to p53-R175H neoantigen by interferon-gamma ELISPOT (left axis) or upregulation of 4-1BB by flow cytometry (right axis).

[0031] Figure 1F is a graph showing interferon- γ secretion as measured by ELISA into 4285-CD4 *TP53*-TMG-IVS and peptide pulsed autologous antigen presenting cell co-culture supernatants. The 4285-CD4 *TP53*-TMG-IVS culture was co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3 technical replicates).

[0032] Figure 2A is a graph showing clonality of the total populations, which is a normalized estimate of sample diversity where numbers closer to 1 are less diverse. *TCRB* sequencing was performed on PBL prior to or after expansion with IVS and 4-1BB/OX40 enrichment with either LP or TMG. The cultures with verified p53 neoantigen responses are highlighted with an asterisk.

[0033] Figure 2B is a graph showing the maximum productive unique CDR3B frequency from each population. *TCRB* sequencing was performed on PBL prior to or after expansion with IVS and 4-1BB/OX40 enrichment with either LP or TMG. The cultures with verified p53 neoantigen responses are highlighted with an asterisk.

[0034] Figure 2C is a graph showing the results of an experiment in which untransduced T cells (a negative control for TCR transduced T cells) were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the

co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0035] Figure 2D is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR1 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0036] Figure 2E is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR2 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0037] Figure 2F is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR3 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0038] Figure 2G is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR5 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0039] Figure 2H is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR6 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0040] Figure 2I is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR7 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0041] Figure 2J is a graph showing the results of an experiment in which T cells transduced with 4285-PBL-TCR9 were co-cultured with immature dendritic cells pulsed with decreasing concentrations of either WT (open circles) or mutated (closed squares) p53-R175 peptides of 25 amino acid length. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0042] Figures 2K-2L are graphs showing the percentage of TCRB clonotypes measured after tracking CDR3B with known specificity to mutated *TP53* before (PBL) and after (IVS/enriched) the IVS and 4-1BB enrichment protocol. P53^{R175H}-specific clonotypes are shown in K. P53^{R248W}-specific clonotypes are shown in L.

[0043] Figure 3A is a graph showing the percentage of 4-1BB positive cells measured following transfection of a COS7 monkey cell line with the indicated HLA and pulsed with the indicated minimal p53 peptide (WT (open bars); mutated (closed bars)). Results from the 4141-CD8 TP53-TMG-IVS culture are shown. The TMG-wtR175 had mutated *TP53* at all positions except for R175H. The sequence HMTEVVRRC is SEQ ID NO: 95. The sequence HMTEVVRHC is SEQ ID NO: 96.

[0044] Figure 3B is a graph showing the amount of IFN- γ measured following transfection of a COS7 monkey cell line with the indicated HLA and pulsed with the indicated minimal p53 peptide (WT (open bars); mutated (closed bars)). Results from 4266-CD8 TP53-TMG-IVS culture are shown. The sequence SSCMGGMNRR is SEQ ID NO: 97. The sequence SSCMGGMNNR is SEQ ID NO: 98.

[0045] Figure 3C is a graph showing the results of an experiment in which a COS7 monkey tumor cell line was transfected with HLA plasmid DNA corresponding to patient 4285's haplotype and either WT TP53 TMG only at R175H position (WT-R175-TMG; open bars) or the mutated TP53-TMG containing the p53-R175H neoantigen (closed bars). The following day, the 4285-CD4 TP53-TMG-IVS culture was added and co-cultured. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0046] Figure 3D shows flow cytometry plots showing the upregulation of 4-1BB on CD8⁺ T cells from TP53-TMG-IVS cultures (4266-CD8 on left and 4141-CD8 on right) following co-culture with TC#4266 (autologous xenograft from patient 4266; A*68:01; p53^{R248W}) and Saos2 cells (A*02:01) overexpressing full length p53^{R175H} gene.

[0047] Figure 4 is a graph showing the results of an experiment in which a COS7 monkey tumor cell line was transfected with DRA1*01:01:01 and DRB1*13:01:01 and either

irrelevant (open bars), WT TP53 TMG only at R175H position (WT-R175-TMG; gray bars) or the mutated TP53-TMG containing the p53-R175H neoantigen (closed bars). The following day, the T cells transduced with 4285-PBL-TCRs or untransduced were added and co-cultured. After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0048] Figure 5 is a graph showing the results of an experiment in which T-cell clones were prepared by limiting dilution after sorting CD8⁺ T cells from the 4259-F1 tumor fragment culture. The 24 cultures were co-cultured with T2 tumor cells (HLA-A*02:01) pulsed with DMSO (peptide vehicle), WT p53-Y220 peptide or MUT p53-Y220C peptide. After overnight incubation, the cells were stained for CD3, CD8 and 4-1BB then analyzed by flow cytometry. The frequencies of CD8⁺4-1BB⁺ T cells are displayed from the cultures.

[0049] Figure 6 is a graph showing the results of an experiment in which the 4259-F1-TCR was transduced into donor peripheral blood T cells then co-cultured with T2 tumor cells (HLA-A*02:01) pulsed with decreasing concentrations of either WT p53-Y220 peptide (VVPYEPPEV) (SEQ ID NO: 112) or MUT p53-Y220C peptide (VVPCEPPEV) (SEQ ID NO: 113). After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. Data are mean $\pm$ SEM (n=3).

[0050] Figure 7 is a graph showing the results of an experiment in which T cells either expressing no TCR (untransduced), a p53-R175H specific TCR or the 4259-F1-TCR were co-cultured with tumor cells, which either with or without expression of HLA-A*02:01, p53-R175H or p53-Y220C. After overnight incubation, the cells were stained for CD3, CD8 and 4-1BB then analyzed by flow cytometry. The frequencies of CD8⁺4-1BB⁺ T cells are displayed from the cultures. Data are mean $\pm$ SEM (n=3).

[0051] Figure 8 shows an alignment of the amino acid sequences of the nine p53 splice variants. SP|P04637|P53_HUMAN (SEQ ID NO: 1); SP|P04637-2|P53_HUMAN (SEQ ID NO: 114); SP|P04637-3|P53_HUMAN (SEQ ID NO: 115); SP|P04637-4|P53_HUMAN (SEQ ID NO: 116); SP|P04637-5|P53_HUMAN (SEQ ID NO: 117); SP|P04637-6|P53_HUMAN (SEQ ID NO: 118); SP|P04637-7|P53_HUMAN (SEQ ID NO: 119); SP|P04637-8|P53_HUMAN (SEQ ID NO: 120); and SP|P04637-9|P53_HUMAN (SEQ ID NO: 121).

[0052] Figure 9 shows an alignment of the amino acid sequences of portions of the sequence of TRBV7-9*03 with that of TRBV7-9*01. "L36092|TRBV7-9*01|Homo" is SEQ ID NO: 129. "AF009663|TRBV7-9*03|Homo" is SEQ ID NO: 130.

[0053] Figure 10 is a graph showing the percentage of 4-1BB positive cells (% of CD8+) (right y-axis; black bars) and IFN- γ (spots per 2x10⁴ cells) (left y-axis; hatched bars) measured following co-culture of TIL from Patient 4141 (fragment culture 12) with autologous APCs transfected with TMG encoding irrelevant mutations (TMG-IRR), WT p53 sequence (TP53-wt-TMG) or mutated p53 sequence including R175H (TP53-mut-TMG). Media alone and PMA and ionomycin were negative and positive controls, respectively.

[0054] Figure 11 is a graph showing the number of IFN- γ -positive spots per 2x10⁴ effector cells measured following co-culture of TIL from Patient 4141 (fragment culture 12) with Cos7 cells co-transfected with the indicated HLA alleles and either no extra gene (HLA only; open bars), WT TP53 TMG (gray hatched bars), or mutated (black bars) TP53 TMG containing the p53-R175H sequence.

[0055] Figure 12 is a graph showing the concentration of IFN- γ (pg/mL) measured following co-culture of T cells expressing mock (no TCR) or 4141-TCR1a2 with T2 tumor cells (expressing HLA-A*02:01). T2 cells were pulsed with peptide vehicle (DMSO; gray bars) or purified (>95% by HPLC) peptides composed of WT p53-R175 peptide (hatched gray bars) or mutated p53-R175H peptide (black bars). Media alone (open bars) and PMA and Ionomycin (lattice bars) were negative and positive controls, respectively. Data are mean $\pm$ SEM (n=3).

[0056] Figure 13 is a graph showing the percentage of cells positive for expression of one of the indicated markers following co-culture of T cells expressing 4141-TCR1a2 with Saos2 cells (p53-NUL and HLA-A*02:01+), which were either unmanipulated (unshaded bars) or made to overexpress full length p53-R175H protein (shaded bars). Data are mean $\pm$ SEM (n=3). Student's two-tailed t-tests were performed for each cytokine between the two cell lines for statistical analyses (**p<0.001).

DETAILED DESCRIPTION OF THE INVENTION

[0057] Tumor Protein P53 (also referred to as "TP53" or "p53") acts as a tumor suppressor by, for example, regulating cell division. The p53 protein is located in the nucleus of the cell, where it binds directly to DNA. When DNA becomes damaged, the p53 protein is involved in determining whether the DNA will be repaired or the damaged cell will undergo apoptosis. If the DNA can be repaired, p53 activates other genes to fix the damage. If the DNA cannot be repaired, the p53 protein prevents the cell from dividing and signals it to undergo apoptosis. By stopping cells with mutated or damaged DNA from dividing, p53

helps prevent the development of tumors. WT (normal) full-length p53 comprises the amino acid sequence of SEQ ID NO: 1.

[0058] Mutations in the p53 protein may reduce or eliminate the p53 protein's tumor suppressor function. Alternatively or additionally, a p53 mutation may be a gain-of-function mutation by interfering with WT p53 in a dominant negative fashion. Mutated p53 protein may be expressed in any of a variety of human cancers such as, for example, cholangiocarcinoma, melanoma, colon cancer, rectal cancer, ovarian cancer, endometrial cancer, non-small cell lung cancer (NSCLC), glioblastoma, uterine cervical cancer, head and neck cancer, breast cancer, pancreatic cancer, or bladder cancer.

[0059] An embodiment of the invention provides an isolated or purified T cell receptor (TCR) having antigenic specificity for mutated human p53 (hereinafter, "mutated p53"). Hereinafter, references to a "TCR" also refer to functional portions and functional variants of the TCR, unless specified otherwise. Mutations of p53 are defined herein by reference to the amino acid sequence of full-length, WT p53 (SEQ ID NO: 1). Mutations of p53 are described herein by reference to the amino acid residue present at a particular position, followed by the position number, followed by the amino acid with which that residue has been replaced in the particular mutation under discussion. A p53 amino acid sequence (e.g., a p53 peptide) may comprise fewer than all of the amino acid residues of the full-length, WT p53 protein. Accordingly, the position numbers are defined herein by reference to the WT full-length p53 protein (namely, SEQ ID NO: 1) with the understanding that the actual position of the corresponding residue in a particular example of a p53 amino acid sequence may be different. Because the positions are as defined by SEQ ID NO: 1, the term "R175" refers to the arginine present at position 175 of SEQ ID NO: 1, "R175H" indicates that the arginine present at position 175 of SEQ ID NO: 1 is replaced by histidine, while "Y220C" indicates that the tyrosine present at position 220 of SEQ ID NO: 1 has been replaced with cysteine. For example, when a particular example of a p53 amino acid sequence is, e.g., YKQSQHMTEVVRRCPHHERCSDSDG (SEQ ID NO: 110) (an exemplary WT p53 peptide corresponding to contiguous amino acid residues 163 to 187 of SEQ ID NO: 1), "R175H" refers to a substitution of the underlined arginine in SEQ ID NO: 110 with histidine, even though the actual position of the underlined arginine in SEQ ID NO: 110 is 13. Human p53 amino acid sequences with the R175H mutation are hereinafter referred to as "R175H" or "p53^{R175H}." Human p53 amino acid sequences with the Y220C mutation are hereinafter

referred to as “Y220C” or “p53^{Y220C}.” As used herein, “mutated p53” refers to human p53^{R175H} or human p53^{Y220C}.

[0060] P53 has nine known splice variants. The p53 mutations described herein are conserved over all nine p53 splice variants. An alignment of the nine p53 splice variants is shown in Figure 8. Accordingly, the inventive TCRs may have antigenic specificity for any mutated p53 amino acid sequence described herein encoded by any of the nine p53 splice variants. Because the positions are as defined by SEQ ID NO: 1, then the actual positions of the amino acid sequence of a particular splice variant of p53 are defined relative to the corresponding positions of SEQ ID NO: 1, and the positions as defined by SEQ ID NO: 1 may be different than the actual positions in a particular splice variant. Thus, for example, mutations refer to a replacement of an amino acid residue in the amino acid sequence of a particular splice variant of p53 corresponding to the indicated position of the 393-amino acid sequence of SEQ ID NO: 1 with the understanding that the actual positions in the splice variant may be different.

[0061] In an embodiment of the invention, the TCR has antigenic specificity for human p53 with a mutation at position 175, as defined by SEQ ID NO: 1. The p53 mutation at position 175 may be any missense mutation. Accordingly, the mutation at position 175 may be a substitution of the native (WT) arginine residue present at position 175 with any amino acid residue other than arginine. In an embodiment of the invention, the TCR has antigenic specificity for human p53 with the R175H mutation. For example, the inventive TCR may have antigenic specificity for one or more mutated p53 amino acid sequences selected from the group consisting of: EVVRHCPHHER (SEQ ID NO: 2), HMTEVVRHC (SEQ ID NO: 96), KQSQHMTEVVRHCPH (SEQ ID NO: 100), QSQHMTEVVRHCPHH (SEQ ID NO: 101), SQHMTEVVRHCPHHE (SEQ ID NO: 102), QHMTEVVRHCPHHER (SEQ ID NO: 103), HMTEVVRHCPHHERC (SEQ ID NO: 104), MTEVVRHCPHHERCS (SEQ ID NO: 105), TEVVRHCPHHERCSD (SEQ ID NO: 106), EVVRHCPHHERCSDS (SEQ ID NO: 107), VVRHCPHHERCSDSD (SEQ ID NO: 108), VRHCPHHERCSDSDG (SEQ ID NO: 109), YKQSQHMTEVVRHCPHHERCSDSDG (SEQ ID NO: 111).

[0062] In an embodiment of the invention, the TCR has antigenic specificity for human p53 with a mutation at position 220, as defined by SEQ ID NO: 1. The p53 mutation at position 220 may be any missense mutation. Accordingly, the mutation at position 220 may be a substitution of the native (WT) tyrosine residue present at position 220 with any amino acid residue other than tyrosine. In an embodiment of the invention, the TCR has antigenic

specificity for human p53 with the Y220C mutation. For example, the inventive TCR may have antigenic specificity for the mutated p53 amino acid sequence of VVPCEPPEV (SEQ ID NO: 113).

[0063] In an embodiment of the invention, the inventive TCRs may be able to recognize mutated p53 in an HLA (human leukocyte antigen)-molecule-dependent manner. “HLA-molecule-dependent manner,” as used herein, means that the TCR elicits an immune response upon binding to mutated p53 within the context of an HLA molecule, which HLA molecule is expressed by the patient from which the TCR was isolated. The inventive TCRs may be able to recognize mutated p53 that is presented by the applicable HLA molecule and may bind to the HLA molecule in addition to mutated p53.

[0064] In an embodiment of the invention, the inventive TCRs are able to recognize R175H presented by an HLA Class II molecule. In this regard, the TCR may elicit an immune response upon binding to R175H within the context of an HLA Class II molecule. The inventive TCRs are able to recognize R175H that is presented by an HLA Class II molecule and may bind to the HLA Class II molecule in addition to R175H.

[0065] In an embodiment of the invention, the HLA Class II molecule is an HLA-DR heterodimer. The HLA-DR heterodimer is a cell surface receptor including an α chain and a β chain. The HLA-DR α chain is encoded by the HLA-DRA gene. In an embodiment, the alpha chain of the HLA Class II molecule is expressed by the HLA-DRA1*01:01:01 allele. The HLA-DR β chain is encoded by the HLA-DRB1 gene, the HLA-DRB3 gene, HLA-DRB4 gene, or the HLA-DRB5 gene. Examples of molecules encoded by the HLA-DRB1 gene may include, but are not limited to, HLA-DR1, HLA-DR2, HLA-DR3, HLA-DR4, HLA-DR5, HLA-DR6, HLA-DR7, HLA-DR8, HLA-DR9, HLA-DR10, HLA-DR11, HLA-DR12, HLA-DR13, HLA-DR14, HLA-DR15, HLA-DR16, and HLA-DR17. The HLA-DRB3 gene encodes HLA-DR52. The HLA-DRB4 gene encodes HLA-DR53. The HLA-DRB5 gene encodes HLA-DR51. In an embodiment of the invention, the HLA Class II molecule is an HLA-DRB1:HLA-DRA heterodimer. The beta chain of the HLA Class II molecule may be expressed by the HLA-DRB1*13:01, HLA-DRB1*13:02, HLA-DRB1*13:03, HLA-DRB1*13:04, HLA-DRB1*13:05, HLA-DRB1*13:06, HLA-DRB1*13:07, HLA-DRB1*13:08, HLA-DRB1*13:09, or HLA-DRB1*13:10 allele. In an especially preferred embodiment, the beta chain of the HLA Class II molecule is expressed by the HLA-DRB1*13:01 allele.

[0066] In an embodiment of the invention, one of the inventive TCRs is able to recognize Y220C presented by an HLA Class I molecule. In this regard, the TCR may elicit an immune response upon binding to Y220C within the context of an HLA Class I molecule. The inventive TCR is able to recognize Y220C that is presented by an HLA Class I molecule and may bind to the HLA Class I molecule in addition to Y220C.

[0067] In an embodiment of the invention, the HLA Class I molecule is an HLA-A molecule. The HLA-A molecule is a heterodimer of an α chain and β 2 microglobulin. The HLA-A α chain may be encoded by an HLA-A gene. β 2 microglobulin binds non-covalently to the alpha1, alpha2 and alpha3 domains of the alpha chain to build the HLA-A complex. The HLA-A molecule may be any HLA-A molecule. In an embodiment of the invention, the HLA Class I molecule is an HLA-A2 molecule. The HLA-A2 molecule may be any HLA-A2 molecule. Examples of HLA-A2 molecules may include, but are not limited to, HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, or HLA-A*02:11. Preferably, the HLA Class I molecule is an HLA-A*02:01 molecule.

[0068] The TCRs of the invention may provide any one or more of many advantages, including when expressed by cells used for adoptive cell transfer. Mutated p53 is expressed by cancer cells and is not expressed by normal, noncancerous cells. Without being bound to a particular theory or mechanism, it is believed that the inventive TCRs advantageously target the destruction of cancer cells while minimizing or eliminating the destruction of normal, non-cancerous cells, thereby reducing, for example, by minimizing or eliminating, toxicity. Moreover, the inventive TCRs may, advantageously, successfully treat or prevent mutated p53-positive cancers that do not respond to other types of treatment such as, for example, chemotherapy, surgery, or radiation. Additionally, the inventive TCRs may provide highly avid recognition of mutated p53, which may provide the ability to recognize unmanipulated tumor cells (e.g., tumor cells that have not been treated with interferon (IFN)- γ , transfected with a vector encoding one or both of mutated p53 and the applicable HLA molecule, pulsed with a p53 peptide with the p53 mutation, or a combination thereof). Roughly half of all tumors harbor a mutation in p53, about half of which will be a missense mutation. The R175H mutation is expressed by about 4.5% of all cancers, and the HLA-DRB1*13:01 allele is expressed by about 15% of the U.S. population. The Y220C mutation occurs in about 1.5% of all cancers, and the HLA-A*02:01 allele is expressed by about 40% to about 50% of the U.S. population. The R175H and Y220C mutations arise in many cancer histologies, suggesting that a diverse group of patients could benefit from the inventive TCRs.

Accordingly, the inventive TCRs may increase the number of patients who may be eligible for treatment with immunotherapy.

[0069] The phrase “antigenic specificity,” as used herein, means that the TCR can specifically bind to and immunologically recognize mutated p53 with high avidity. For example, a TCR may be considered to have “antigenic specificity” for mutated p53 if about 1×10^4 to about 1×10^5 T cells expressing the TCR secrete at least about 200 pg/mL or more (e.g., 200 pg/mL or more, 300 pg/mL or more, 400 pg/mL or more, 500 pg/mL or more, 600 pg/mL or more, 700 pg/mL or more, 1000 pg/mL or more, 5,000 pg/mL or more, 7,000 pg/mL or more, 10,000 pg/mL or more, 20,000 pg/mL or more, or a range defined by any two of the foregoing values) of IFN- γ upon co-culture with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with a low concentration of mutated p53 peptide (e.g., about 0.05 ng/mL to about 5 ng/mL, 0.05 ng/mL, 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, or a range defined by any two of the foregoing values) or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53. Cells expressing the inventive TCRs may also secrete IFN- γ upon co-culture with antigen-negative, applicable HLA molecule positive target cells pulsed with higher concentrations of mutated p53 peptide.

[0070] Alternatively or additionally, a TCR may be considered to have “antigenic specificity” for mutated p53 if T cells expressing the TCR secrete at least twice as much IFN- γ upon co-culture with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with a low concentration of mutated p53 peptide or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53 as compared to the amount of IFN- γ expressed by a negative control. The negative control may be, for example, (i) T cells expressing the TCR, co-cultured with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with the same concentration of an irrelevant peptide (e.g., some other peptide with a different sequence from the mutated p53 peptide) or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding an irrelevant peptide has been introduced such that the target cell expresses the irrelevant peptide, or (ii) untransduced T cells (e.g., derived from PBMC, which do not express the TCR) co-cultured with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with the same concentration of mutated p53 peptide or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide

sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53. IFN- γ secretion may be measured by methods known in the art such as, for example, enzyme-linked immunosorbent assay (ELISA).

[0071] Alternatively or additionally, a TCR may be considered to have “antigenic specificity” for mutated p53 if at least twice as many of the numbers of T cells expressing the TCR secrete IFN- γ upon co-culture with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with a low concentration of mutated p53 peptide or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53 as compared to the numbers of negative control T cells that secrete IFN- γ . The concentration of peptide and the negative control may be as described herein with respect to other aspects of the invention. The numbers of cells secreting IFN- γ may be measured by methods known in the art such as, for example, enzyme-linked immunospot (ELISOT) assay.

[0072] Alternatively or additionally, a TCR may be considered to have “antigenic specificity” for mutated p53 if at least twice as many spots are detected by ELISPOT for the T cells expressing the TCR upon co-culture with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with a low concentration of mutated p53 peptide or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53 as compared to the number of spots detected by ELISPOT for negative control T cells co-cultured with the same target cells. The concentration of peptide and the negative control may be as described herein with respect to other aspects of the invention.

[0073] Alternatively or additionally, a TCR may be considered to have “antigenic specificity” for mutated p53 if greater than about 50 spots are detected by ELISPOT for the T cells expressing the TCR upon co-culture with (a) antigen-negative, applicable HLA molecule positive target cells pulsed with a low concentration of mutated p53 peptide or (b) antigen-negative, applicable HLA molecule positive target cells into which a nucleotide sequence encoding mutated p53 has been introduced such that the target cell expresses mutated p53. The concentration of peptide may be as described herein with respect to other aspects of the invention.

[0074] Alternatively or additionally, a TCR may be considered to have “antigenic specificity” for mutated p53 if T cells expressing the TCR upregulate expression of one or

both of 4-1BB and OX40 as measured by, for example, flow cytometry after stimulation with target cells expressing mutated p53.

[0075] An embodiment of the invention provides a TCR comprising two polypeptides (i.e., polypeptide chains), such as an alpha (α) chain of a TCR, a beta (β) chain of a TCR, a gamma (γ) chain of a TCR, a delta (δ) chain of a TCR, or a combination thereof. The polypeptides of the inventive TCR can comprise any amino acid sequence, provided that the TCR has antigenic specificity for mutated p53.

[0076] In an embodiment of the invention, the TCR comprises two polypeptide chains, each of which comprises a variable region comprising a complementarity determining region (CDR)1, a CDR2, and a CDR3 of a TCR. In an embodiment of the invention, the TCR comprises a first polypeptide chain comprising an α chain CDR1 (CDR1 α), an α chain CDR2 (CDR2 α), and an α chain CDR3 (CDR3 α), and a second polypeptide chain comprising a β chain CDR1 (CDR1 β), a β chain CDR2 (CDR2 β), and a β chain CDR3 (CDR3 β). In an embodiment of the invention, the TCR comprises the amino acid sequences of: (1) all of SEQ ID NOs: 3-8; (2) all of SEQ ID NOs: 14-19; (3) all of SEQ ID NOs: 25-30; (4) all of SEQ ID NOs: 36-41; (5) all of SEQ ID NOs: 47-52; (6) all of SEQ ID NOs: 58-63; (7) all of SEQ ID NOs: 69-74; (8) all of SEQ ID NOs: 80-85; or (9) all of SEQ ID NOs: 131-136. Each one of the foregoing nine collections of amino acid sequences in this paragraph sets forth the six CDR regions of each of nine different TCRs having antigenic specificity for mutated human p53. The six amino acid sequences in each collection correspond to the CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β of a TCR, respectively.

[0077] In an embodiment of the invention, the TCR comprises an α chain variable region amino acid sequence and a β chain variable region amino acid sequence, which together comprise one of the collections of CDRs set forth above. In this regard, the TCR can, e.g., comprise the amino acid sequences of any one of SEQ ID NOs: 9, 10, 20, 21, 31, 32, 42, 43, 53, 54, 64, 65, 75, 76, 86, 87, 137, 138, 142-159, 178, 181, 184, 187, 190, 193, 196, 199, 202, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, and 226. For example, the TCR can comprise: (1) both of SEQ ID NOs: 9 and 10; (2) both of SEQ ID NOs: 20 and 21; (3) both of SEQ ID NOs: 31 and 32; (4) both of SEQ ID NOs: 42 and 43; (5) both of SEQ ID NOs: 53 and 54; (6) both of SEQ ID NOs: 64 and 65; (7) both of SEQ ID NOs: 75 and 76; (8) both of SEQ ID NOs: 86 and 87; (9) both of SEQ ID NOs: 137 and 138; (10) both of SEQ ID NOs: 142 and 143; (11) both of SEQ ID NOs: 144 and 145; (12) both of SEQ ID NOs: 146 and 147; (13) both of SEQ ID NOs: 148 and 149; (14) both of SEQ ID NOs: 150 and 151; (15)

both of SEQ ID NOs: 152 and 153; (16) both of SEQ ID NOs: 154 and 155; (17) both of SEQ ID NOs: 156 and 157; (18) both of SEQ ID NOs: 159 and 158; (19) both of SEQ ID NOs: 178 and 10; (20) both of SEQ ID NOs: 181 and 21; (21) both of SEQ ID NOs: 184 and 32; (22) both of SEQ ID NOs: 187 and 43; (23) both of SEQ ID NOs: 190 and 54; (24) both of SEQ ID NOs: 193 and 65; (25) both of SEQ ID NOs: 196 and 76; (26) both of SEQ ID NOs: 199 and 87; (27) both of SEQ ID NOs: 137 and 202; (28) both of SEQ ID NOs: 9 and 205; (29) both of SEQ ID NOs: 20 and 207; (30) both of SEQ ID NOs: 31 and 209; (31) both of SEQ ID NOs: 42 and 211; (32) both of SEQ ID NOs: 53 and 213; (33) both of SEQ ID NOs: 64 and 215; (34) both of SEQ ID NOs: 75 and 217; (35) both of SEQ ID NOs: 86 and 219; (36) both of SEQ ID NOs: 137 and 221; (37) both of SEQ ID NOs: 223 and 202; (38) both of SEQ ID NOs: 223 and 221; (39) both of SEQ ID NOs: 20 and 226; or (40) both of SEQ ID NOs: 181 and 226. Each one of the foregoing collections of amino acid sequences in this paragraph sets forth the two variable regions of each of the different TCRs having antigenic specificity for mutated human p53. The two amino acid sequences in each collection correspond to the variable region of the α chain and the variable region of the β chain of a TCR, respectively.

[0078] The inventive TCRs may further comprise a constant region. The constant region may be derived from any suitable species such as, e.g., human or mouse. In an embodiment of the invention, the TCRs further comprise a murine constant region. As used herein, the term “murine” or “human,” when referring to a TCR or any component of a TCR described herein (e.g., complementarity determining region (CDR), variable region, constant region, alpha chain, and/or beta chain), means a TCR (or component thereof) which is derived from a mouse or a human, respectively, i.e., a TCR (or component thereof) that originated from or was, at one time, expressed by a mouse T cell or a human T cell, respectively. In an embodiment of the invention, the TCR may comprise a murine α chain constant region and a murine β chain constant region. The murine α chain constant region may be modified or unmodified. A modified murine α chain constant region may be, e.g., cysteine-substituted, LVL-modified, or both cysteine-substituted and LVL-modified, as described, for example, in U.S. Patent No. 10,174,098. The murine β chain constant region may be modified or unmodified. A modified murine β chain constant region may be, e.g., cysteine-substituted, as described, for example, in U.S. Patent No. 10,174,098. In an embodiment of the invention, the TCR comprises a cysteine-substituted, LVL-modified murine α chain constant region comprising the amino acid sequence of SEQ ID NO: 91 or 92. In an embodiment of the

invention, the TCR comprises a cysteine-substituted murine β chain constant region comprising the amino acid sequence of SEQ ID NO: 93.

[0079] In an embodiment of the invention, the inventive TCR can comprise an α chain of a TCR and a β chain of a TCR. The α chain of the TCR may comprise a variable region of an α chain and a constant region of an α chain. An α chain of this type can be paired with any β chain of a TCR. The β chain may comprise a variable region of a β chain and a constant region of a β chain.

[0080] In some embodiments, the amino acid sequence of any of the α chains and/or β chains disclosed herein further comprises the amino acid sequence RAKR (SEQ ID NO: 230) at the C-terminal end.

[0081] In an embodiment of the invention, the TCR comprises the amino acid sequences of any one of SEQ ID NOs 11, 12, 22, 23, 33, 34, 44, 45, 55, 56, 66, 67, 77, 78, 88, 89, 139, 140, 160-177, 179, 182, 185, 188, 191, 194, 197, 200, 203, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, and 227. For example, the TCR can comprise: (1) both of SEQ ID NOs: 11 and 12; (2) both of SEQ ID NOs: 22 and 23; (3) both of SEQ ID NOs: 33 and 34; (4) both of SEQ ID NOs: 44 and 45; (5) both of SEQ ID NOs: 55 and 56; (6) both of SEQ ID NOs: 66 and 67; (7) both of SEQ ID NOs: 77 and 78; (8) both of SEQ ID NOs: 88 and 89; (9) both of SEQ ID NOs: 139 and 140; (10) both of SEQ ID NOs: 160 and 161; (11) both of SEQ ID NOs: 162 and 163; (12) both of SEQ ID NOs: 164 and 165; (13) both of SEQ ID NOs: 166 and 167; (14) both of SEQ ID NOs: 168 and 169; (15) both of SEQ ID NOs: 170 and 171; (16) both of SEQ ID NOs: 172 and 173; (17) both of SEQ ID NOs: 174 and 175; (18) both of SEQ ID NOs: 176 and 177; (19) both of SEQ ID NOs: 179 and 12; (20) both of SEQ ID NOs: 182 and 23; (21) both of SEQ ID NOs: 185 and 34; (22) both of SEQ ID NOs: 188 and 45; (23) both of SEQ ID NOs: 191 and 56; (24) both of SEQ ID NOs: 194 and 67; (25) both of SEQ ID NOs: 197 and 78; (26) both of SEQ ID NOs: 200 and 89; (27) both of SEQ ID NOs: 139 and 203; (28) both of SEQ ID NOs: 11 and 206; (29) both of SEQ ID NOs: 22 and 208; (30) both of SEQ ID NOs: 33 and 210; (31) both of SEQ ID NOs: 44 and 212; (32) both of SEQ ID NOs: 55 and 214; (33) both of SEQ ID NOs: 66 and 216; (34) both of SEQ ID NOs: 77 and 218; (35) both of SEQ ID NOs: 88 and 220; (36) both of SEQ ID NOs: 139 and 222; (37) both of SEQ ID NOs: 224 and 203; (38) both of SEQ ID NOs: 224 and 222; (39) both of SEQ ID NOs: 22 and 227; or (40) both of SEQ ID NOs: 182 and 227. Each one of the foregoing collections of amino acid sequences in this paragraph sets forth the α chain and β chain of each of different TCRs having antigenic specificity for mutated human p53. The

two amino acid sequences in each collection correspond to the α chain and the β chain of a TCR, respectively.

[0082] Included in the scope of the invention are functional variants of the inventive TCRs described herein. The term “functional variant,” as used herein, refers to a TCR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent TCR, polypeptide, or protein, which functional variant retains the biological activity of the TCR, polypeptide, or protein of which it is a variant. Functional variants encompass, for example, those variants of the TCR, polypeptide, or protein described herein (the parent TCR, polypeptide, or protein) that retain the ability to specifically bind to mutated p53 for which the parent TCR has antigenic specificity or to which the parent polypeptide or protein specifically binds, to a similar extent, the same extent, or to a higher extent, as the parent TCR, polypeptide, or protein. In reference to the parent TCR, polypeptide, or protein, the functional variant can, for instance, be at least about 30%, at least about 50%, at least about 75%, at least about 80%, 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 more identical in amino acid sequence to the parent TCR, polypeptide, or protein, respectively.

[0083] The functional variant can, for example, comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one conservative amino acid substitution. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic amino acid substituted for another acidic amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino acid with a nonpolar side chain (e.g., Ala, Gly, Val, Ile, Leu, Met, Phe, Pro, Trp, Val, etc.), a basic amino acid substituted for another basic amino acid (Lys, Arg, etc.), an amino acid with a polar side chain substituted for another amino acid with a polar side chain (Asn, Cys, Gln, Ser, Thr, Tyr, etc.), etc.

[0084] Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent TCR, polypeptide, or protein with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. Preferably, the non-conservative amino acid substitution enhances the biological activity of

the functional variant, such that the biological activity of the functional variant is increased as compared to the parent TCR, polypeptide, or protein.

[0085] The TCR, polypeptide, or protein can consist essentially of the specified amino acid sequence or sequences described herein, such that other components of the TCR, polypeptide, or protein, e.g., other amino acids, do not materially change the biological activity of the TCR, polypeptide, or protein.

[0086] Also provided by the invention is a polypeptide comprising a functional portion of any of the TCRs described herein. The term “polypeptide,” as used herein, includes oligopeptides and refers to a single chain of amino acids connected by one or more peptide bonds.

[0087] With respect to the inventive polypeptides, the functional portion can be any portion comprising contiguous amino acids of the TCR of which it is a part, provided that the functional portion specifically binds to mutated p53. The term “functional portion,” when used in reference to a TCR, refers to any part or fragment of the TCR of the invention, which part or fragment retains the biological activity of the TCR of which it is a part (the parent TCR). Functional portions encompass, for example, those parts of a TCR that retain the ability to specifically bind to mutated p53 (e.g., in an applicable HLA molecule-dependent manner), or detect, treat, or prevent cancer, to a similar extent, the same extent, or to a higher extent, as the parent TCR. In reference to the parent TCR, the functional portion can comprise, for instance, about 10%, about 25%, about 30%, about 50%, about 68%, about 80%, about 90%, about 95%, or more, of the parent TCR.

[0088] The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent TCR. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, e.g., specifically binding to mutated p53; and/or having the ability to detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent TCR.

[0089] The polypeptide can comprise a functional portion of either or both of the α and β chains of the TCRs of the invention, such as a functional portion comprising one or more of CDR1, CDR2, and CDR3 of the variable region(s) of the α chain and/or β chain of a TCR of the invention. In an embodiment of the invention, the polypeptide can comprise a functional portion comprising the amino acid sequences of: (1) all of SEQ ID NOs: 3-8; (2) all of SEQ

ID NOs: 14-19; (3) all of SEQ ID NOs: 25-30; (4) all of SEQ ID NOs: 36-41; (5) all of SEQ ID NOs: 47-52; (6) all of SEQ ID NOs: 58-63; (7) all of SEQ ID NOs: 69-74; (8) all of SEQ ID NOs: 80-85; or (9) all of SEQ ID NOs: 131-136.

[0090] In an embodiment of the invention, the inventive polypeptide can comprise, for instance, the variable region of the inventive TCR comprising a combination of the CDR regions set forth above. In this regard, the polypeptide can comprise, e.g., the amino acid sequences of: (1) both of SEQ ID NOs: 9 and 10; (2) both of SEQ ID NOs: 20 and 21; (3) both of SEQ ID NOs: 31 and 32; (4) both of SEQ ID NOs: 42 and 43; (5) both of SEQ ID NOs: 53 and 54; (6) both of SEQ ID NOs: 64 and 65; (7) both of SEQ ID NOs: 75 and 76; (8) both of SEQ ID NOs: 86 and 87; (9) both of SEQ ID NOs: 137 and 138; (10) both of SEQ ID NOs: 142 and 143; (11) both of SEQ ID NOs: 144 and 145; (12) both of SEQ ID NOs: 146 and 147; (13) both of SEQ ID NOs: 148 and 149; (14) both of SEQ ID NOs: 150 and 151; (15) both of SEQ ID NOs: 152 and 153; (16) both of SEQ ID NOs: 154 and 155; (17) both of SEQ ID NOs: 156 and 157; (18) both of SEQ ID NOs: 159 and 158; (19) both of SEQ ID NOs: 178 and 10; (20) both of SEQ ID NOs: 181 and 21; (21) both of SEQ ID NOs: 184 and 32; (22) both of SEQ ID NOs: 187 and 43; (23) both of SEQ ID NOs: 190 and 54; (24) both of SEQ ID NOs: 193 and 65; (25) both of SEQ ID NOs: 196 and 76; (26) both of SEQ ID NOs: 199 and 87; (27) both of SEQ ID NOs: 137 and 202; (28) both of SEQ ID NOs: 9 and 205; (29) both of SEQ ID NOs: 20 and 207; (30) both of SEQ ID NOs: 31 and 209; (31) both of SEQ ID NOs: 42 and 211; (32) both of SEQ ID NOs: 53 and 213; (33) both of SEQ ID NOs: 64 and 215; (34) both of SEQ ID NOs: 75 and 217; (35) both of SEQ ID NOs: 86 and 219; (36) both of SEQ ID NOs: 137 and 221; (37) both of SEQ ID NOs: 223 and 202; (38) both of SEQ ID NOs: 223 and 221; (39) both of SEQ ID NOs: 20 and 226; or (40) both of SEQ ID NOs: 181 and 226.

[0091] In an embodiment of the invention, the inventive polypeptide can further comprise the constant region of the inventive TCR set forth above. In this regard, the polypeptide can comprise, e.g., the amino acid sequence of (i) one of SEQ ID NOs 91-93 or (ii) SEQ ID NO: 93 and one of SEQ ID NOs: 91 and 92.

[0092] In an embodiment of the invention, the inventive polypeptide may comprise an α chain and a β chain of the inventive TCR. In this regard, the polypeptide can comprise, e.g., the amino acid sequences of: (1) both of SEQ ID NOs: 11 and 12; (2) both of SEQ ID NOs: 22 and 23; (3) both of SEQ ID NOs: 33 and 34; (4) both of SEQ ID NOs: 44 and 45; (5) both of SEQ ID NOs: 55 and 56; (6) both of SEQ ID NOs: 66 and 67; (7) both of SEQ ID NOs: 77

and 78; (8) both of SEQ ID NOs: 88 and 89; (9) both of SEQ ID NOs: 139 and 140; (10) both of SEQ ID NOs: 160 and 161; (11) both of SEQ ID NOs: 162 and 163; (12) both of SEQ ID NOs: 164 and 165; (13) both of SEQ ID NOs: 166 and 167; (14) both of SEQ ID NOs: 168 and 169; (15) both of SEQ ID NOs: 170 and 171; (16) both of SEQ ID NOs: 172 and 173; (17) both of SEQ ID NOs: 174 and 175; (18) both of SEQ ID NOs: 176 and 177; (19) both of SEQ ID NOs: 179 and 12; (20) both of SEQ ID NOs: 182 and 23; (21) both of SEQ ID NOs: 185 and 34; (22) both of SEQ ID NOs: 188 and 45; (23) both of SEQ ID NOs: 191 and 56; (24) both of SEQ ID NOs: 194 and 67; (25) both of SEQ ID NOs: 197 and 78; (26) both of SEQ ID NOs: 200 and 89; (27) both of SEQ ID NOs: 139 and 203; (28) both of SEQ ID NOs: 11 and 206; (29) both of SEQ ID NOs: 22 and 208; (30) both of SEQ ID NOs: 33 and 210; (31) both of SEQ ID NOs: 44 and 212; (32) both of SEQ ID NOs: 55 and 214; (33) both of SEQ ID NOs: 66 and 216; (34) both of SEQ ID NOs: 77 and 218; (35) both of SEQ ID NOs: 88 and 220; (36) both of SEQ ID NOs: 139 and 222; (37) both of SEQ ID NOs: 224 and 203; (38) both of SEQ ID NOs: 224 and 222; (39) both of SEQ ID NOs: 22 and 227; or (40) both of SEQ ID NOs: 182 and 227.

[0093] The invention further provides a protein comprising at least one of the polypeptides described herein. By “protein” is meant a molecule comprising one or more polypeptide chains. In an embodiment, the protein of the invention can comprise: (1) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 3-5 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 6-8; (2) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 14-16 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 17-19; (3) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 25-27 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 28-30; (4) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 36-38 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 39-41; (5) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 47-49 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 50-52; (6) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 58-60 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 61-63; (7) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 69-71 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 72-74; (8) a

first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 80-82 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 83-85; or (9) a first polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 131-133 and a second polypeptide chain comprising the amino acid sequences of all of SEQ ID NOs: 134-136.

[0094] In an embodiment of the invention, the protein comprises: (1) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 9 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 10; (2) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 21; (3) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 31 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 32; (4) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 42 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 43; (5) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 53 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 54; (6) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 64 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 65; (7) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 75 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 76; (8) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 86 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 87; (9) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 137 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 138; (10) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 142 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 143; (11) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 144 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 145; (12) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 146 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 147; (13) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 148 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 149; (14) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 150 and a second polypeptide chain

comprising the amino acid sequence of SEQ ID NO: 151; (15) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 152 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 153; (16) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 154 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 155; (17) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 156 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 157; (18) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 158 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 159; (19) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 178 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 10; (20) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 181 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 21; (21) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 184 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 32; (22) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 187 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 43; (23) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 190 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 54; (24) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 193 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 65; (25) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 196 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 76; (26) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 199 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 87; (27) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 137 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 202; (28) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 9 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 205; (29) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 207; (30) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 31 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 209; (31) a first polypeptide chain

comprising the amino acid sequence of SEQ ID NO: 42 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 211; (32) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 53 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 213; (33) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 64 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 215; (34) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 75 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 217; (35) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 86 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 219; (36) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 137 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 221; (37) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 223 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 202; (38) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 223 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 221; (39) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 20 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 226; or (40) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 181 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 226.

[0095] In an embodiment of the invention, the protein comprises: (1) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 11 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 12; (2) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 23; (3) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 33 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 34; (4) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 44 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 45; (5) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 55 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 56; (6) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 66 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 67; (7) a first polypeptide chain

comprising the amino acid sequence of SEQ ID NO: 77 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 78; (8) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 88 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 89; (9) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 140; (10) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 160 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 161; (11) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 162 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 163; (12) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 164 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 165; (13) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 166 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 167; (14) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 168 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 169; (15) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 170 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 171; (16) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 172 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 173; (17) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 174 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 175; (18) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 176 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 177; (19) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 179 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 12; (20) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 182 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 23; (21) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 185 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 34; (22) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 188 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 45; (23) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 191 and a second polypeptide chain

comprising the amino acid sequence of SEQ ID NO: 56; (24) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 194 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 67; (25) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 197 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 78; (26) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 200 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 89; (27) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 203; (28) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 11 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 206; (29) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 208; (30) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 33 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 210; (31) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 44 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 212; (32) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 55 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 214; (33) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 66 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 216; (34) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 77 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 218; (35) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 88 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 220; (36) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 139 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 222; (37) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 224 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 203; or (38) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 224 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 222; (39) a first polypeptide chain comprising the amino acid sequence of SEQ ID NO: 22 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 227; or (40) a first polypeptide chain

comprising the amino acid sequence of SEQ ID NO: 182 and a second polypeptide chain comprising the amino acid sequence of SEQ ID NO: 227.

[0096] The protein of the invention may be a TCR. Alternatively, if the first and/or second polypeptide chain(s) of the protein further comprise(s) other amino acid sequences, e.g., an amino acid sequence encoding an immunoglobulin or a portion thereof, then the inventive protein can be a fusion protein. In this regard, the invention also provides a fusion protein comprising at least one of the inventive polypeptides described herein along with at least one other polypeptide. The other polypeptide can exist as a separate polypeptide of the fusion protein, or can exist as a polypeptide, which is expressed in frame (in tandem) with one of the inventive polypeptides described herein. The other polypeptide can encode any peptidic or proteinaceous molecule, or a portion thereof, including, but not limited to an immunoglobulin, CD3, CD4, CD8, an MHC molecule, a CD1 molecule, e.g., CD1a, CD1b, CD1c, CD1d, etc.

[0097] The fusion protein can comprise one or more copies of the inventive polypeptide and/or one or more copies of the other polypeptide. For instance, the fusion protein can comprise 1, 2, 3, 4, 5, or more, copies of the inventive polypeptide and/or of the other polypeptide. Suitable methods of making fusion proteins are known in the art, and include, for example, recombinant methods.

[0098] In some embodiments of the invention, the TCRs, polypeptides, and proteins of the invention may be expressed as a single protein comprising a linker peptide linking the α chain and the β chain. In this regard, the TCRs, polypeptides, and proteins of the invention may further comprise a linker peptide. The linker peptide may advantageously facilitate the expression of a recombinant TCR, polypeptide, and/or protein in a host cell. The linker peptide may comprise any suitable amino acid sequence. For example, the linker peptide may comprise the amino acid sequence of SEQ ID NO: 94. Upon expression of the construct including the linker peptide by a host cell, the linker peptide may be cleaved, resulting in separated α and β chains. In an embodiment of the invention, the TCR, polypeptide, or protein may comprise an amino acid sequence comprising a full-length α chain, a full-length β chain, and a linker peptide positioned between the α and β chains.

[0099] In some embodiments, the TCR, polypeptide or protein disclosed herein comprises an α chain and/or a β chain, as disclosed herein, comprising a signal peptide. In some embodiments, the sequence of the signal peptide of any of the α chains and/or β chains

disclosed herein comprises an alanine or histidine residue substituted for the wild-type residue at position 2.

[0100] In some embodiments, the TCR, polypeptide or protein disclosed herein comprises a mature version of an α chain and/or a β chain, as disclosed herein, that lacks a signal peptide.

[0101] The protein of the invention can be a recombinant antibody, or an antigen binding portion thereof, comprising at least one of the inventive polypeptides described herein. As used herein, “recombinant antibody” refers to a recombinant (e.g., genetically engineered) protein comprising at least one of the polypeptides of the invention and a polypeptide chain of an antibody, or an antigen binding portion thereof. The polypeptide of an antibody, or antigen binding portion thereof, can be a heavy chain, a light chain, a variable or constant region of a heavy or light chain, a single chain variable fragment (scFv), or an Fc, Fab, or F(ab)₂' fragment of an antibody, etc. The polypeptide chain of an antibody, or an antigen binding portion thereof, can exist as a separate polypeptide of the recombinant antibody. Alternatively, the polypeptide chain of an antibody, or an antigen binding portion thereof, can exist as a polypeptide, which is expressed in frame (in tandem) with the polypeptide of the invention. The polypeptide of an antibody, or an antigen binding portion thereof, can be a polypeptide of any antibody or any antibody fragment, including any of the antibodies and antibody fragments described herein.

[0102] The TCRs, polypeptides, and proteins of the invention can be of any length, i.e., can comprise any number of amino acids, provided that the TCRs, polypeptides, or proteins retain their biological activity, e.g., the ability to specifically bind to mutated p53; detect cancer in a mammal; or treat or prevent cancer in a mammal, etc. For example, the polypeptide can be in the range of from about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length. In this regard, the polypeptides of the invention also include oligopeptides.

[0103] The TCRs, polypeptides, and proteins of the invention of the invention can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α -amino n-decanoic acid, homoserine, S-acetylaminoethylcysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β -phenylserine β -hydroxyphenylalanine, phenylglycine, α -naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-

carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N^ε-benzyl-N^ε-methyl-lysine, N^ε,N^ε-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine.

[0104] The TCRs, polypeptides, and proteins of the invention can be, e.g., glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

[0105] The TCR, polypeptide, and/or protein of the invention can be obtained by methods known in the art such as, for example, *de novo* synthesis. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Green and Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Press, Cold Spring Harbor, NY (2012). Alternatively, the TCRs, polypeptides, and/or proteins described herein can be commercially synthesized by companies, such as Synpep (Dublin, CA), Peptide Technologies Corp. (Gaithersburg, MD), and Multiple Peptide Systems (San Diego, CA). In this respect, the inventive TCRs, polypeptides, and proteins can be synthetic, recombinant, isolated, and/or purified.

[0106] An embodiment of the invention provides a nucleic acid comprising a nucleotide sequence encoding any of the TCRs, polypeptides, or proteins described herein. “Nucleic acid,” as used herein, includes “polynucleotide,” “oligonucleotide,” and “nucleic acid molecule,” and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, which can contain natural, non-natural or altered nucleotides, and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In an embodiment, the nucleic acid comprises complementary DNA (cDNA). It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0107] Preferably, the nucleic acids of the invention are recombinant. As used herein, the term “recombinant” refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0108] The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Green and Sambrook et al., *supra*. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (e.g., phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that can be used to generate the nucleic acids include, but are not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N⁶-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N⁶-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N⁶-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids of the invention can be purchased from companies, such as Macromolecular Resources (Fort Collins, CO) and Synthegen (Houston, TX).

[0109] In an embodiment of the invention, the nucleic acid comprises a codon-optimized nucleotide sequence encoding any of the TCRs, polypeptides, or proteins described herein. Without being bound to any particular theory or mechanism, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency.

Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

[0110] The invention also provides a nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

[0111] The nucleotide sequence which hybridizes under stringent conditions preferably hybridizes under high stringency conditions. By “high stringency conditions” is meant that the nucleotide sequence specifically hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (e.g., 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive TCRs. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

[0112] The invention also provides a nucleic acid comprising a nucleotide sequence that is at least about 70% or more, e.g., about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to any of the nucleic acids described herein. In this regard, the nucleic acid may consist essentially of any of the nucleotide sequences described herein.

[0113] The nucleic acids of the invention can be incorporated into a recombinant expression vector. In this regard, the invention provides a recombinant expression vector comprising any of the nucleic acids of the invention. In an embodiment of the invention, the recombinant expression vector comprises a nucleotide sequence encoding the α chain, the β chain, and linker peptide.

[0114] For purposes herein, the term “recombinant expression vector” means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The inventive recombinant expression vectors can comprise any type of nucleotide, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0115] The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the transposon/transposase series, pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λ GT10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NM1149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-C1, pMAM and pMAMneo (Clontech). Preferably, the recombinant expression vector is a transposon vector or a viral vector, e.g., a retroviral vector.

[0116] The recombinant expression vectors of the invention can be prepared using standard recombinant DNA techniques described in, for example, Green and Sambrook et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEI, 2 μ plasmid, λ , SV40, bovine papillomavirus, and the like.

[0117] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0118] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host cell to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0119] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the TCR, polypeptide, or protein, or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the TCR, polypeptide, or protein. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter, e.g., a human elongation factor-1 α promoter, or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, and a promoter found in the long-terminal repeat of the murine stem cell virus.

[0120] The inventive recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0121] Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

[0122] Another embodiment of the invention further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term “host cell” refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5 α *E. coli* cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5 α cell. For purposes of producing a recombinant TCR, polypeptide, or protein, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell preferably is a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell.

[0123] For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. Preferably, the T cell is a human T cell. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4⁺/CD8⁺ double positive T cells, CD4⁺ helper T cells, e.g., Th₁ and Th₂ cells, CD4⁺ T cells, CD8⁺ T cells (e.g., cytotoxic T cells), tumor infiltrating lymphocytes (TILs), memory T cells (e.g., central memory T cells and effector memory T cells), naïve T cells, and the like.

[0124] Also provided by the invention is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of host cells (e.g.,

consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0125] In an embodiment of the invention, the numbers of cells in the population may be rapidly expanded. Expansion of the numbers of T cells can be accomplished by any of a number of methods as are known in the art as described in, for example, U.S. Patent 8,034,334; U.S. Patent 8,383,099; U.S. Patent Application Publication No. 2012/0244133; Dudley et al., *J. Immunother.*, 26:332-42 (2003); and Riddell et al., *J. Immunol. Methods*, 128:189-201 (1990). In an embodiment, expansion of the numbers of T cells is carried out by culturing the T cells with OKT3 antibody, IL-2, and feeder PBMC (e.g., irradiated allogeneic PBMC).

[0126] The inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, and host cells (including populations thereof), can be isolated and/or purified. The term “isolated” as used herein means having been removed from its natural environment. The term “purified” as used herein means having been increased in purity, wherein “purity” is a relative term, and not to be necessarily construed as absolute purity. For example, the purity can be at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or can be about 100%.

[0127] The inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, and host cells (including populations thereof), all of which are collectively referred to as “inventive TCR materials” hereinafter, can be formulated into a composition, such as a pharmaceutical composition. In this regard, the invention provides a pharmaceutical composition comprising any of the TCRs, polypeptides, proteins, nucleic acids, expression vectors, and host cells (including populations thereof), described herein, and a pharmaceutically acceptable carrier. The inventive pharmaceutical compositions containing any of the inventive TCR materials can comprise more than one inventive TCR material, e.g., a polypeptide and a nucleic acid, or two or more different TCRs. Alternatively, the pharmaceutical composition can comprise an inventive TCR material in combination with another pharmaceutically active agent(s) or drug(s), such as a chemotherapeutic agent, e.g.,

asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc.

[0128] Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used for the particular inventive TCR material under consideration. Methods for preparing administrable compositions are known or apparent to those skilled in the art and are described in more detail in, for example, *Remington: The Science and Practice of Pharmacy*, 22nd Ed., Pharmaceutical Press (2012). It is preferred that the pharmaceutically acceptable carrier be one which has no detrimental side effects or toxicity under the conditions of use.

[0129] The choice of carrier will be determined in part by the particular inventive TCR material, as well as by the particular method used to administer the inventive TCR material. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. Suitable formulations may include any of those for parenteral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal, intratumoral, or interperitoneal administration. More than one route can be used to administer the inventive TCR materials, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0130] Preferably, the inventive TCR material is administered by injection, e.g., intravenously. When the inventive TCR material is a host cell expressing the inventive TCR, the pharmaceutically acceptable carrier for the cells for injection may include any isotonic carrier such as, for example, normal saline (about 0.90% w/v of NaCl in water, about 300 mOsm/L NaCl in water, or about 9.0 g NaCl per liter of water), NORMOSOL R electrolyte solution (Abbott, Chicago, IL), PLASMA-LYTE A (Baxter, Deerfield, IL), about 5% dextrose in water, or Ringer's lactate. In an embodiment, the pharmaceutically acceptable carrier is supplemented with human serum albumen.

[0131] For purposes of the invention, the amount or dose (e.g., numbers of cells when the inventive TCR material is one or more cells) of the inventive TCR material administered should be sufficient to effect, e.g., a therapeutic or prophylactic response, in the subject or animal over a reasonable time frame. For example, the dose of the inventive TCR material should be sufficient to bind to a cancer antigen (e.g., mutated p53), or detect, treat or prevent cancer in a period of from about 2 hours or longer, e.g., 12 to 24 or more hours, from the time of administration. In certain embodiments, the time period could be even longer. The dose will be determined by the efficacy of the particular inventive TCR material and the condition

of the animal (e.g., human), as well as the body weight of the animal (e.g., human) to be treated.

[0132] Many assays for determining an administered dose are known in the art. For purposes of the invention, an assay, which comprises comparing the extent to which target cells are lysed or IFN- γ is secreted by T cells expressing the inventive TCR, polypeptide, or protein upon administration of a given dose of such T cells to a mammal among a set of mammals of which each is given a different dose of the T cells, could be used to determine a starting dose to be administered to a mammal. The extent to which target cells are lysed or IFN- γ is secreted upon administration of a certain dose can be assayed by methods known in the art.

[0133] The dose of the inventive TCR material also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular inventive TCR material. Typically, the attending physician will decide the dosage of the inventive TCR material with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inventive TCR material to be administered, route of administration, and the severity of the cancer being treated. In an embodiment in which the inventive TCR material is a population of cells, the number of cells administered per infusion may vary, e.g., from about 1×10^6 to about 1×10^{12} cells or more. In certain embodiments, fewer than 1×10^6 cells may be administered.

[0134] One of ordinary skill in the art will readily appreciate that the inventive TCR materials of the invention can be modified in any number of ways, such that the therapeutic or prophylactic efficacy of the inventive TCR materials is increased through the modification. For instance, the inventive TCR materials can be conjugated either directly or indirectly through a bridge to a chemotherapeutic agent. The practice of conjugating compounds to a chemotherapeutic agent is known in the art. One of ordinary skill in the art recognizes that sites on the inventive TCR materials, which are not necessary for the function of the inventive TCR materials, are ideal sites for attaching a bridge and/or a chemotherapeutic agent, provided that the bridge and/or chemotherapeutic agent, once attached to the inventive TCR materials, do(es) not interfere with the function of the inventive TCR materials, i.e., the ability to bind to mutated p53 or to detect, treat, or prevent cancer.

[0135] It is contemplated that the inventive pharmaceutical compositions, TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or

populations of cells can be used in methods of treating or preventing cancer. Without being bound to a particular theory, the inventive TCRs are believed to bind specifically to mutated p53, such that the TCR (or related inventive polypeptide or protein), when expressed by a cell, is able to mediate an immune response against a target cell expressing mutated p53. In this regard, an embodiment of the invention provides a method of treating or preventing cancer in a mammal, comprising administering to the mammal any of the pharmaceutical compositions, TCRs, polypeptides, or proteins described herein, any nucleic acid or recombinant expression vector comprising a nucleotide sequence encoding any of the TCRs, polypeptides, proteins described herein, or any host cell or population of cells comprising a recombinant vector which encodes any of the TCRs, polypeptides, or proteins described herein, in an amount effective to treat or prevent cancer in the mammal.

[0136] An embodiment of the invention provides any of the pharmaceutical compositions, TCRs, polypeptides, or proteins described herein, any nucleic acid or recombinant expression vector comprising a nucleotide sequence encoding any of the TCRs, polypeptides, proteins described herein, or any host cell or population of cells comprising a recombinant vector which encodes any of the TCRs, polypeptides, or proteins described herein, for use in the treatment or prevention of cancer in a mammal.

[0137] The terms “treat,” and “prevent” as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of cancer in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the cancer being treated or prevented. For example, treatment or prevention can include promoting the regression of a tumor. Also, for purposes herein, “prevention” can encompass delaying the onset of the cancer, or a symptom or condition thereof. Alternatively or additionally, “prevention” may encompass preventing or delaying the recurrence of cancer, or a symptom or condition thereof.

[0138] Also provided by an embodiment of the invention is a method of detecting the presence of cancer in a mammal. The method comprises (i) contacting a sample comprising one or more cells from the mammal with any of the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or

pharmaceutical compositions described herein, thereby forming a complex, and detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the mammal.

[0139] With respect to the inventive method of detecting cancer in a mammal, the sample of cells can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction.

[0140] For purposes of the inventive detecting method, the contacting can take place *in vitro* or *in vivo* with respect to the mammal. Preferably, the contacting is *in vitro*.

[0141] Also, detection of the complex can occur through any number of ways known in the art. For instance, the inventive TCRs, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, or populations of cells, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles).

[0142] For purposes of the inventive methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal.

[0143] With respect to the inventive methods, the cancer can be any cancer, including, e.g., any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vagina, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, colorectal cancer, endometrial cancer, esophageal cancer, uterine cervical cancer, gastrointestinal carcinoid tumor, glioma, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, cancer of the oropharynx, ovarian cancer, cancer of the penis, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, cancer of the uterus, ureter cancer, and urinary bladder cancer. In a preferred embodiment, the cancer is a cancer which expresses mutated p53. The cancer may express

p53 with a mutation at one or both of positions 175 and 220, as defined by SEQ ID NO: 1. The cancer may express p53 with one or both of the following human p53 mutations: R175H and Y220C. Preferably, the cancer is an epithelial cancer or cholangiocarcinoma, melanoma, colon cancer, rectal cancer, ovarian cancer, endometrial cancer, non-small cell lung cancer (NSCLC), glioblastoma, uterine cervical cancer, head and neck cancer, breast cancer, pancreatic cancer, or bladder cancer.

[0144] The mammal referred to in the inventive methods can be any mammal. As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboidea, or Simiiformes (monkeys) or of the order Anthropoidea (humans and apes). An especially preferred mammal is the human.

[0145] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLES

[0146] The following materials and methods were employed in the experiments described in Examples 1-7.

Subjects and samples

[0147] Leukapheresis products and tumor samples were obtained from individuals with metastatic epithelial cancers enrolled on National Institute of Health protocol NCT01174121, which was approved by the institutional-review board (IRB) of National Cancer Institute (NCI). Patients were chosen on basis of availability of pre-treatment leukapheresis and TIL screening results and had received prior therapies (surgery, chemotherapy, radiation therapy) per standard of care (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019); Deniger et al., *Clin. Cancer Res.*, 24(22):5562-73 (2018)). Ficoll-Hypaque was used to isolated PBL from leukapheresis and cells were cryopreserved for further use. All patients studied had a confirmed *TP53* mutation by whole-exome sequencing in line with clinical protocols as previously described (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)).

Antibodies and FACS

[0148] Fluorescently labeled antibodies flow cytometry are detailed in Table 1.

Analytical flow cytometry was performed on FACS CANTO II system (BD Biosciences, San Jose, CA) with analysis by FLOWJO software (TreeStar, Ashland, OR). All cells were gated by lymphocytes and live cells by exclusion of cells stained with propidium iodide (PI). Cells were sorted for IVS and 4-1BB/OX40 on a FACS ARIA II cell sorter (BD Biosciences). 4-1BB⁺ and/or OX40⁺ cells were sorted separately through CD3⁺CD4⁺CD8⁻ (CD4) and CD3⁺CD4⁻CD8⁺ (CD8) gates. Co-cultures were sorted by SH800S cell sorter (Sony Biotechnology) for single-cell PCR to identify TCR genes.

TABLE 1

Antigen	Catalog #	Clone	Fluorochrome	Vendor
CD3	560176	SK7	APC-H7	BD Pharminogen
CD3	349201	SK7	FITC	BD Biosciences
CD3	130-093-387	OKT3	purified	Miltenyi Biotec
CD4	555347	RPA-T4	PE	BD Pharminogen
CD4	340133	SK3	FITC	BD Biosciences
CD4	317438	OKT4	BV605	BioLegend
CD8	557746	RPA-T8	PE-Cy7	BD Pharminogen
CD134	555837	ACT35	FITC	BD Pharminogen
CD137	550890	4B4-1	APC	BD Pharminogen
CD62L	555544	DREG-56	PE	BD Pharminogen
CD45RO	559865	UCHL1	APC	BD Pharminogen
TCRb	MA5-17652	H57-597	FITC	eBioscience/ ThermoFisher
TCRb	560656	H57-597	APC	eBioscience/ ThermoFisher
Live/Dead	P4864	n/a	Propidium Iodide	Sigma-Aldrich

TP53 “hotspot” mutation screening reagents

[0149] The WT (TMG-WT-TP53) and mutated TP53 (TMG-MUT-TP53) TMG constructs were generated for TIL screening as previously described (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). In brief, each TP53 “hotspot” mutation (R175H, Y220C, G245S, G245D, R248L, R248Q, R248W, R249S, R273C, R273H, R273L and

R282W) was composed into a minigene with mutated codon in the middle and 12 normal codons upstream and downstream and the minigenes were concatenated into a TMG. A similar sequence corresponding to WT sequences was also derived. TMGs were synthesized as DNA and cloned in frame to LAMP signal sequence and DC-LAMP localization sequence then *in vitro* transcribed to mRNA using mMESSAGE mMACHINE T7 Ultra Kit according to manufacturer's instructions (Thermo-Fisher; Waltham, MA). In addition, WT and mutated peptides were synthesized for p53^{R175H}, p53^{R248W} and p53^{Y220C} and purified to >95% by high-performance liquid chromatography (Genscript; Piscataway, NJ). All peptides were reconstituted in DMSO.

Antigen presenting cells

[0150] Monocyte-derived immature dendritic cells (DCs) were generated by adherence method (Tran et al., *Science*, 350(6266):1387-90 (2015)). Briefly, PBL were plated in AIM-V media (Life Technologies, Waltham, MA) containing DNase (Genentech Inc., San Francisco, CA) and incubated for 1.5-2 hours at 37 °C. Non-adherent cells were removed and used fresh or cryopreserved. Adherent cells were washed in AIM-V, incubated one hour at 37 °C and media was exchanged to DC media (RPMI-1640, 2 mM L-glutamine, with 5% human serum, 100 U/mL penicillin, 100 µg/mL streptomycin, amphotericin B, 800 IU/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) and 200 U/mL interleukin-4 (IL-4) (Peprotech, Rocky Hill, NJ)). Cells were fed every 2-3 days and harvested on days 5-6.

IVS, mutant TP53 co-culture, 4-1BB/OX40 enrichment and REP

[0151] To perform the antigen experienced sort, pre-treatment PBL were processed as previously described (Cafri et al., *Nat. Commun.*, 10(1):449 (2019)). Cryopreserved apheresis samples were thawed, washed, set to 5-10x10⁶ cells/ml with AIM-V media containing DNase and 1.75-2x10⁸ viable cells were plated per T175 flasks (Corning Inc., Corning, NY) and incubated at 37 °C, 5% CO₂ for 90 minutes. After 90 minutes the non-adherent monocyte-depleted PBL were harvested, centrifuged, and incubated overnight at 37 °C and 5% CO₂ in 50/50 media (AIM-V media, RPMI-1640 media (Lonza, Walkersville, MD), 5% human AB serum, 100 U/mL penicillin and 100 µg/mL streptomycin (Life Technologies), 2 mM L-glutamine (Life Technologies), 10 µg/mL gentamicin (Quality Biological Inc., Gaithersburg, MD), 12.5 mM HEPES (Life Technologies)). Adherent monocytes were differentiated into immature DCs as described above. After resting the non-adherent cells overnight, the cells

were harvested, and $1-2 \times 10^8$ cells were resuspended in 50 μ l of staining buffer (PBS, 0.5% BSA, 2 mM EDTA) with CD3, CD8, CD4, CD62L, CD45RO antibodies. Cells were incubated for 30 minutes at 4°C and washed twice before acquisition. To determine the sorting population, gating was performed on live cells (propidium iodide negative), single cells, CD3⁺ T cells then antigen experienced cells (CD62L⁻CD45RO⁺, CD62L⁺CD45RO⁺, CD62L⁻CD45RO⁻), which were further subdivided into CD4⁺ or CD8⁺. The CD8⁺ and CD4⁺ antigen experienced memory T cells were sorted separately, collected, counted and resuspended in 50/50 media containing concentration of 60 ng/mL interleukin-21 (IL-21). Autologous DCs were electroporated 18-24 hours in advance with TMG-MUT-*TP53* or pulsed for 2-4 hours the day of the FACS sort with patient-specific mutant p53-LP. Target cells (DCs) were washed in 50/50 media twice and resuspended in 50/50 media with no cytokines. *In vitro* stimulation (IVS) was performed with a 1:3 to 1:6 ratio (DC:T cell) co-culture in a final concentration of 30 ng/ml IL-21 after the addition of the DCs. Following 14 days of growth and 3 feedings with IL-21 and interleukin-2 (IL-2; aldesleukin) as previously described (Cafri et al., *Nat. Commun.*, 10(1):449 (2019)), autologous DCs were again electroporated or pulsed with TMG-MUT-*TP53* or mutated long peptide (LP), respectively, and co-cultured IVS cultures for 18-24 hours at 37 °C and 5% CO₂. In parallel, IVS cultures were co-cultured with DCs electroporated with irrelevant TMG or pulsed with DMSO for negative controls during the 4-1BB/OX40 enrichment sort. Following co-culture, the cells were harvested and resuspended in 50 μ l of staining buffer (PBS, 0.5% BSA, 2 mM EDTA) containing CD3, CD4, CD8, 4-1BB and OX40 antibodies, incubated for 30 minutes at 4 °C and washed twice before acquisition. The sorted 4-1BB⁺/OX40⁺ enriched T cells were expanded by REP using irradiated PBL feeders, 30 ng/mL OKT3 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) and 3,000 IU/mL IL-2 in 50/50 media. The rapidly expanded (REP) cultures were fed 3 times and were tested for reactivity or cryopreserved on day 14.

Co-culture

[0152] Screening of IVS and enriched T cells was accomplished through same strategy used to screen TIL fragments (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). Briefly, autologous DCs were electroporated with TMG (10^5 cells/well) and rested overnight or pulsed with peptide or DMSO (8×10^4 cells/well) for 2-4 hours. Target cells were washed twice and resuspended in 50/50 media and co-cultured with 2×10^4 T cells in interferon- γ (IFN γ) Enzyme-Linked ImmunoSpot (ELISPOT) plates (EMD Millipore, Burlington, MA).

Phorbol 12-myristate 13-acetate (PMA) and ionomycin (Thermo Fisher) were used as a positive control and media only was a negative control. The co-cultured cells were removed, stained and analyzed by flow cytometry as above whilst the ELISPOT plate was processed according to manufacturer's instructions. Tumor cell lines were grown for at least a week from cryopreserved stocks before co-culture and their generation is described elsewhere (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). Tumor cells were co-cultured at 1:1 ratio with T cells (2×10^5 total cells) overnight in round-bottom 96 well plates. Following harvesting of co-culture supernatant to assess IFN γ secretion by enzyme linked immunosorbent assay (ELISA; ThermoFisher), the co-cultured cells were and analyzed by flow cytometry.

Minimal peptide assay and HLA restriction mapping

[0153] A similar method previously described was used to identify minimal peptides and determine HLA restrictions (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). In short, NetMHC peptide binding affinity algorithm (v3.4) was used (Lundegaard et al., *Bioinformatics*, 24(11): 1397-8 (2008)) to predict neopeptides for HLA Class-I alleles. Candidate 9-11 amino acid were co-cultured as described above. To investigate the CD4⁺ minimal neoantigens, 15 amino acid peptides overlapping 14 amino acids were co-cultured as described above. To determine the HLA restrictions, COS7 tumor cells were plated at 2.5×10^4 cells/well in RPMI-1640, 2 mM L-glutamine and 10% fetal bovine serum in flat-bottom 96 well plates and incubated overnight at 37 °C. Patient specific individual HLA Class-I alleles (300 ng/well) or both HLA Class-II α and β chains (150 ng/well each) in DNA plasmids (pcDNA3.1 backbone) were transfected with LIPOFECTAMINE 2000 transfection reagent according to manufacturer's instructions (ThermoFisher). When TMGs were co-transfected with HLA, the concentration of HLAs reduced to 150 ng/well for Class-I and to 100ng/well for Class-II. The WT TMGs used for these experiments was the TMG-MUT-TP53 reverted to WT only at the position of interest, e.g., R175H. Following 24-hour incubation, transfection media was removed, peptides or DMSO were pulsed for 2-4 hours in 50/50 media, wells were washed twice with 50/50 media and 10^5 T cells were incubated overnight. Co-culture supernatants were analyzed for IFN γ secretion by ELISA and cells were stained for upregulation of 4-1BB and analyzed by flow cytometry.

TCRB sequencing

[0154] *TCRB* survey sequencing was performed from genomic DNA by Adaptive Biotechnologies (Seattle, WA). A minimum 5×10^4 cells were sent for sequencing. Analysis of productive TCR rearrangements was performed using IMMUNSEQ ANALYZER 3.0 tool (Adaptive Biotechnologies).

TCR identification and reconstruction

[0155] TCRs were identified by sorting co-cultures of T cells and DCs expressing p53 neoantigens into single wells followed by single cell reverse transcriptase polymerase chain reaction (RT-PCR) of TCR genes similar to previous studies (Pasetto et al., *Cancer Immunol. Res.*, doi 10.1158/2326-6066.CIR-16-0001 (2016); Deniger et al., *Clin. Cancer Res.*, 23(2): 351-62 (2017)). The PCR products were kept separate for TCR α and TCR β and were analyzed by Sanger sequencing. These partial TCR sequences were analyzed with IMGT/V-Quest (imgt.org/IMGT) and IGBLAST (ncbi.nlm.nih.gov/igblast) websites which identified the exact CDR3 and J or D/J regions and the most likely TRAV and TRBV family. The human full-length variable sequences were fused to murine constant chains as was done in other studies (Deniger et al., *Clin. Cancer Res.*, 23(2): 351-62 (2017); Cohen et al., *J. Clin. Invest.*, 125(10): 3981-91 (2015). The murinized TCR α and TCR β genes were linked with a RAKR-SGSG (SEQ ID NO: 128) and P2A ribosomal slip sequence to result in stoichiometric expression of the TCR chains in a single cistron. This sequence was synthesized and cloned into MSGV1 vector for generation of transient retroviral supernatants.

TCR transduction

[0156] PBL donors were adjusted to 3×10^6 cells/ml in 50/50 media supplemented 50 ng/ml soluble OKT3 and 300 IU/ml IL-2 and were activated on low adherence plates for two days prior to retroviral transduction. The pMSGV1 plasmid encoding mutation-specific TCR (1.5 μ g/well) and the envelope-encoding plasmid RD114 (0.75 μ g/well) were co-transfected into 10^6 HEK293GP cells/well of a 6-well poly-D-lysine-coated plate using LIPOFECTAMINE 2000 transfection reagent (Life Technologies). Retroviral supernatants were collected two days after transfection, diluted 1:1 with DMEM media, and centrifuged onto non-tissue culture-treated 6-well plate coated with RETRONECTIN reagent (10 μ g/well, Takara, Rockville, MD) at 2000xg for 2 hours at 32 °C. Supernatants were aspirated and

2×10^6 stimulated T-cells at 5×10^5 cells/mL were added to each well in 50/50 media with 300 IU/mL IL-2. The T cells were centrifuged onto the RETRONECTIN reagent coated plates for 10 min at 300xg. The media was exchanged 3-4 days later with 300 IU/mL IL-2 and transduced cells were assayed 10-14 days post-transduction.

EXAMPLE 1

[0157] This example demonstrates the IVS of antigen experienced peripheral blood T cells with *TP53* mutations.

[0158] Antigen experienced T cells from PBL were evaluated in 7 colon, 1 rectal and 1 ovarian cancer patients with a *TP53* mutated tumor (Table 2). Patients 4217 (p53^{R175H}), 4213 (p53^{R248Q}), 4257 (p53^{R248W}) and 4254 (p53^{R273H}) did not have TIL responses to p53 neoantigens. In contrast, TIL were reactive to the autologous *TP53* mutation in patients 4141 (p53^{R175H}), 4285 (p53^{R175H}), 4149 (p53^{Y220C}), 4266 (p53^{R248W}) and 4273 (p53^{R248W}) (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019); Deniger et al., *Clin. Cancer Res.*, 24(22):5562-73 (2018)). Cryopreserved aphereses (prior to any ACT) were used to sort antigen experienced CD4⁺ or CD8⁺ T cells from PBL (Fig. 1A left). Sorted CD4⁺ or CD8⁺ T cells were *in vitro* stimulated with DCs expressing mutated *TP53*-TMG mRNA (*TP53*-TMG-IVS) or pulsed with patient-specific mutated p53-LP (p53-LP-IVS). After 12 days of culture in the presence of IL-21 and IL-2, *in vitro* stimulated CD4⁺ and CD8⁺ memory cells were co-cultured with DCs electroporated with mutated *TP53*-TMG mRNA, in the case of *TP53*-TMG-IVS, or co-cultured with patient-specific mutated p53-LP, in the case of p53-LP-IVS, and sorted the following day based on expression of T cell activation markers 4-1BB and/or OX40 (Fig. 1A middle). The cell yields after *TP53*-TMG-IVS and p53-LP-IVS were comparable for both CD4⁺ and CD8⁺ T cells ranging from 0.9×10^7 - 18.3×10^7 cells from 0.3×10^7 - 15.6×10^7 starting T cells (Table 3). A portion of CD4⁺4-1BB⁺/OX40⁺ and CD8⁺4-1BB⁺/OX40⁺ T cells were sorted from all populations for completeness and symmetry of the experiment, which ranged from 2×10^2 - 1.7×10^5 cells and 0.1% - 6.9% from the parent CD4 or CD8 gate (Table 3). The sorted T cells underwent a REP and were analyzed after 14 days of rapid expansion. The final cell yields ranged from 7×10^6 - 4.2×10^8 T cells, which were likely influenced by the input cell numbers into the REP (Table 3).

TABLE 2

Patient #	Age/Sex	Cancer type	p53 amino acid substitution	p53 neoepitope HLA restriction	TIL response	PBL response
4141	52M	Colon	R175H	A*02:01	CD8	CD8
4217	51M	Colon	R175H	n/a	-	-
4285	46M	Colon	R175H	DRB1*13:01	CD4	CD4
4149	36F	Ovarian	Y220C	Class-II	CD4	CD4
4213	65M	Colon	R248Q	n/a	-	-
4257	65M	Colon	R248W	n/a	-	-
4266	41F	Colon	R248W	A*68:01	CD8	CD8
4273	49M	Rectal	R248W	DPB1*02:01	CD4	CD4
4254	55F	Colon	R273H	n/a	-	-

TABLE 3

Patient #	p53 a.a. sub	T cell type	IVS	# cells to start IVS (x10 ⁶)	# cells after IVS (x10 ⁶)	% 4-1BB/OX40 sorted	# cells to start REP (x10 ⁶)	# cells after REP (x10 ⁶)
4141	R175H	CD4	p53-LP	13.2	12.6	0.9	0.0146	15.9
			TP53-TMG	15.6	18.3	2.3	0.0179	42.2
		CD8	p53-LP	1	9.1	0.1	0.0002	12.9
			TP53-TMG	1.5	7.9	4.9	0.1185	44.2
4285	R175H	CD4	p53-LP	5	31	2.0	0.0360	360
			TP53-TMG	5	36	2.2	0.0428	420
		CD8	p53-LP	1.5	10	2.4	0.0192	34

Patient #	p53 a.a. sub	T cell type	IVS	# cells to start IVS (x10 ⁶)	# cells after IVS (x10 ⁶)	% 4-1BB/OX40 sorted	# cells to start REP (x10 ⁶)	# cells after REP (x10 ⁶)
			TP53-TMG	1.5	19	0.2	0.0788	10
4149	Y220C	CD4	p53-LP	1	14	0.7	0.0127	19.3
			TP53-TMG	1	5.7	0.1	0.0002	7.1
		CD8	p53-LP	1	6.6	0.2	0.0040	45.4
			TP53-TMG	1	7.2	0.1	0.0011	48.4
4266	R248W	CD4	p53-LP	5	0.9	0.1	0.0041	305
			TP53-TMG	7.8	11.5	0.1	0.0351	294
		CD8	p53-LP	0.3	0.5	0.2	0.0010	136.8
			TP53-TMG	0.4	13.3	0.1	0.0123	131.2
4273	R248W	CD4	p53-LP	8.4	1.6	1.1	0.0642	15.1
			TP53-TMG	8.4	3.3	2.2	0.1719	68.6
		CD8	p53-LP	0.4	3.9	6.9	0.0084	66.6
			TP53-TMG	0.8	5.6	2.9	0.0419	44.8

Table 3. T-cell yields after IVS, 4-1BB/OX40 enrichment and rapid expansion in patients with a p53 neoantigen-reactive culture. The numbers of cells to start the IVS depended upon the frequencies of CD4 and CD8 antigen experienced T cells in the PBL. The percentage of 4-1BB/OX40 T cells sorted was based on control gating to eliminate as many non-specific T cells as possible and is likely an underestimate of the percentage of reactive T cells in the population. All enriched cells were placed in a REP.

EXAMPLE 2

[0159] This example demonstrates that *TP53* mutation-reactive T cells were present in PBL of patients with intratumoral TIL responses to p53 neoantigens.

[0160] An analytical screen was performed on the cultures of Example 1 after IVS, 4-1BB/OX40 enrichment and REP where reactivities were evaluated by cell surface marker 4-1BB upregulation via flow cytometry and IFN γ secretion using an ELISPOT assay (Fig. 1A right). Peripheral blood T cells were not reactive to p53 neoantigens in patients 4213, 4217, 4254, and 4257, which corroborated the TIL screening results (Table 2). These patients may not have had an immunogenic combination of HLA and p53 neoepitope. In contrast, *TP53* mutation specific T cells were identified in antigen experienced T cells from PBL of 5 patients (4141 and 4285: p53^{R175H}, 4149: p53^{Y220C}, 4266 and 4273: p53^{R248W}) who had intratumoral T-cell responses to mutated *TP53* by TIL (Figs. 1B-1C and Table 2) (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). *TP53*-TMG-IVS resulted in p53 neoantigen-specific T cells in 4141-CD8, 4285-CD4, 4149-CD4 and 4266-CD8 cultures (Fig. 1B), and p53-LP-IVS resulted in p53 neoantigen-specific T cells in 4285-CD4 and 4273-CD4 cultures (Fig. 1C). The specificity of the responses to mutated *TP53* (Figs. 1B-1C closed shapes) was exemplified by the lack of response to the WT counterpart (Figs. 1B-1C open shapes). The highest frequency of *TP53* mutation-reactive cells was 78% in the 4141-CD8 *TP53*-TMG-IVS culture against p53^{R175H} (Fig. 1D). The 4285-CD4 *TP53*-TMG-IVS culture exemplified a positive IFN γ secretion screening result as T cells were reactive to the mutant TMG-MUT-*TP53* and p53^{R175H} LP but not against WT TMG-MUT-*TP53*, irrelevant TMG, DMSO (peptide vehicle) or WT p53^{R175} LP (Fig. 1E). The p53^{R175H} LP had the amino acid sequence YKQSQHMTEVVRHCPHHERCSDSDG (SEQ ID NO: 111). The WT p53^{R175} LP had the amino acid sequence YKQSQHMTEVVRRCPHHERCSDSDG (SEQ ID NO: 110). Selected cultures were deemed reactive based on 4-1BB upregulation and/or IFN γ secretion and were studied further (Tables 4A-4B). The 4285-CD4 *TP53*-TMG-IVS culture showed specific recognition of the cognate p53^{R175H} LP with peptide concentrations down to 10 ng/mL (Fig. 1F). Thus, through IVS and 4-1BB/OX40 enrichment, highly specific CD8⁺ and CD4⁺ T cells targeting public *TP53* mutations could be identified.

TABLE 4A

				Interferon-gamma ELISPOT						CD8- 4-1BB+					
Patient #	p53 amino acid substitution	T cell population	IVS	TMG #s	TMG FC	cal I	PEP #s	PEP FC	cal I	TMG %	TMG FC	cal I	PEP %	PEP FC	cal I
4141	R175H	CD4	p53-LP	>1000	<2	no	>1000	<2	no	13.6	0.9	no	5.28	1.0	no
4141	R175H	CD4	TP53-TMG	>1000	<2	no	>1000	<2	no	28.3	1.8	no	6.56	0.8	no
4141	R175H	CD8	p53-LP	66	1.8	no	95	0.7	no	19.7	7.1	yes	6.72	1.5	no
4141	R175H	CD8	TP53-TMG	>1000	>69	yes	617	19.3	yes	1.29	5.6	yes	1.94	4.5	Yes
4285	R175H	CD4	p53-LP	>1000	<2	no	>1000	<2	no	24.0	20.5	yes	22.93	5.5	no
4285	R175H	CD4	TP53-TMG	>1000	>5	yes	>1000	>3	yes	37.6	151.2	yes	27.43	19.7	yes
4285	R175H	CD8	p53-LP	0	0.0	no	0	0.0	no	0.72	2.4	no	0.37	1.3	no
4285	R175H	CD8	TP53-TMG	456	2.9	yes	304	1.8	no	0.32	3.9	no	0.2	1.6	no
4149	Y220C	CD4	p53-LP	>1000	<2	no	>1000	<2	no	0.88	1.8	no	0.51	1.5	no
4149	Y220C	CD4	TP53-TMG	794	23.7	yes	0	0.0	no	11.25	18.3	yes	0.15	1.2	no
4149	Y220C	CD8	p53-LP	>1000	<2	no	>1000	<2	no	0.13	1.7	no	0.2	1.6	no
4149	Y220C	CD8	TP53-TMG	0	<2	no	216	<2	no	0.023	2.2	no	0.026	2.4	no
4266	R248W	CD4	p53-LP	570	>2	yes	>1000	<2	no	2.34	2.5	yes	0.78	1.2	no
4266	R248W	CD4	TP53-TMG	390	2.6	yes	>1000	<2	no	1.84	7.1	yes	0.15	1.1	no
4266	R248W	CD8	p53-LP	576	>2	no	>1000	<2	no	0.942	14.9	no	0.34	1.6	no
4266	R248W	CD8	TP53-TMG	668	>3	yes	0	<2	no	1.2	14.3	no	0.46	1.7	no

Patient #	p53 amino acid substitution	T cell population	Interferon-gamma ELISPOT					CD8- 4-1BB+				
			TMG #s	TMG FC	cal I	PEP #s	PEP FC	cal I	TMG %	TMG FC	cal I	PEP %
4273	R248W	CD4	>1000	>2	yes	>1000	<2	yes	9.3	4.2	yes	4.81
4273	R248W	CD4	>1000	>2	no	>1000	<2	no	4.24	5.2	yes	0.91
4273	R248W	CD8	251	2.0	yes	151	1.3	no	0.577	8.1	no	0.24
4273	R248W	CD8	343	4.3	yes	51	1.2	no	1.04	>2	no	0.67

TABLE 4B

Patient #	p53 amino acid substitution	T cell population	IVS	CD8+ 4-1BB+					call	Selected
				TMG %	TMG FC	PEP %	PEP FC	call		
4141	R175H	CD4	p53-LP	0	0.0	0.19	0.8	no	no	no
4141	R175H	CD4	7P53-TMG	0.25	0.3	0.21	1.4	no	no	no
4141	R175H	CD8	p53-LP	3.35	2.7	0.48	1.0	Yes	no	no
4141	R175H	CD8	7P53-TMG	76.3	165.9	1.89	8.2	Yes	Yes	Yes
4285	R175H	CD4	p53-LP	0.14	>2	0.047	2.0	no	no	Yes
4285	R175H	CD4	7P53-TMG	0.26	>2	0	0.0	no	no	Yes
4285	R175H	CD8	p53-LP	0.71	1.8	0.49	1.3	no	no	no
4285	R175H	CD8	7P53-TMG	2.47	16.4	1.59	3.1	yes	yes	no
4149	Y220C	CD4	p53-LP	0	0.0	0	0.0	no	no	no
4149	Y220C	CD4	7P53-TMG	0	0.0	0	0.0	no	0	Yes
4149	Y220C	CD8	p53-LP	0.06	1.2	0.03	1.1	no	no	no
4149	Y220C	CD8	7P53-TMG	0.042	1.5	0.017	1.2	no	no	no
4266	R248W	CD4	p53-LP	0.012	1.3	0	0.0	no	no	no

Patient #	p53 amino acid substitution	T cell population	IVS	CD8+ 4-1BB+						Selected
				TMG %	TMG FC	call	PEP %	PEP FC	call	
4266	R248W	CD4	7P53-TMG	0.013	>2	no	0	0.0	no	no
4266	R248W	CD8	p53-LP	10.92	13.4	yes	0.2	1.0	no	no
4266	R248W	CD8	7P53-TMG	20.85	22.9	yes	0.52	1.1	no	Yes
4273	R248W	CD4	p53-LP	0.03	>2	no	0.0	0.0	no	Yes
4273	R248W	CD4	7P53-TMG	0.01	>2	no	0.0	>2	no	no
4273	R248W	CD8	p53-LP	7.77	6.8	yes	3.00	1.5	no	no
4273	R248W	CD8	7P53-TMG	3.14	29.5	yes	0.9	2.0	no	no

EXAMPLE 3

[0161] This example demonstrates that *TCRB* tracking demonstrated enrichment of p53 neoantigen-reactive T cells from PBL.

[0162] It was desired to further characterize the TCR diversity for each patient's *TP53* mutation reactive T-cell population and determine whether the IVS and 4-1BB/OX40 enrichment altered the T-cell repertoire. To accomplish this, *TCRB* deep sequencing (Pasetto et al., *Cancer Immunol. Res.*, 4(9):734-43 (2016)) was performed and productive T cell clonotype frequencies were measured based on unique CDR3B sequences and overall sample clonality, which is a normalized measurement of the population diversity where more oligoclonal samples approach 1 (Boyd et al., *Sci. Transl. Med.*, 1(12):12ra23) (2009); Howie et al., *Sci. Transl. Med.*, 7(301):301ra131 (2015)). *TCRB* clonality significantly increased in *TP53*-TMG-IVS and p53-LP-IVS cultures generated from all patient PBL samples relative to the pre-IVS PBL (Fig. 2A). Similarly, the most frequent unique *TCRB* clonotype in each *TP53*-TMG-IVS and p53-LP-IVS sample was of higher frequency than in the pre-IVS PBL (Fig. 2B). The ranking of p53 neoantigen-reactive *TCRB* sequences ranged from 1-167 in the final p53-LP-IVS or *TP53*-TMG-IVS cultures but were not detected or rank 5,020 in the original PBL (Table 5). The increased clonality or maximum *TCRB* clonotype frequencies were not restricted to cultures with T-cell responses to p53 neoantigens (Figs. 2A-2B; asterisks denoted positive cultures). This suggested that there was T-cell repertoire skewing through the IVS and 4-1BB/OX40 enrichment but assessments of top frequency and clonality were not sufficient to predict a culture's response to mutated *TP53*.

TABLE 5

Patient #	p53 amino acid substitution	CDR3B	pre-IVS PBL		post-IVS, 4-1BB/OX40 enrichment and REP	
			Rank	%	Rank	%
4141	R175H	CASSIQQGADTQYF (SEQ ID NO: 122)	n/a	< 0.001	1	71.553
4285	R175H	CASGLVGFNQPQHF (SEQ ID NO: 8)	n/a	< 0.001	3	10.861
		CASLQFNEQFF (SEQ ID NO: 19)	n/a	< 0.001	9	2.317

Patient #	p53 amino acid substitution	CDR3B	pre-IVS PBL		post-IVS, 4-1BB/OX40 enrichment and REP	
			Rank	%	Rank	%
		CASSEYQSQSNEQFF (SEQ ID NO: 30)	n/a	< 0.001	1	29.844
		CASSINRYTEAFF (SEQ ID NO: 123)	n/a	< 0.001	6	4.944
		CASSIRTEAFF (SEQ ID NO: 41)	n/a	< 0.001	15	0.941
		CASSPFVIGQINEQYF (SEQ ID NO: 52)	n/a	< 0.001	34	0.223
		CASSYAGLAAPREQFF (SEQ ID NO: 63)	n/a	< 0.001	10	1.676
		CASSYPSMDRDRYGYTF (SEQ ID NO: 124)	n/a	< 0.001	72	0.048
		CATRTGNEAFF (SEQ ID NO: 74)	n/a	< 0.001	23	0.435
		CASNLGGGSTDTQYF (SEQ ID NO: 125)	n/a	< 0.001	3	7.450
4266	R248W	CASSFGSGSTDTQYF (SEQ ID NO: 126)	n/a	< 0.001	11	2.578
		CASSSRDYEYF (SEQ ID NO: 127)	5020	0.002	167	0.017

Table 5. TCRB tracking of p53 neoantigen-reactive cells before and after IVS, co-culture, 4-1BB/OX40 sorting and REP. Adaptive Biotechnologies TCRB survey sequencing was performed with at least 50,000 cells.

EXAMPLE 4

[0163] This example demonstrates the isolation of p53 neoantigen-reactive TCRs and tracking by *TCRB* sequencing.

[0164] TCRs were then identified from the p53 neoantigen-reactive IVS populations for potential therapeutic and research use and to track the *TCRB* clonotypes during the culture period to evaluate the extent of *TP53* mutation-reactive T-cell enrichment. The TCRs were

identified following a co-culture of reactive IVS cultures with the cognate p53 neoantigen (TMG or LP), sorting 4-1BB⁺ T cells and single-cell RT-PCR of TCR alpha and beta genes, similar to previous studies (Pasetto et al., *Cancer Immunol. Res.*, 4(9):734-43 (2016)). TCR pairs were reconstructed, cloned into retroviral vectors and transduced into donor PBL. The second amino acid residue was changed to alanine (A) to have a stronger kozak sequence for highly efficient translation. The TCR α chain constant regions were replaced with a cysteine-substituted, LVL-modified murine α chain constant region. The TCR β chain constant regions were replaced with a cysteine-substituted murine β chain constant region.

[0165] A total of 11 TCRs targeting p53^{R175H} (patients 4141 and 4285) and p53^{R248W} (patients 4266 and 4273) neoantigen were identified (Table 5). The TCR for patient 4149 was unable to be determined due to limited availability of T cells. The same p53 neoantigen-reactive TCRs from TIL (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)) were identified in PBL after IVS and 4-1BB/OX40 enrichment in patients 4141, 4266 and 4273 targeting p53^{R175H}/HLA-A*02:01, p53^{R248W}/HLA-A*68:01 and p53^{R248W}/HLA-DPB1*02:01, respectively. No additional *TP53* mutation-reactive T cells were identified in these patients. In contrast, 7 unique p53^{R175H} neoantigen-specific TCRs from patient 4285 were identified from PBL, which were not present in the TIL study, and the TCR derived from TIL was not found in the PBL populations. The functional avidities of the PBL-derived TCRs (4285-PBL-TCR) were comparable to the TIL-derived TCR (4285-TIL-TCR) with recognition of p53^{R175H} LP to 10 ng/mL and no response to the WT p53^{R175} LP (Figs. 2C-2J). Tracking of p53^{R175H} neoantigen-specific *TCRB* clonotypes from patients 4141 and 4285 demonstrated exponential expansion after IVS and 4-1BB/OX40 enrichment compared to the starting PBL (Fig. 2K). Moreover, the CDR3B sequences from patient 4285 were below the limit of detection (<0.001% from 2x10⁵ reads) in bulk PBL but were of sufficient frequencies following IVS and 4-1BB/OX40 enrichment, including four TCRs in the top 10 total CDR3B clonotypes (Table 5). The p53^{R248W} neoantigen-specific TCRs from patient 4266 were also below the limit of detection in the PBL but were 2.6% (4266-PBL-TCR3) and 7.5% (4266-PBL-TCR2) in the 4266-CD8 *TP53*-TMG-IVS culture (Fig. 2L). The TCR with specificity to p53^{R248W} neoantigen enriched from 0.002% to 0.017% in the 4273-CD4 p53-LP-IVS culture (Fig. 2L). Collectively, the data demonstrated that PBL can be source of public *TP53* mutation-reactive TCRs identical or comparable to intratumoral TCRs.

EXAMPLE 5

[0166] This example demonstrated that the common HLA restriction elements and p53 neoepitopes were immunogenic.

[0167] The recognized minimal p53 neoepitopes and corresponding HLA restrictions were then assessed. HLA mapping was accomplished by transfecting DNA plasmids corresponding to each of the patient's individual HLA alleles into COS7 monkey cell line (lacking HLA) and pulsing peptides or co-transfecting with TMG, similar to previous reports (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019); Deniger et al., *Clin. Cancer Res.*, 24(22):5562-73 (2018)). The 4141-CD8 TP53-TMG-IVS culture was specific for the p53^{R175H} HMTEVVRHC (SEQ ID NO: 96) neoepitope in the context of HLA-A*02:01, a highly frequent HLA in the USA population (Gonzales et al., *Hisp. Health Care Int.*, 15(4):180-8 (2017)), as measured by 4-1BB expression (Fig. 3A). HLA-A*68:01 restricted the p53^{R248W} neoepitope SSCMGGMNWR (SEQ ID NO: 98) recognized by the CD8 TP53-TMG-IVS culture as measured by IFN γ secretion (Fig. 3B). This was expected as the TCRs from TIL in patients 4141 and 4266 were found in these IVS cultures and had already established minimal neoepitopes and HLA restriction elements (Malekzadeh et al., *J. Clin. Invest.* 129(3):1109-14 (2019)). Similarly, the 4273-PBL-TCR was found in the 4273-CD4 p53-LP-IVS culture and had a known p53^{R248W} and HLA-DPB1*02:01 combination from the TIL studies. Even though the TCRs were different between PBL and TIL for patient 4285, the 4285-CD4 TP53-TMG-IVS culture was specific for the same p53^{R175H} and HLA-DBR1*13:01 combination found in the TIL (Fig. 3C). Furthermore, all 4285-PBL-TCRs were specific for p53^{R175H} and HLA-DBR1*13:01 and were all present in the 4285-CD4 TP53-TMG-IVS culture by *TCRB* sequencing (Table 5; Figure 4). Fifteen amino acid p53^{R175H} peptides overlapping 14 amino acids were pulsed on DCs from patient 4285 and co-cultured with TCR transduced T cells, and the core peptide EVVRHCPHHER (SEQ ID NO: 2) was determined to be the common sequence recognized by 4285-PBL-TCRs in the context of HLA-DRB1*13:01 (Table 6). In sum, the same TCRs from TIL in PBL that recognized the same HLA and minimal p53 neoepitopes were found in three cases and in one case additional TCRs with the same p53 neoantigen specificity as the intratumoral T cells were found.

TABLE 6

peptide	UT	4285- PBL- TCR1	4285- PBL- TCR2	4285- PBL- TCR3	4285- PBL- TCR5	4285- PBL- TCR6	4285- PBL- TCR7	4285- PBL- TCR9
YKQSQHMTTEVVRHCP (SEQ ID NO: 99)	-	-	-	-	-	-	-	-
KQSQHMTTEVVRHCPH (SEQ ID NO: 100)	-	-	-	+	-	-	-	+
QSQHMTTEVVRHCPHH (SEQ ID NO: 101)	-	-	++	+	++	-	-	+
SQHMTTEVVRHCPHHE (SEQ ID NO: 102)		-	++	++	++	-	-	++
QHMTTEVVRHCPHHER (SEQ ID NO: 103)	-	+	++	++	++	+	+	++
HMTEVVRHCPHHERC (SEQ ID NO: 104)	-	++	++	++	++	++	++	++
MTEVVRHCPHHERCS (SEQ ID NO: 105)	-	++	++	++	++	++	++	++
TEVVRHCPHHERCSD (SEQ ID NO: 106)	-	++	++	++	++	++	++	++
EVVRHCPHHERCSDS (SEQ ID NO: 107)	-	++	++	++	++	++	++	++
VVRHCPHHERCSDSD (SEQ ID NO: 108)	-	++	-	+	-	++	++	-
VRHCPHHERCSDSDG (SEQ ID NO: 109)	-	++	-	-	-	+	++	-
Vehicle (DMSO)	-	-	-	-	-	-	-	-

EXAMPLE 6

[0168] This example demonstrates that tumor cells process and present p53 neoepitopes on HLA which are recognized by PBL-derived T cells following IVS.

[0169] *TP53* mutation reactive T cells were evaluated for the capacity to recognize naturally processed and presented antigen expressed on the tumor cell surface. Saos2-R175H osteosarcoma tumor cell line (HLA-A*02:01 and overexpressing full length p53^{R175H}) and TC#4266 human xenograft tumor cell line (p53^{R248W} and HLA-A*68:01:02 colon cancer) were used as models. Following overnight co-culture, CD8⁺ T cells from the 4141-CD8

TP53-TMG-IVS and 4266-CD8 *TP53*-TMG-IVS cultures upregulated 4-1BB in response to Saos2-R175H and TC#4266 cell lines, respectively, with minimal activation the cross-matched cell line (Fig. 3D). Thus, *TP53* mutation-specific T cells from PBL could recognize tumor cells with naturally processed and presented p53 neoepitopes.

EXAMPLE 7

[0170] This example demonstrates the amino acid sequences of the TCRs constructed in Example 4 and named in the header of Table 6. The amino acid sequences of the TCR alpha and beta chain variable regions of these TCRs are shown in Table 7. The CDRs are underlined.

TABLE 7

TCR Name	TCR chain	Amino acid sequence
4285-PBL-TCR1	Alpha chain variable region (with N-terminal signal peptide)	MHLVTSITVLLSLGIMGDAKTTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSSSTLILHRATLRDAAVYYCILRDNNARLMFGDGTQLVVKP (SEQ ID NO: 9)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MKLVTSITVLLSLGIMGDAKTTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSSSTLILHRATLRDAAVYYCILRDNNARLMFGDGTQLVVKP (SEQ ID NO: 178)
	Beta chain variable region (with N-terminal signal peptide)	MAPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSKEKRNFPLILESPSPNQTSLYFCASGLVGFNQPHFGDGTLSIL (SEQ ID NO: 10)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSKEKRNFPLILESPSPNQTSLYFCASGLVGFNQPHFGDGTLSIL (SEQ ID NO: 205)
	Alpha chain variable region (predicted sequence without	KTQPNSMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNNRMASLAIAEDRKSSSTLILHRATLRDAAVYYCILRDNNARLMFGDGTQLVVKP (SEQ ID NO: 142)

TCR Name	TCR chain	Amino acid sequence
	N-terminal signal peptide)	
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	QVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYK VSRKEKRNFLILESPSPNQTSLYFCASGLV GFNQPOHFGDGTLSIL (SEQ ID NO: 143)
4285-PBL-TCR2	Alpha chain variable region (with N-terminal signal peptide)	MHLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNE YVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSSTLILPHATL RDTAVYYCIVRARANAGGTSYGKLTFGQGTLTVHP (SEQ ID NO: 20)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MRLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNE YVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSSTLILPHATL RDTAVYYCIVRARANAGGTSYGKLTFGQGTLTVHP (SEQ ID NO: 181)
	Beta chain variable region (with N-terminal signal peptide)	MAMSNQVLCCVVL CFLGANTVDGGITQSPKYLFRKEGQNVTLSC EQ NLNHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKK ESFPLTVTSAQKNPTAFYLCASLOFNEQFFGPGTRLTVL (SEQ ID NO: 21)
	Beta chain variable region (with N-terminal signal peptide)	MANQVLCCVVL CFLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNL NHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKES FPLTVTSAQKNPTAFYLCASLOFNEQFFGPGTRLTVL (SEQ ID NO: 226)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MSNQVLCCVVL CFLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNL NHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKES FPLTVTSAQKNPTAFYLCASLOFNEQFFGPGTRLTVL (SEQ ID NO: 207)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	KTTPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIH GLKNNETNEMASLIITEDRKSSTLILPHATLRDTAVYYCIVRARANAG GTSYGKLTFGQGTLTVHP (SEQ ID NO: 144)

TCR Name	TCR chain	Amino acid sequence
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	GITQSPKYLFRKEGQNVTLSC EQNLNHDAMYWYRQDPGQGLRLIYY SQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASLQF NEOFFGPGTRLTVL (SEQ ID NO: 145)
4285-PBL- TCR3	Alpha chain variable region (with N-terminal signal peptide)	MHLQSTLGAVWLGLLLNSLWKVAESKDQVFQPSTVASSEGAVVEIF CNHSVSNAYNFFWYLHFGCAPRLLVKGSKPSQQGRYNMTYERFSSS LLILQVREADAAVYYCAVEDRRRTALIFGKGTTLSVSS (SEQ ID NO: 31)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MALQSTLGAVWLGLLLNSLWKVAESKDQVFQPSTVASSEGAVVEIF CNHSVSNAYNFFWYLHFGCAPRLLVKGSKPSQQGRYNMTYERFSSS LLILQVREADAAVYYCAVEDRRRTALIFGKGTTLSVSS (SEQ ID NO: 184)
	Beta chain variable region (with N-terminal signal peptide)	MATWLCWAIFSLKAGLTEPEVTQTPSHQVTQMGEVILRCVPI SN HLYFYWYRQILGQKVEFLVSFYNNEISEKSEIFDDQFSVERPDGSNFT LKIRSTKLEDSAMYFCASSEYQSOSNEOFFGPGTRLTVL (SEQ ID NO: 32)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MDTWLCWAIFSLKAGLTEPEVTQTPSHQVTQMGEVILRCVPI SN HLYFYWYRQILGQKVEFLVSFYNNEISEKSEIFDDQFSVERPDGSNFT LKIRSTKLEDSAMYFCASSEYQSOSNEOFFGPGTRLTVL (SEQ ID NO: 209)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	KDQVFQPSTVASSEGAVVEIFCNHSVSNAYNFFWYLHFGCAPRLLV KGSKPSQQGRYNMTYERFSSSLLILQVREADAAVYYCAVEDRRRTAL IFGKGTTLSVSS (SEQ ID NO:146)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	EPEVTQTPSHQVTQMGEVILRCVPI SNHLYFYWYRQILGQKVEFLVS FYNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSEY QSOSNEOFFGPGTRLTVL (SEQ ID NO: 147)

TCR Name	TCR chain	Amino acid sequence
4285-PBL-TCR5	Alpha chain variable region (with N-terminal signal peptide)	MHLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSSSTLILPHATLRDTAVYYCIVRGSPGAGGTSYGKLTFGQGTILTVHP (SEQ ID NO: 42)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MRLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSSSTLILPHATLRDTAVYYCIVRGSPGAGGTSYGKLTFGQGTILTVHP (SEQ ID NO: 187)
	Beta chain variable region (with N-terminal signal peptide)	MANQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSCQNLSNHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSIRTEAFFGQGTRLTVV (SEQ ID NO: 43)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MSNQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSCQNLSNHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSIRTEAFFGQGTRLTVV (SEQ ID NO: 211)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	KTTPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSSSTLILPHATLRDTAVYYCIVRGSPGAGGTSYGKLTFGQGTILTVHP (SEQ ID NO: 148)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	GITQSPKYLFRKEGQNVTLSCQNLSNHDAMYWYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSIRTEAFFGQGTRLTVV (SEQ ID NO: 149)
	Alpha chain variable region (with N-terminal signal peptide)	MHLITSMVLWMQLSQVNGQQVMQIPQYQHVGEGEDFTTYCNSSTTLSNIQWYKQRPGGHPVFLIQLVKSGEVKKQKRLTFQFGEAKKNSSLHITATQTTDVGTIFYCAGPGGAGSYQLTFGKGTKLSVIP (SEQ ID NO: 53)
	Alpha chain variable region	MLLITSMVLWMQLSQVNGQQVMQIPQYQHVGEGEDFTTYCNSSTTLSNIQWYKQRPGGHPVFLIQLVKSGEVKKQKRLTFQFGEAKKNSSLH

TCR Name	TCR chain	Amino acid sequence
4285-PBL-TCR6	(with wild-type N-terminal signal peptide)	ITATQTTDVGTYFCAGPGGAGSYQLTFGKGTKLSVIP (SEQ ID NO: 190)
	Beta chain variable region (with N-terminal signal peptide)	MAPQLLGYYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNM NHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYK VSRKEKR NFPLILESPSPNQTSLYFCASSPFVVIGQINEQYFGPGTRLTVT (SEQ ID NO: 54)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MGPQLLGYYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNM NHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYK VSRKEKR NFPLILESPSPNQTSLYFCASSPFVVIGQINEQYFGPGTRLTVT (SEQ ID NO: 213)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	QQVMQIPQYQHVQEGEDFTTYCNSSTLTSNIQWYKQRPGGHPVFLIQ LVKSGEVKKQKRLTFQFGEAKKNSSLHITATQTTDVGTYFCAGPGGA GSYQLTFGKGTKLSVIP (SEQ ID NO: 150)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	QVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYK VSRKEKRNFP LILESPSPNQTSLYFCASSPF VVIGQINEQYFGPGTRLTVT (SEQ ID NO: 151)
4285-PBL-TCR7	Alpha chain variable region (with N-terminal signal peptide)	MHKLLAMILWLQDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSSTS DRLYWYRQDPGKSLESFLVLLSNGAVKQEGRLMASLDTKARLSTLHI TAAVHDL SATYFC AVAHMDSNYQLIWGAGTKLIKP (SEQ ID NO: 64)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MKKLLAMILWLQDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSSTS DRLYWYRQDPGKSLESFLVLLSNGAVKQEGRLMASLDTKARLSTLHI TAAVHDL SATYFC AVAHMDSNYQLIWGAGTKLIKP (SEQ ID NO: 193)
	Beta chain variable region (with N-terminal signal peptide)	MALGLLCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSMTLCAQD MNHEYMYWYRQDPGMGLRLIHYSVGEGTTAKGEVPDGYNVSRLLK QNFLGLESAAPSQTSVYFCASSYAGLAAPREQFFGPGTRLTVL (SEQ ID NO: 65)

TCR Name	TCR chain	Amino acid sequence
	Beta chain variable region (with wild-type N-terminal signal peptide)	MSLGLLCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSM TLLCAQD <u>MNHEYMYWYRQDPGMGLRLIHYSVGEGTTAKGEVPDGYNVSRLLKQNFLLGLESAAPSQTSVYFCASSYAGLAAPREQFFGPGTRLT</u> TVL (SEQ ID NO: 215)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	ELKVEQNPLFLSMQEGKNYTIYCNYSTTSDRLYWYRQDPGKSLES LF <u>VLLSNGAVKQEGRLMASLDTKARLSTLHITAAVHDL SATYFC</u> AVAH <u>MDSNYQLIWGAGTKLIKP</u> (SEQ ID NO: 152)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	GVTQTPKFRVLKTGQSM TLLCAQD <u>MNHEYMYWYRQDPGMGLRLIHYSVGEGTTAKGEVPDGYNVSRLLKQNFLLGLESAAPSQTSVYFCASSYAGLAAPREQFFGPGTRLT</u> TVL (SEQ ID NO: 153)
4285-PBL-TCR9	Alpha chain variable region (with N-terminal signal peptide)	MHLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNE <u>YVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSS TLILPHATL</u> RDTAVYY <u>CIVRARANAGGTSYGKLTFGQGTILTVHP</u> (SEQ ID NO: 75)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MRLVARVTVFLTFGTIIDAKTTQPPSMDCAEGRAANLPCNHSTISGNE <u>YVYWYRQIHSQGPQYIIHGLKNNETNEMASLIITEDRKSS TLILPHATL</u> RDTAVYY <u>CIVRARANAGGTSYGKLTFGQGTILTVHP</u> (SEQ ID NO: 196)
	Beta chain variable region (with N-terminal signal peptide)	MANQVLCCVVL CFLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNL <u>NHD</u> AMYWYRQDPGQGLRLIYY <u>SQIVNDFQKGDIAEGYSVSREKKES</u> FPLTVTSAQKNPTAFYLCATRTGNEAFFGQGTRLTVV (SEQ ID NO: 76)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MSNQVLCCVVL CFLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNL <u>NHD</u> AMYWYRQDPGQGLRLIYY <u>SQIVNDFQKGDIAEGYSVSREKKES</u> FPLTVTSAQKNPTAFYLCATRTGNEAFFGQGTRLTVV (SEQ ID NO: 217)

TCR Name	TCR chain	Amino acid sequence
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	KTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIH <u>GLKNN</u> ETNEMASLIITEDRKSSSTLILPHATLRDTAVVYVCIVRARANAG <u>GTSYGKLT</u> FGQGTILTVHP (SEQ ID NO:154)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	GITQSPKYLFRKEGQNVTLSCQNLNHDAMYWYRQDPGQGLRLIYY <u>SQIVNDFQKGDIAEGYSVSREKESFPLTVTSAQKNPTAFYLCATRTG</u> <u>NEAFFGQ</u> GTRLTVV (SEQ ID NO: 155)

[0171] TCR name: 4285-PBL-TCR1

MAPQLLGYYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYR
QDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSREKERNFPLILESPSPNQTSLYFCAS
GLVGFNQPQHFGDGTRL *SILEDLRNVTPPKVSLFEP*SKAEIANKQKATLVCLARGFFPDH
*VELSWWWNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFR*QVQFHGL
SEEDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTL
*VVMAMVKKRKN***RAKRSGGATNFSLLKQAGDVEENPGPMHLVTSITVLLSLGIMGD**
AKTTQPNMESNEEEPVHLPCNHSTISGTDYIHWYRQLPSQGPEYVIHGLTSNVNRR
MASLAIAEDRKSSSTLILHRATLRDAAVYYCILRDNNARLMFGDGTQLVVKPNIQNPE
*PAVYQLKDP*RSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMD~~SKSNGA~~IAWS
*NQTSFTCQDIFKET*NATYPSSDVPCDATLTEKSFETDMNLFQNLVIVLRILLKLVAGFNL
LMTLRWSS (SEQ ID NO: 13)

[0172] The sequence of TCR **4285-PBL-TCR1**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 6), the second underlined region is the CDR2beta (SEQ ID NO: 7), the third underlined region is the CDR3beta (SEQ ID NO: 8), the fourth underlined region is the CDR1alpha (SEQ ID NO: 3), the fifth underlined region is the CDR2alpha (SEQ ID NO: 4), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 5). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is

the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 10) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 9) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 12) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 11) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0173] A variant of 4285-PBL-TCR1 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 180. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 178. The full-length alpha chain of the variant is set forth in SEQ ID NO: 179.

[0174] Another variant of 4285-PBL-TCR1 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 205. The full-length beta chain of the variant is set forth in SEQ ID NO: 206.

[0175] The predicted 4285-PBL-TCR1 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 142. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 160. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 143. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 161.

[0176] TCR name: 4285-PBL-TCR2

MAMSNQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSC~~EQNLNHD~~AMYW
YRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLC
SLQFNEQFFGPGTRLTVLEDLRNVTPPKVSLFEP~~SKAEIANKQKATLVCLARGFFPDHVE~~
~~LSWWVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSE~~
~~EDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTLV~~
~~VMAMVKKRKN~~**RAKRSGSGATNFSLLKQAGDVEENPGPMHLVARVTVFLTFGTIDA**
KTTQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEM

ASLIITEDRKSSTLILPHATLRDTAVYYCIVRARANAGGTSYGKLTFGQGTLTVHPM/
 QNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMDSSKNGA
 LAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQNLLVIVLRILLKVA
 GFNLLMTLRLWSS (SEQ ID NO: 24)

[0177] The sequence of TCR **4285-PBL-TCR2**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 17), the second underlined region is the CDR2beta (SEQ ID NO: 18), the third underlined region is the CDR3beta (SEQ ID NO: 19), the fourth underlined region is the CDR1alpha (SEQ ID NO: 14), the fifth underlined region is the CDR2alpha (SEQ ID NO: 15), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 16). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 21) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 20) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 23) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 22) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0178] A variant of 4285-PBL-TCR2 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 183. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 181. The full-length alpha chain of the variant is set forth in SEQ ID NO: 182.

[0179] Another variant of 4285-PBL-TCR2 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 207. The full-length beta chain of the variant is set forth in SEQ ID NO: 208.

[0180] The predicted 4285-PBL-TCR2 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 144. The predicted full-length alpha chain (inclusive of the alpha chain

variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 162. The predicted beta chain variable region without the N-terminal signal peptides is shown in SEQ ID NO: 145. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 163.

[0181] TCR name: 4285-PBL-TCR3

MATWLVCAWAFSLLKAGLTEPEVTQTPSHQVTQMGQEVILRCVPISNHLYFYWYRQI
 LGQKVEFLVSFYNNISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSEY
QSQSNEQFFGPGTRLTTVLEDLRNVTPPKVSLFEPSCAEIANKQKATLVCLARGFFPDHVE
 LSWWWNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSE
 EDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTLV
 VMAMVKRKNS**RAKRSGSGATNFSLLKQAGDVEENPGPMHLQSTLGAVWLGLLN**
 SLWKVAESKDQVFQPS TVASSEGAVVEIFCNHVSSNAYNFFWYLHFPGCAPRLLVK
GSKPSQQGRYNMTYERFSSLLILQVREADAAVYYCAVEDRRRTALIFGKGTTLSVS
 SNIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMDSKS
 NGALAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLFQNLVIVLRILL
 KVAGFNLLMTLRLWSS (SEQ ID NO: 35)

[0182] The sequence of TCR **4285-PBL-TCR3**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 28), the second underlined region is the CDR2beta (SEQ ID NO: 29), the third underlined region is the CDR3beta (SEQ ID NO: 30), the fourth underlined region is the CDR1alpha (SEQ ID NO: 25), the fifth underlined region is the CDR2alpha (SEQ ID NO: 26), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 27). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 32) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 31) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 34) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 33)

includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0183] A variant of 4285-PBL-TCR3 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 186. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 184. The full-length alpha chain of the variant is set forth in SEQ ID NO: 185.

[0184] Another variant of 4285-PBL-TCR3 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 209. The full-length beta chain of the variant is set forth in SEQ ID NO: 210.

[0185] The predicted 4285-PBL-TCR3 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 146. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 164. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 147. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 165.

[0186] TCR name: 4285-PBL-TCR5

MANQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSCNQNLNHDAMYWYR
 QDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASSI
RTEAFFGQGTRLTVVEDLRNVTTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHVELSW
 WVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFHGLSEEDK
 WPEGSPKPVTQNISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTLVVMA
 MVKRKNS**RAKRSGSGATNFSLLKQAGDVEENPGPMHLVARVTVFLTFGTIIDAKTT**
 QPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEMASL
 IITEDRKSSTLILPHATLRDTAVYYCIVRGSPGAGGTSYGKLTFGQGTLTVHPNIQNPE
 PAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMDSKSNALAWS
 NQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLNFQNLVIVLRILLKLVAGFNL
 LMTLRLWSS (SEQ ID NO: 46)

[0187] The sequence of TCR **4285-PBL-TCR5**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is

the CDR1beta (SEQ ID NO: 39), the second underlined region is the CDR2beta (SEQ ID NO: 40), the third underlined region is the CDR3beta (SEQ ID NO: 41), the fourth underlined region is the CDR1alpha (SEQ ID NO: 36), the fifth underlined region is the CDR2alpha (SEQ ID NO: 37), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 38). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 43) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 42) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 45) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 44) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0188] A variant of 4285-PBL-TCR5 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 189. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 187. The full-length alpha chain of the variant is set forth in SEQ ID NO: 188.

[0189] Another variant of 4285-PBL-TCR5 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 211. The full-length beta chain of the variant is set forth in SEQ ID NO: 212.

[0190] The predicted 4285-PBL-TCR5 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 148. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 166. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 149. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 167.

[0191] TCR name: 4285-PBL-TCR6

MAPQLLGYYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMMNHEYMSWYR
 QDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASS
PFVVIGQINEQYFGPGTRLTVTEDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFP
 DHVELSWVWNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQFH
 GLSEEDKWPEGSPKPVTQNISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLV
 STLVVMMAMVKRKNS**RAKRSGSGATNFSLLKQAGDVEENPGPM**HLITSMLVLWMQL
 SQVNGQQVMQIPQYQHVEGEDFTTYCNSSTLSNIQWYKQRPGGHPVFLIQLVKSG
EVKKQKRLTFQFGEAKKNSSLHITATQTTDVGTYFCAGPGGAGSYQLTFGKGTKLSV
 IPNIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMDSKS
 NGAIAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLFQNLLVIVLRILL
 KVAGFNLLMTLRLWSS (SEQ ID NO: 57)

[0192] The sequence of TCR **4285-PBL-TCR6**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 50), the second underlined region is the CDR2beta (SEQ ID NO: 51), the third underlined region is the CDR3beta (SEQ ID NO: 52), the fourth underlined region is the CDR1alpha (SEQ ID NO: 47), the fifth underlined region is the CDR2alpha (SEQ ID NO: 48), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 49). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 54) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 53) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 56) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 55) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0193] A variant of 4285-PBL-TCR6 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 192. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 190. The full-length alpha chain of the variant is set forth in SEQ ID NO: 191.

[0194] Another variant of 4285-PBL-TCR6 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 213. The full-length beta chain of the variant is set forth in SEQ ID NO: 214.

[0195] The predicted 4285-PBL-TCR6 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 150. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 168. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 151. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 169.

[0196] **TCR name: 4285-PBL-TCR7**

MALGLGCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSM TLLCAQDMNHEYMYWY
 RQDPGMGLRLIHYSVGE GTTAKGEVPDGYNVSRLLKQNFLLGLESAAPSQTSVYFC
ASSYAGLAAPREQFFGPGTRLTVLEDLRNVTPPKVSLFEP SKAEIANKQKATLVCLARGF
FPDHVELSWVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFRCQVQ
FHGLSEEDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAV
LVSTLVMMAMVKRKNSRAKRSGSGATNFSLLKQAGDVEENPGPMHKLLAMILWLQ
 LDRLSGELKVEQNPLFLSMQEGKNYTIYCNYSTTSDRLYWYRQDPGKSLESFLVLLS
NGAVKQEGRLMASLDTKARLSTLHITAAVHDLSATYFC CAVAHMDSNYQLIWGAGT
 KLIHKPNIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAM
 DSKSNGALAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLFQNLLVIVLR
 ILLKVGAFNLLMTLRLWSS (SEQ ID NO: 68)

[0197] The sequence of TCR **4285-PBL-TCR7**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 61), the second underlined region is the CDR2beta (SEQ ID NO: 62), the third underlined region is the CDR3beta (SEQ ID NO: 63), the fourth underlined region is the CDR1alpha (SEQ ID NO: 58), the fifth underlined region is the CDR2alpha (SEQ ID NO: 59), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 60). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second

italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 65) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 64) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 67) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 66) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0198] A variant of 4285-PBL-TCR7 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 195. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 193. The full-length alpha chain of the variant is set forth in SEQ ID NO: 194.

[0199] Another variant of 4285-PBL-TCR7 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 215. The full-length beta chain of the variant is set forth in SEQ ID NO: 216.

[0200] The predicted 4285-PBL-TCR7 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 152. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 170. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 153. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 171.

[0201] TCR name: 4285-PBL-TCR9

MANQVLCCVVLCLGANTVDGGITQSPKYLFRKEGQNVTLSC EQNLNHDAMYWYR
QDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCATR
TGNEAFFGQGTRLTVVEDLRNVTPPKVSLFEP SKAEIANKQKATLVCLARGFFPDHVELS
WWVNGKEVHSGVCTDPQAYKESNYSYCLSSRLRV SATFWHNP RNHFRCQVQFHGLSEED
KWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTLVVM
AMVKRKNSRAKRSGSGATNFSLLKQAGDVEENPGPMHLVARVTVFLTFGTIIDAKT
TQPPSMDCAEGRAANLPCNHSTISGNEYVYWYRQIHSQGPQYIIHGLKNNETNEMAS

LIITEDRKSSTLILPHATLRDTAVYYCIVRARANAGGTSYGKLTFGQGTILTVHPNIQN
 PEPAYYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKAMDSKSNAGA
 WSNQTSFTCQDIFKETNATYPSSDVPCDATLTEKSFETDMNLFQNLLVIVLRILLKLVAGF
 NLLMTLRLWSS (SEQ ID NO: 79)

[0202] The sequence of TCR **4285-PBL-TCR9**, which was isolated from Patient 4285, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 72), the second underlined region is the CDR2beta (SEQ ID NO: 73), the third underlined region is the CDR3beta (SEQ ID NO: 74), the fourth underlined region is the CDR1alpha (SEQ ID NO: 69), the fifth underlined region is the CDR2alpha (SEQ ID NO: 70), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 71). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 76) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 75) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 78) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 77) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0203] A variant of 4285-PBL-TCR9 comprising a wild-type alpha chain signal peptide is set forth in SEQ ID NO: 198. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 196. The full-length alpha chain of the variant is set forth in SEQ ID NO: 197.

[0204] Another variant of 4285-PBL-TCR9 comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 217. The full-length beta chain of the variant is set forth in SEQ ID NO: 218.

[0205] The predicted 4285-PBL-TCR9 alpha and beta chain variable region mature sequences without N-terminal signal peptides are shown in Table 7. The predicted alpha chain variable region for the mature sequence without the N-terminal signal peptide is shown in SEQ ID NO: 154. The predicted full-length alpha chain (inclusive of the alpha chain

variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 172. The predicted beta chain variable region without the N-terminal signal peptide is shown in SEQ ID NO: 155. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 173.

EXAMPLE 8

[0206] This example demonstrates the frequencies of CD8⁺4-1BB⁺ T cells detected following co-culture of T cells from a tumor sample from Patient 4259 with mutated p53-Y220C peptide.

[0207] T-cell clones were prepared by limiting dilution after sorting CD8⁺ T cells from 4259-F1 tumor fragment culture. The 24 cultures were co-cultured with T2 tumor cells (HLA-A*02:01) pulsed with DMSO (peptide vehicle), WT p53-Y220 peptide (VVPYEPPEV) (SEQ ID NO: 112) or MUT p53-Y220C peptide (VVPCEPPEV) (SEQ ID NO: 113). After overnight incubation, the cells were stained for CD3, CD8 and 4-1BB then analyzed by flow cytometry. The frequencies of CD8⁺4-1BB⁺ T cells from the cultures are shown in Figure 5. As shown in Figure 5, reactive T-cell clones were obtained.

EXAMPLE 9

[0208] This example demonstrates the isolation of the p53-Y220C neoantigen-reactive T-cell receptor 4259-F1-TCR.

[0209] The sequence of the TCR with specificity to p53-Y220C and HLA-A*02:01 (from the fragment culture F1 of patient 4259 of Example 8) was difficult to determine. The sequence was unable to be determined using the classical single cell PCR technique. Two limitations of the single cell PCR technique are that (1) it only sequences a short part of the TCR gene surrounding the hypervariable CDR3 region leading the investigator to infer the rest of the TCR using less polymorphic regions of the TCR, e.g., the variable family, and (2) only one sequence can be present to have a functional sequencing readout by Sanger sequencing method. These issues were circumvented by making T-cell clones by limiting dilution and then sequencing the full length TCRalpha and TCRbeta genes as expressed mRNA transcripts by 5'RACE. It was then determined that the T-cell clonotype with specificity for p53-Y220C and HLA-A*02:01 expressed two TCRalpha and two TCRbeta chains. This is in contrast with most T cells which only express one TCRalpha and one TCRbeta. Only one of the TCRbeta chains was functional because the other one had a

frameshift that resulted in a premature stop codon. Moreover, this TCRbeta chain was of the TRBV7-9*03 family instead of the TRBV7-9*01 family that was reported by single cell PCR and TRBV7-9 family (low resolution) Adaptive Biotechnologies TCRB survey sequencing. There is a non-conserved amino acid substitution between the two variable chains (N or D at position 26) indicating that the choice of TRBV7-9*03 was useful (see Figure 9). There were two identified functional TCRalpha chains and only one of them was paired correctly with the functional TCRbeta to confer specificity to p53-Y220C and HLA-A*02:01.

[0210] TCR pairs were reconstructed and cloned into retroviral vectors. The second amino acid residue was changed to alanine (A) to have a stronger kozak sequence for highly efficient translation. The TCR α chain constant regions were replaced with a cysteine-substituted, LVL-modified murine α chain constant region. The TCR β chain constant regions were replaced with a cysteine-substituted murine β chain constant region.

[0211] **TCR name: 4259-F1-TCR**

MATSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPISEHNRLYWYR
 QTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGFSSTLEIQRTEQGDSAMYL**CAS**
SPGLAYEQYFGPGTRLTVTEDLRNVTPPKVSLFEPSKAEIANKQKATLVCLARGFFPDHV
*ELSWVWNGKEVHSGVCTDPQAYKESNYSYCLSSRLRV*SATFWHNPRNHFR**CQVQFHGLS**
EEDKWPEGSPKPVTONISAEAWGRADCGITSASYQQGVLSATILYEILLGKATLYAVLVSTL
*VVMAMVKRKNS***RAKRSGSGATNFSLLKQAGDVEENPGPMHSAPISMLAMLFTLSG**
 LRAQSVAPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRGLQFLLKYITGD
NLVKGSYGFEAEFNKSQTSFHLKKPSALVSDSALYFCAVRDGSATSGTYKYIFGTGT
 RLKVLANIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKTMESGTFITDKCVLDMKA
 MDSKSNGAIAWSNQTSFTCQDIFKETNATYPSSDVPCD**ATLTEKSFETDMNLNFQNLLVIV**
LRILLKVAGFNLLMTLRLWSS (SEQ ID NO: 90)

[0212] The sequence of the p53-Y220C neoantigen-reactive TCR **4259-F1-TCR**, which was isolated from Patient 4259, is set forth immediately above. Starting from the amino terminus, the first underlined region is the CDR1beta (SEQ ID NO: 83), the second underlined region is the CDR2beta (SEQ ID NO: 84), the third underlined region is the CDR3beta (SEQ ID NO: 85), the fourth underlined region is the CDR1alpha (SEQ ID NO: 80), the fifth underlined region is the CDR2alpha (SEQ ID NO: 81), and the sixth underlined region is the CDR3alpha (SEQ ID NO: 82). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the beta chain constant

region (SEQ ID NO: 93) and the second italicized region is the alpha chain constant region (SEQ ID NO: 91). The beta chain variable region (SEQ ID NO: 87) includes the sequence starting from the amino terminus and ending immediately prior to the start of the beta chain constant region. The alpha chain variable region (SEQ ID NO: 86) includes the sequence starting immediately after the linker and ending immediately prior to the start of the alpha chain constant region. The full-length beta chain (SEQ ID NO: 89) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length alpha chain (SEQ ID NO: 88) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0213] A variant of 4259-F1-TCR comprising an alpha chain with a wild-type signal peptide is set forth in SEQ ID NO: 201. The variant comprises an alpha chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 199. The full-length alpha chain of the variant is set forth in SEQ ID NO: 200.

[0214] Another variant of 4259-F1-TCR comprises a beta chain variable region (with a wild-type signal peptide) as set forth in SEQ ID NO: 219. The full-length beta chain of the variant is set forth in SEQ ID NO: 220.

[0215] The amino acid sequences of the 4259-F1-TCR alpha and beta chain variable regions are shown in Table 8. The CDRs are underlined. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 174. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 175.

TABLE 8

TCR Name	TCR chain	Amino acid sequence
	Alpha chain variable region (with N-terminal signal peptide)	MHSAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYSVS <u>GNPYLFWYVQYPNRGLQFLKYITGDNLVKGSYGFEAEFNKSQTSFH</u> LKKPSALVSDSALYFC ~~AVRDGS~~ ATSGTYKYIFGTGTRLKVLA (SEQ ID NO: 86)
	Alpha chain variable region	MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYSVS <u>GNPYLFWYVQYPNRGLQFLKYITGDNLVKGSYGFEAEFNKSQTSFH</u> LKKPSALVSDSALYFC ~~AVRDGS~~ ATSGTYKYIFGTGTRLKVLA (SEQ ID NO: 199)

TCR Name	TCR chain	Amino acid sequence
4259-F1-TCR	(with wild-type N-terminal signal peptide)	
	Beta chain variable region (with N-terminal signal peptide)	MATSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPIS <u>EHNR</u> LYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERP ~~KGSF~~ STLEIQRTEQGDSAMYL <u>CASSPGLAYEQYFGPGTR</u> TLVT (SEQ ID NO: 87)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPIS <u>EHNR</u> LYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERP ~~KGSF~~ STLEIQRTEQGDSAMYL <u>CASSPGLAYEQYFGPGTR</u> TLVT (SEQ ID NO: 219)
	Alpha chain variable region (predicted sequence without N-terminal signal peptide)	QSVAPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYVQYPNRLQFLK <u>YITG</u> DNLVKGSYGFEAEFNKSQTSFHLKKPSALVSDSALYFC <u>AVR</u> <u>DGSATSGTYKYIFGTG</u> TRLKVL (SEQ ID NO: 156)
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	DTGVSQDPRHKITKRGQNVTFRCDPIS <u>EHNR</u> LYWYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERP ~~KGSF~~ STLEIQRTEQGDSAMYL <u>CASSPGLAYEQYFGPGTR</u> TLVT (SEQ ID NO: 157)

EXAMPLE 10

[0216] This example demonstrates that the 4259-F1-TCR of Example 9 specifically recognizes mutated p53-Y220C peptide and not the corresponding WT peptide.

[0217] The 4259-F1-TCR of Example 9 was transduced into donor peripheral blood T cells then co-cultured with T2 tumor cells (HLA-A*02:01) pulsed with decreasing concentrations of either WT p53-Y220 peptide (VVPYEPPEV) (SEQ ID NO: 112) or MUT p53-Y220C peptide (VVPCEPPEV) (SEQ ID NO: 113). After overnight incubation, the co-culture supernatants were analyzed by ELISA for interferon-gamma secretion. The results are shown in Figure 6.

EXAMPLE 11

[0218] This example demonstrates that the 4259-F1-TCR specifically recognizes tumor cells expressing p53-Y220C presented by HLA-A*02:01.

[0219] T cells either expressing no TCR (untransduced), a p53-R175H specific TCR or the 4259-F1-TCR of Example 9 were co-cultured with tumor cells, either with or without expression of HLA-A*02:01, p53-R175H or p53-Y220C. After overnight incubation, the cells were stained for CD3, CD8 and 4-1BB then analyzed by flow cytometry. The frequencies of CD8⁺4-1BB⁺ T cells from the cultures are shown in Figure 7.

EXAMPLE 12

[0220] This example demonstrates the isolation and specific reactivity of a TCR from patient 4141.

[0221] A summary of the treatment of patients with p53 mutation-reactive TIL is provided in Table 9.

TABLE 9

Tumor Type	Patient	p53 mut	Infusion bag screening	Total # of Cells (x10 ⁹)	% of p53 reactive TIL	# of p53 reactive TIL (x10 ⁹)	Res- ponse	Duration (months)
Colon	4141	R175H	Y	69	0.8	0.6	N.R.	-

[0222] Autologous APCs were transfected with TMG encoding irrelevant mutations, WT p53 sequence, or mutated p53 sequence including R175H. Media alone and PMA and ionomycin were negative and positive controls, respectively. The following day, TIL from patient 4141 (fragment culture 12) were co-cultured overnight at 37 °C with TMG-transfected APCs. Secretion of IFN-γ was evaluated by ELISPOT. Expression of 4-1BB was evaluated by flow cytometry after gating for lymphocytes → living cells (PI negative) → CD3⁺ (T cells) → CD4-CD8⁺. The results are shown in Figure 10.

[0223] Cos7 cells (2.5x10⁴ per well) were plated on wells of flat-bottom 96 well plates. The following day, cells were co-transfected with individual HLA alleles from patient 4141 and either no extra gene, WT TP53 TMG, or mutated TP53 TMG containing the p53-R175H

sequence. TIL with specificity to p53-R175H from Patient 4141 (fragment culture 12) were co-cultured the following day with transfected Cos7 cells and were incubated overnight at 37 °C. Secretion of IFN- γ was evaluated by ELISPOT. The results are shown in Figure 11.

[0224] T cells expressing mock (no TCR) or 4141-TCR1a2 were co-cultured with T2 tumor cells (expressing HLA-A*02:01). T2 cells were pulsed for 2 hours at 37 °C with peptide vehicle (DMSO) or purified (>95% by HPLC) peptides composed of WT p53-R175 peptide HMTEVVRRC (SEQ ID NO: 95) or mutated p53-R175H peptide HMTEVVRHC (SEQ ID NO: 96). Media alone and PMA and Ionomycin were negative and positive controls, respectively. Co-cultures were performed overnight at 37 °C. Secretion of IFN- γ was evaluated by ELISA. The results are shown in Figure 12.

[0225] T cells expressing 4141-TCR1a2 were co-cultured overnight at 37 °C with Saos2 cells (p53-NUL and HLA-A*02:01+), which were either unmanipulated or made to overexpress full length p53-R175H protein. Inhibitors of secretion (monensin and brefeldin A) were added to co-cultures to trap cytokines within T cells. After 6 hours of co-culture, cells were fixed and permeabilized then stained for IL-2, CD107a, IFN- γ and tumor necrosis factor- α (TNF α). Flow cytometry was used to analyze co-cultures based on a lymphocyte gate. The results are shown in Figure 13.

[0226] The sequence of TCR **4141-TCR1a2**, which was isolated from Patient 4141, is set forth below. Starting from the amino terminus, the first underlined region is the CDR1alpha (SEQ ID NO: 131), the second underlined region is the CDR2alpha (SEQ ID NO: 132), the third underlined region is the CDR3alpha (SEQ ID NO: 133), the fourth underlined region is the CDR1beta (SEQ ID NO: 134), the fifth underlined region is the CDR2beta (SEQ ID NO: 135), and the sixth underlined region is the CDR3beta (SEQ ID NO: 136). The bold region is the linker (SEQ ID NO: 94). Starting from the amino terminus, the first italicized region is the alpha chain constant region (SEQ ID NO: 91) and the second italicized region is the beta chain constant region (SEQ ID NO: 93). The alpha chain variable region (SEQ ID NO: 137) includes the sequence starting from the amino terminus and ending immediately prior to the start of the alpha chain constant region. The beta chain variable region (SEQ ID NO: 138) includes the sequence starting immediately after the linker and ending immediately prior to the start of the beta chain constant region. The full-length alpha chain (SEQ ID NO: 139) includes the sequence starting from the amino terminus and ending immediately prior to the start of the linker. The full-length beta chain (SEQ ID NO: 140) includes the sequence starting immediately after the linker and ending with the carboxyl terminus.

[0227] Cancer reactive T cells were identified as described below. The TCR was isolated as described below.

TCR name: 4141-TCR1a2

Recognition of p53 mutation: R175H

Screening method: p53 “hotspot” mutation universal screening

Co-culture to identify TCR: Co-culture 4141 infusion bag TIL with p53mutTMG and sorted CD8+41BB+ T cells

Method to identify TCR: single-cell RT-PCR then TA TOPO cloning kit (Thermo Fisher Scientific, Waltham, MA) for alpha chain

TCR orientation: alpha-beta

Expression vector: SB transposon

MAKSLRVLLVILWLQLSWVWSQQKEVEQDPGPLSVPEGAIIVSLNCTYSNSAFQYFM
WYRQYSRKGPPELLMYTYSSGNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAM
SGLKEDSSYKLIFGSGTRLLVRPNIQNPEPAVYQLKDPRSQDSTLCLFTDFDSQINVPKT
MESGTFITDKCVLDMKAMDSKSNGAIAWSNQTSFTCQDIFKETNATYPSSDVPCDATLTE
KSFETDMNLNFQNLVIVLRILLKVAGFNLLMTLRLWSSRAKRSGSGATNFSLLKQAG
DVEENPGPMHPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMN
HEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVS RKEKRNFLILESPSPN
QTSLYFCASSIQQGADTQYFGPGTRLTVLEDLRNVTTPKVSLEFPSKAEIANKQKATLVC
LARGFFPDHVELSWWWNGKEVHSGVCTDPQAYKESNYSYCLSSRLRVSATFWHNPRNHFR
CQVQFHGLSEEDKWPEGSPKPTQNISAEAWGRADCGITSASYQQGVLSATILYEILLGKA
TLYAVLVSTLVVMAMVKRKNS (SEQ ID NO: 141)

[0228] A variant of 4141-TCR1a2 comprising a beta chain with a wild-type signal peptide is set forth in SEQ ID NO: 204. The variant comprises a beta chain variable region (with the wild-type signal peptide) as set forth in SEQ ID NO: 202. The full-length beta chain of the variant is set forth in SEQ ID NO: 203.

[0229] Another variant of 4141-TCR1a2 comprising an alpha chain with a wild-type signal peptide is set forth in SEQ ID NO: 225. The variant comprises a beta chain variable region as set forth in SEQ ID NO: 221. The full-length beta chain of the variant is set forth in SEQ ID NO: 222. The variant comprises an alpha chain variable region (with the wild-type

signal peptide) as set forth in SEQ ID NO: 223. The full-length alpha chain of the variant is set forth in SEQ ID NO: 224.

[0230] The amino acid sequences of the 4141-TCR1a2 alpha and beta chain variable regions are shown in Table 10. The CDRs are underlined. The predicted full-length alpha chain (inclusive of the alpha chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 176. The predicted full-length beta chain (inclusive of the beta chain variable and constant regions) without the N-terminal signal peptide is shown in SEQ ID NO: 177.

TABLE 10

TCR Name	TCR chain	Amino acid sequence
4141-TCR1a2	Alpha chain variable region (with N-terminal signal peptide)	MAKSLRVLLVILWLQLSWVWSQQKEVEQDPGLSVPEGAIIVSLNCT YSNSAFQYFMWYRQYSRKGPPELLMYTYSSGNKEDGRFTAQVDKSSK YISLFIRDSQPSDSATYLCAMSGLKEDSSYKLIFGSGTRLLVRP (SEQ ID NO: 137)
	Alpha chain variable region (with wild-type N-terminal signal peptide)	MMKSLRVLLVILWLQLSWVWSQQKEVEQDPGLSVPEGAIIVSLNCT YSNSAFQYFMWYRQYSRKGPPELLMYTYSSGNKEDGRFTAQVDKSSK YISLFIRDSQPSDSATYLCAMSGLKEDSSYKLIFGSGTRLLVRP (SEQ ID NO: 223)
	Beta chain variable region (with N-terminal signal peptide)	MHPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNM NHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSREKR NFPLILESPSPNQTSLYFCASSIQQGADTQYFGPGTRLTVL (SEQ ID NO: 138)
	Beta chain variable region (with N-terminal signal peptide)	MAPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNM NHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSREKR NFPLILESPSPNQTSLYFCASSIQQGADTQYFGPGTRLTVL (SEQ ID NO: 221)
	Beta chain variable region (with wild-type N-terminal signal peptide)	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNM NHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSREKR NFPLILESPSPNQTSLYFCASSIQQGADTQYFGPGTRLTVL (SEQ ID NO: 202)
	Alpha chain variable region	QQKEVEQDPGLSVPEGAIIVSLNCTYSNSAFQYFMWYRQYSRKGPPELLMYTYSSGNKEDGRFTAQVDKSSKYISLFIRDSQPSDSATYLCAMSGLKEDSSYKLIFGSGTRLLVRP (SEQ ID NO: 159)

TCR Name	TCR chain	Amino acid sequence
	(predicted sequence without N-terminal signal peptide)	
	Beta chain variable region (predicted sequence without N-terminal signal peptide)	QVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYY SMNVEVTDKGDVPEGYK VSRKEKRNFLILESPSPNQTSLYFCASSIQ QGADTQYFGPGTRLTVL (SEQ ID NO: 158)

[0231] The statistics for 4141-TCR1a2 from patient 4141 are set forth in Table 11 below.

TABLE 11

Parameter	#	Frequency
Total wells	96	100%
CDR3alpha	Unknown (TA TOPO cloning)	Not applicable
CDR3beta	58	60.4%

[0232] The TCR 4141-TCR1a2 was isolated, expressed in T cells and tested against the relevant antigen. A summary of the results is shown in Table 12.

TABLE 12

TCR name	p53 a.a. substitution	TCR tested?	p53 mutation reactive?
4141-TCR1a2	R175H	yes	yes

[0233] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0234] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following

claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0235] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.