

HUMAN ALPHA FETOPROTEIN-SPECIFIC T CELL RECEPTORS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims benefit of and priority to US Provisional Patent Applications 62/505,406 filed on May 12, 2017, 62/609,614 filed on December 22, 2017, and 62/625,051 filed on February 1, 2018, and where permitted all of which are incorporated by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

10 This invention was made with government support under R01CA168912 awarded by the National Institutes of Health. The government has certain rights in the invention.

SEQUENCE LISTING

15 The Sequence Listing submitted May 1 2018, as a text file named "Sequence listing 064466.067_ST25" created on April 26, 2018, and having a size of 171,818 bytes is hereby incorporated by reference pursuant to 37 C.F.R. § 1.52(e)(5).

TECHNICAL FIELD OF THE INVENTION

20 The invention is generally directed to immunology, in particular to T cell receptors and methods of their use in treating immune disorders including cancer.

BACKGROUND OF THE INVENTION

25 With ~800,000 new cases each year, hepatocellular carcinoma (HCC) is the 5th most common cancer in the world. In the US, according to American Cancer Society, the number of liver cancers (the majority of them are HCC) has doubled in last decade in the United States, representing one of the fast-growing malignancies - mainly due to the prevalence of obesity. The HCC incidence will likely remain high due to the large number of existing chronic HBV and HCV patients and the pandemic obesity. To make matters worse, the lack of effective
30 management makes HCC the 2nd leading cause of cancer death in adult men. Thus, there is an urgent need to develop novel therapies. Adoptive transfer of tumor-specific T cells has great

potential of controlling tumor growth without significant toxicity. Because of the difficulty of isolating tumor-specific T cells from most solid tumors (other than the melanoma), genetically engineering patient's autologous T cells with tumor antigen-specific TCR genes will likely provide the functional tumor-specific T cells for adoptive cell transfer immunotherapy.

5 HCC frequently re-expresses human glypican 3 (hGPC3) and human alpha fetoprotein (hAFP) as tumor associated antigens (TAAs). These antigens not only serve as biomarkers for diagnosis, but can also be targets for immunotherapy. Recently, hGPC3-specific human TCR genes were cloned and demonstrated antitumor efficacy using a xenografted HCC model in immune compromised mice (Dargel et al., 2015). However, because TAAs are often not
10 expressed at equal levels throughout an HCC, anti-hGPC3 treatment may select hGPC3-negative cells, causing relapse. In theory, using a combination of TCRs toward different epitopes and different tumor antigens can avoid or delay tumor immune escape. Four HLA-A2 restricted hAFP epitopes have been identified (Butterfield et al., 2001). The epitope hAFP₁₅₈ is frequently presented by HCC tumor cells and the hAFP₁₅₈-specific immune cells are found in HCC patients
15 (Butterfield et al., 2003) though antitumor effect was weak (Butterfield et al., 2006) possibly because the human hAFP₁₅₈-specific T cells are low affinity. Thus, finding high affinity hAFP₁₅₈-specific TCR may increase antitumor efficacy of targeting AFP antigen.

There are two hAFP₁₅₈-specific TCR genes that have been reported. One recent patent directed to human TCR specific for the hAFP₁₅₈ epitope (CN 104087592 A) showed limited
20 antitumor effect (Sun et al., 2016). The weaker antitumor effect was further confirmed by another patent application from Adaptimmune Co (US 2016/0137715 A1), in which the wild type human TCR specific for hAFP₁₅₈ epitope did not produce any effector function when co-cultured with human HCC tumor cells. Thus, hAFP₁₅₈-specific high affinity TCRs were created and patented via mutating the CDR regions of wild type human TCR to increase the recognition
25 of HCC tumor cells (US 2016/0137715 A1). But, no clinical data is available to show that such TCR modified human T cells (TCR-T) can indeed generate antitumor effect *in vivo*. Furthermore, in light of the recent reports that adoptive transfer of high affinity TCR-T cells has been associated with severe off-target toxicity (Cameron et al., 2013; Linette et al., 2013; Morgan et al., 2013), it is desirable to have more TCRs available to increase the chance of
30 finding the optimal TCRs with high antitumor efficacy and low toxicity. Thus, there is a true

demand for identifying additional TCRs specific for TAAs that can be used to engineer a patient's autologous T cells for immunotherapy.

SUMMARY OF THE INVENTION

5 T cell receptors that specifically recognize hAFP₁₅₈ are provided. One embodiment provides an engineered murine T cell receptor (mTCR) V α chain polypeptide having at least 90% sequence identity to SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, or 10, wherein the mTCR specifically recognizes hAFP₁₅₈ (SEQ ID NO:1).

Another embodiment provides an engineered murine T cell receptor (mTCR) V β chain polypeptide having at least 90% sequence identity to SEQ ID NO:11, 12, 13, or 14, wherein the
10 mTCR specifically recognizes hAFP₁₅₈ (SEQ ID NO:1).

Still another embodiment provides an engineered murine T cell receptor (mTCR) full length α chain polypeptide having at least 90% sequence identity to SEQ ID NO:15, 16, 17, 18, 19, 20, 21, 22, or 23, wherein the mTCR specifically recognizes hAFP₁₅₈ (SEQ ID NO:1).

Another embodiment provides an engineered murine T cell receptor (mTCR) full length β
15 chain polypeptide having at least 90% sequence identity to SEQ ID NO:24, 25, 26, or 27, wherein the mTCR specifically recognizes hAFP₁₅₈ (SEQ ID NO:1).

One embodiment provides an engineered murine T cell receptor (mTCR) α chain polypeptide having a CDR3 region with at least 90% sequence identity to SEQ ID NO:28, 29, 30, 31, 32, 33, 34, 35, or 36, wherein the mTCR specifically recognizes hAFP₁₅₈ (SEQ ID
20 NO:1).

Another embodiment provides an engineered murine T cell receptor (mTCR) β chain polypeptide having a CDR3 region with at least 90% sequence identity to SEQ ID NO:37, 38, 39, or 40, wherein the mTCR specifically recognizes hAFP₁₅₈ (SEQ ID NO:1).

The leader sequence of any one of the disclosed polypeptide sequences can be removed.

25 One embodiment provides an engineered murine T cell receptor (mTCR) having a V α domain having at least 90% sequence identity to SEQ ID NO:2, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:28, and a V β domain having at least 90% sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37.

30 Another embodiment provides an engineered murine T cell receptor (mTCR) having a V α domain having at least 90% sequence identity to SEQ ID NO:3, wherein the CDR3 region

has at least 90% sequence identity to SEQ ID NO:29, and a V β domain having at least 90% sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37.

Another embodiment provides an engineered murine T cell receptor (mTCR) having a
5 V α domain having at least 90% sequence identity to SEQ ID NO:4, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:30, and a V β domain having at least 90% sequence identity to SEQ ID NO:12, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:38.

Still another embodiment provides an engineered murine T cell receptor (mTCR) having
10 a V α domain having at least 90% sequence identity to SEQ ID NO:5, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:31, and a V β domain having at least 90% sequence identity to SEQ ID NO:13, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:39.

One embodiment provides an engineered murine T cell receptor (mTCR) having a V α
15 domain having at least 90% sequence identity to SEQ ID NO:6, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:32, and a V β domain having at least 90% sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37.

Some embodiment provide an engineered murine T cell receptor (mTCR) having a V α
20 domain with at least 90% sequence identity to SEQ ID NO:7, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:33, and a V β domain having at least 90% sequence identity to SEQ ID NO:14, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:40.

Another embodiment provides an engineered murine T cell receptor (mTCR) having a
25 V α domain having at least 90% sequence identity to SEQ ID NO:8, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:34, and a V β domain having at least 90% sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37.

Yet another embodiment provides an engineered murine T cell receptor (mTCR) having a
30 V α domain having at least 90% sequence identity to SEQ ID NO:9, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:35, and a V β domain having at least 90%

sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37.

One embodiment provides an engineered murine T cell receptor (mTCR) having a V α domain having at least 90% sequence identity to SEQ ID NO:10, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:36, and a V β domain having at least 90% sequence identity to SEQ ID NO:11, wherein the CDR3 region has at least 90% sequence identity to SEQ ID NO:37. Any of the disclosed mTCRs can be humanized.

One embodiment provides a T cell engineered to express any of the disclosed mTCRs. The T cell can be human. The T cell can also be an autologous T cell.

Another embodiment provides a soluble mTCR α chain comprising Q21 to L242 of SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 16, 17, 18, 19, 20, 21, 22, or 23.

Another embodiment provides the extracellular domain of an engineered mTCR β chain containing SEQ ID NO:50, 51, 52, or 53, or E18 to Y276 of SEQ ID NO:24, 25, 26, or 27.

One embodiment provides a fusion protein having at least 90% sequence identity to SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, or 62.

Another embodiment provides a vector encoding any one of the mTCR proteins or polypeptides.

Another embodiment provides a nucleic acid having at least 90% sequence identity to SEQ ID NO:63, 64, 65, 66, 67, 68, 69, 70, or 71.

Another embodiment provides a non-naturally occurring hAFP₁₅₈ epitope-specific murine T cell receptor including:

a). an α chain variable domain (V α) having at least 90% sequence identity to amino acid residues 21-132 of SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, or 10; and

b). a β chain variable domain (V β) having at least 90% sequence identity to amino acid residues 18-131 of SEQ ID NO:11, 12, 13, or 14.

Another embodiment provides a fusion protein including a first polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, or 10, linked to a second polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:11, 12, 13, or 14.

Another embodiment provides a fusion protein including a first polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:15, 16, 17, 18,

19, 20, 21, 22, or 23, linked to a second polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:24, 25, 26, or 27.

Another embodiment provides a T cell engineered to express the TCR encoded by SEQ ID NO: 63, 64, 65, 66, 67, 68, 69, 70, or 71. The T cell can be human.

5 Another embodiment provides a hybridoma having a CD8+Tet₁₅₈+ cell fused to a donor cell lacking TCR α and β chains. The hybridoma typically responds to hAFP+ tumor cells. In certain embodiments, the hybridoma secretes IL-2.

Another embodiment provides a fusion protein having a first polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:2, 3, 4, 5, 6, 7, 8, 9, 10 or 10 linked to a second polypeptide having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO:11, 12, 13, 14, 24, 25, 26, or 27, wherein in the fusion protein is linked to a single chain anti-CD3 antibody.

Another embodiment provides a method for treating tumors in a subject in need thereof by genetically engineering human T cells to express a disclosed mTCR and administering the 15 engineered T cells to the subject in an amount effective to reduce tumor burden in the subject. In certain embodiments the tumors are hepatocellular carcinoma. The T cells can be autologous T cells.

Another embodiment provides a method for detecting hepatocellular carcinoma by contacting a disclosed polypeptide or protein to a sample of cells, wherein specific binding of the 20 polypeptide or protein to a cell is indicative of a hepatocellular carcinoma cell.

Another embodiment provides an antibody or antigen binding fragment thereof that specifically binds to a disclosed polypeptide or protein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1 to 1A-4 are dot plots of mouse peripheral blood cells stimulated with 25 hAFP158 peptide to analyze for CD8 and IFN- γ by gating on the Thy1.2+ T cells. Figure 1A-5 is a graph of % of CD8+IFN γ + of total spleen cells with or without lv prime. Figures 1B-1 to 1B-3 are dot plots of splenic T cells stimulated with HepG2(AFP-)(Fig. 1B-1), HepG2(AFP+)(Fig. 1B-2) and hAFP158 peptide (Fig. 1B-3). Figure 1B-4 is a graph of % of CD8+IFN γ + of total spleen cells stimulated with HepG2(AFP-), HepG2(AFP+) and hAFP158 30 peptide. Figures 1C-1 to 1C-3 are photomicrographs of HepG2 cells co-cultured with

splenocytes. Figure 1C-4 is a graph of Remaining alive HepGe cells (OD595) versus E/T ratio, (●) is Lv + Pep and (■) is Pep + Pep.

Figures 2A-2E show the adoptive transfer of immunized AAD mouse splenocytes prevents mice from tumor challenge and eradicates HepG2 tumor xenographs in NSG mice.

5 Figure 2A is a dot plot showing approximately 10% of the immunized mouse splenocytes produced IFN γ in response to hAFP158 peptide. Figure 2B illustrates fifteen million total splenocytes (1.5 million of hAFP158-specific CD8 T cells) of naïve or immunized mice were injected into NSG mice, which were then challenged with HepG2 tumor cells. Figure 2C is a diagram representing NSG mice injected with 1.5 million splenocytes of the immunized mice
10 when tumor size reaches 2cm in diameter. Figure 2D is a line graph of tumor size (mm³) versus days after tumor inoculation in control (●) and ACT (■). Figure 2E is a line graph of Tumor Volume (mm³) versus days after tumor inoculation in mouse 1 (□), mouse 2 (□), mouse 3 (□), and mouse 4 (□),

Figure 3A is a dot plot showing the purity of hAFP158 specific CD8 T cells. Figure 3B
15 is a dot plot showing the percent of hAFP158 specific IFN γ -producing cells. Figure 3C is a dot plot showing the purity of Flu M1 specific CD8 T cells. Figure 3D is a dot plot showing percent of Flu M1 specific IFN γ -producing cells. Figure 3E is a line graph of Tumor Volume (mm³) versus days after tumor inoculation for mice treated with CD8 of Lv+hAFP158 (□) or CD8 of Flu+M1. Figures 3F to 3H are dot plots of purified CD8 T cells from hAFP immunized mice
20 further separated into Tet158+ and Tet158- cells by Tet158 tetramer staining and cell sorter. The purity before and after sorting is presented. Figure 3I is a line graph of tumor volume (mm³) versus days after tumor inoculation for mice treated with Tet158+ (□) or Tet158- (□) cells. Figures 3J-3O are images of the mice from Figure 3I.

Figure 4A is a bar graph of IL-2 (pg/ml) from T cell hybridoma clones. BW-Lyt2.4
25 fusion partner cells and 5 different hybridoma clones were co-cultured in triplicate with hAFP+ (□) or hAFP- (□) HepG2 tumor cells and the IL-2 production was detected by ELISA. Figures 4B to 4G are histograms of the hybridoma clones were stained with anti-V β 8.3 antibody. Figures 4H to 4M are histograms of the hybridoma clones were stained with Tet158 tetramer.

Figure 5A is a schematic of the recombinant lv expressing TCR genes. The paired TCR α
30 and β chain genes were expressed as a single molecule under the control of EF1 α promoter. A P2A sequence was inserted in between to allow generation of equal number of TCR α and β

chains. Figures 5B to 5E are histograms Tet158 tetramer staining of the human T cell line Jurkat cells after transduction with three different TCR-lv. Histogram and MFI were presented. Figures 5G to 5J, 5L to 5O, and 5Q to 5T are dot plots of primary human T cells from 3 different donors transduced with TCR-lvs showing the percent and MFI of Tet158+ CD8 and CD4 T cells. Mock transduced cells had undergone same CD3/CD28 treatment without lv transduction or with GFP-lv transduction. Figures 5F, 5K, 5P, and 5U are bar graphs showing MFI of Tet158 on transduced T cells from three different donors. TCR-T cells are shown. Only the CD8 or CD4 T cells were gated and shown in the representative plots.

Figure 6A is a bar graph of IFN- γ (pg/ml) from primary human T cells transduced with TCR genes and treated with AFP-HepG2 (grey shading), Huh7 (solid shading) or AFP+HepG2 (no shading). Figures 6B to 6E are dot plots showing intracellular staining of IFN γ and IL-2 by CD8. Figures 6F to 6I are dot plots showing intracellular staining of IFN γ and IL-2 by CD4. Figures 6J to 6O are dot plots and histograms showing the induction of CD8 and CD4 TCR-T cell proliferation by hAFP+ HepG2 tumor cells was shown. The experiment was repeated twice with similar data.

Figure 7A is a bar graph of percent killing by HLA-A2+ human primar T cells transduced with TCR genes and treated with AFP- HepG2 (grey shading), Huh7 (solid shading), or AFP+HepG2 (no shading). Figure 7B is a line graph of percent killing versus E:T ratios for TCR ($\square$) and Mock ($\square$). Figure 7C, 7D, and 7E are dot plots showing donor CD8 and CD4 TCR-T cells separated by magnetic beads after TCR gene transduction. Figures 7F to 7K are photomicrographs of the co-culture of the mock- transduced, CD4, or CD8 TCR-T cells with HepG2 tumor cells. Photographs were taken at 2 hours and 24 hours after co-culture. Figure 7E is a bar graph showing the results of an LDH assay to measure percents killing in CD4 cells (black bar), CD8 cells (white bar), and total T cells (gray bar) in TCR or mock transduced human T cells. Figure 7M to 7O are photomicrographs of representative cells from Figure L.

Figure 8 is a map of TCR-1 expressing lentivector, pCDH-EF1-coTCR-1.

Figure 9 is a map of TCR-1 expressing lentivector, pCDH-EF1-coTCR-2.

Figure 10 is a map of TCR-1 expressing lentivector, pCDH-EF1-coTCR-3.

Figure 11A is a dot plot showing the percent of Tet158+ CD8+ splenocytes from immunized mice. Figures 11B-11P are histograms showing Vbeta expression in CD8+, Tet158+ cells using different anti-Vbeta chain antibodies.

Figure 12A-D show the transduction of primary human T-cells with recombinant lv. Figure 12A is an illustration of the GFP-lv promoter. Figure 12B and 12C are representative images of GFP expressing primary T-cells. Figure 12D is a histogram of GFP+ T cells.

Figure 13A is a histogram showing the percent of TCR-T cells stained with anti-Vbeta chain antibody. Figure 13B is a histogram showing the percent of TCR-T cells stained with Tet158 tetramer.

Figures 14A-14I show that human TCR-T cells specifically recognize AFP158 peptide pulsed HLA-A2 mouse splenocytes. Figure 14A is a bar graph showing IFN-g production (pg/ml) in mock T-cells, TCR1 T-cells, TCR2, T-cells, or TCR3 T-cells, stimulated with AAD mouse splenocytes and pulsed with no peptide (grey bar), influenza M1 peptide (black bar), or AFP158 peptide (white bar). Figure 14B-14E are histograms showing percent of IFN γ staining human T-cells (containing both CD4 and CD8) stimulated with hAFP158 peptide pulsed HLA-A2 cells. Figure 14F-14I are histograms showing percent of IL-2 staining human T-cells (containing both CD4 and CD8) stimulated with hAFP158 peptide pulsed HLA-A2 cells.

Figures 15A-15T show that the adoptive transfer of human TCR-T generates antitumor effect against HepG2 tumor in NSG mice. Figure 15A is a dot plot showing the % Tet158+ cells out of total human TCR-T cells. Figure 15B is a line graph representing tumor outgrowth (% tumor free mice) versus days after tumor inoculation in control and TCR-T groups. Figure 15C is a line graph representing tumor size (mm³) versus days after tumor inoculation in control (●) and TCR-T (■). Figure 15D-15K are pictures of HepG2 tumors at day 31 after the inoculation in Mock-T treated (D1-4) and TCR-T treated (D5-8) mice. Figure 15L-15O are representative dot plots showing the percent of human CD45+ cells among total mouse blood cells. Figure 15P is a line graph representing the percent human CD45+ cells among total mouse blood cells for control (●) and TCR-T (■). Figure 15Q-15R is representative dot plots showing the percentage of Tet158+ cells out of the transferred human T cells in the NSG mice. Figure 15S is a line graph representing the percent Tet158+ cells out of the total transferred human T cells versus days after ACT. Figure 15T is a line graph representing the percent of Tet158+ cells out of the total transferred CD8 (□) and CD4 (□) T cells versus days after ACT.

Figure 16 is a schematic comparison of the V-region of the 9 mTCR sequences.

Figure 17 is a schematic comparison of the amino acid sequences of CDR3 of the 9 mTCR α chains (Figure 17A) and β chains (Figure 17B).

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The phrase “having antigenic specificity” as used herein means that the TCR can specifically bind to and immunologically recognize the cancer antigen, such that binding of the TCR to the cancer antigen elicits an immune response.

The term “Tet₁₅₈” refers to HLA-A2/hAFP₁₅₈ tetramers.

The term “hAFP₁₅₈” refers to human alpha fetoprotein polypeptide having the amino acid sequence of FMNKFIYEI (SEQ ID NO:1).

The initialism “TCR” refers to a T cell receptor which is a specific receptor on the surface of T cells that is responsible for identifying the antigen presented by the major histocompatibility complex (MHC).

As used herein, the term “antibody” is intended to denote an immunoglobulin molecule that possesses a “variable region” antigen recognition site. The term “variable region” is intended to distinguish such domain of the immunoglobulin from domains that are broadly shared by antibodies (such as an antibody Fc domain). The variable region includes a “hypervariable region” whose residues are responsible for antigen binding. The hypervariable region includes amino acid residues from a “Complementarity Determining Region” or “CDR” (*i.e.*, typically at approximately residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and at approximately residues 27-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (*i.e.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917). “Framework Region” or “FR” residues are those variable domain residues other than the hypervariable region residues as herein defined. The term antibody includes monoclonal antibodies, multi-specific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, camelized antibodies (*See e.g.*, Muyldermans *et al.*, 2001, *Trends Biochem. Sci.* 26:230; Nuttall *et al.*, 2000, *Cur. Pharm. Biotech.* 1:253; Reichmann and Muyldermans, 1999, *J. Immunol. Meth.* 231:25; International Publication Nos. WO 94/04678 and WO 94/25591; U.S. Patent No. 6,005,079), single-chain Fvs (scFv) (*see, e.g., see Pluckthun in The Pharmacology of Monoclonal*

Antibodies, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994)), single chain antibodies, disulfide-linked Fvs (sdFv), intrabodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id and anti-anti-Id antibodies to antibodies). In particular, such antibodies include immunoglobulin molecules of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass.

As used herein, the term “antigen binding fragment” of an antibody refers to one or more portions of an antibody that contain the antibody’s Complementarity Determining Regions (“CDRs”) and optionally the framework residues that include the antibody’s “variable region” antigen recognition site, and exhibit an ability to immunospecifically bind antigen. Such fragments include Fab', F(ab')₂, Fv, single chain (ScFv), and mutants thereof, naturally occurring variants, and fusion proteins including the antibody’s “variable region” antigen recognition site and a heterologous protein (*e.g.*, a toxin, an antigen recognition site for a different antigen, an enzyme, a receptor or receptor ligand, *etc.*).

As used herein, the term “fragment” refers to a peptide or polypeptide including an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues.

As used herein the term “modulate” relates to a capacity to alter an effect, result, or activity (*e.g.*, signal transduction). Such modulation can be agonistic or antagonistic. Antagonistic modulation can be partial (*i.e.*, attenuating, but not abolishing) or it can completely abolish such activity (*e.g.*, neutralizing). Modulation can include internalization of a receptor following binding of an antibody or a reduction in expression of a receptor on the target cell. Agonistic modulation can enhance or otherwise increase or enhance an activity (*e.g.*, signal transduction). In a still further embodiment, such modulation can alter the nature of the interaction between a ligand and its cognate receptor so as to alter the nature of the elicited signal

transduction. For example, the molecules can, by binding to the ligand or receptor, alter the ability of such molecules to bind to other ligands or receptors and thereby alter their overall activity. Preferably, such modulation will provide at least a 10% change in a measurable immune system activity, more preferably, at least a 50% change in such activity, or at least a 2-fold, 5-fold, 10-fold, or still more preferably, at least a 100-fold change in such activity.

The term “substantially,” as used in the context of binding or exhibited effect, is intended to denote that the observed effect is physiologically or therapeutically relevant. Thus, for example, a molecule is able to substantially block an activity of a ligand or receptor if the extent of blockage is physiologically or therapeutically relevant (for example if such extent is greater than 60% complete, greater than 70% complete, greater than 75% complete, greater than 80% complete, greater than 85% complete, greater than 90% complete, greater than 95% complete, or greater than 97% complete). Similarly, a molecule is said to have substantially the same immunospecificity and/or characteristic as another molecule, if such immunospecificities and characteristics are greater than 60% identical, greater than 70% identical, greater than 75% identical, greater than 80% identical, greater than 85% identical, greater than 90% identical, greater than 95% identical, or greater than 97% identical).

As used herein, a “chimeric antibody” is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region.

As used herein, the term “humanized antibody” refers to an immunoglobulin including a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the “donor” and the human immunoglobulin providing the framework is called the “acceptor.” Constant regions need not be present, but if they are, they should be substantially identical to human immunoglobulin constant regions, *i.e.*, at least about 85-99%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A humanized antibody is an antibody including a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody, because, *e.g.*, the entire variable region of a chimeric antibody is non-human.

As used herein, the term “endogenous concentration” refers to the level at which a molecule is natively expressed (i.e., in the absence of expression vectors or recombinant promoters) by a cell (which cell can be a normal cell, a cancer cell or an infected cell).

As used herein, the terms “treat,” “treating,” “treatment” and “therapeutic use” refer to the elimination, reduction or amelioration of one or more symptoms of a disease or disorder.

As used herein, a “therapeutically effective amount” refers to that amount of a therapeutic agent sufficient to mediate a clinically relevant elimination, reduction or amelioration of such symptoms. An effect is clinically relevant if its magnitude is sufficient to impact the health or prognosis of a recipient subject. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to delay or minimize the onset of disease, e.g., delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of a disease.

As used herein, the term “prophylactic agent” refers to an agent that can be used in the prevention of a disorder or disease prior to the detection of any symptoms of such disorder or disease. A “prophylactically effective” amount is the amount of prophylactic agent sufficient to mediate such protection. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the prevention of disease.

As used herein, the term “cancer” refers to a neoplasm or tumor resulting from abnormal uncontrolled growth of cells. As used herein, cancer explicitly includes leukemias and lymphomas. The term “cancer” refers to a disease involving cells that have the potential to metastasize to distal sites and exhibit phenotypic traits that differ from those of non-cancer cells, for example, formation of colonies in a three-dimensional substrate such as soft agar or the formation of tubular networks or web-like matrices in a three-dimensional basement membrane or extracellular matrix preparation. Non-cancer cells do not form colonies in soft agar and form distinct sphere-like structures in three-dimensional basement membrane or extracellular matrix preparations.

As used herein, an “immune cell” refers to any cell from the hemopoietic origin including, but not limited to, T cells, B cells, monocytes, dendritic cells, and macrophages.

As used herein, “valency” refers to the number of binding sites available per molecule.

As used herein, the terms “immunologic,” “immunological” or “immune” response is the development of a beneficial humoral (antibody mediated) and/or a cellular (mediated by antigen-

specific T cells or their secretion products) response directed against a peptide in a recipient patient. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules to activate antigen-specific CD4⁺ T helper cells and/or CD8⁺ cytotoxic T cells. The response may also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils, activation or recruitment of neutrophils or other components of innate immunity. The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4⁺ T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating antibodies and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second subject.

As used herein, an “immunogenic agent” or “immunogen” is capable of inducing an immunological response against itself on administration to a mammal, optionally in conjunction with an adjuvant.

As used herein, the terms “individual,” “host,” “subject,” and “patient” are used interchangeably herein, and refer to a mammal, including, but not limited to, humans, rodents, such as mice and rats, and other laboratory animals.

As used herein, the term “polypeptide” refers to a chain of amino acids of any length, regardless of modification (*e.g.*, phosphorylation or glycosylation). The term polypeptide includes proteins and fragments thereof. The polypeptides can be “exogenous,” meaning that they are “heterologous,” *i.e.*, foreign to the host cell being utilized, such as human polypeptide produced by a bacterial cell. Polypeptides are disclosed herein as amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

As used herein, the term “variant” refers to a polypeptide or polynucleotide that differs from a reference polypeptide or polynucleotide, but retains essential properties. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are
5 closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more modifications (*e.g.*, substitutions, additions, and/or deletions). A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally.

10 Modifications and changes can be made in the structure of the polypeptides of the disclosure and still obtain a molecule having similar characteristics as the polypeptide (*e.g.*, a conservative amino acid substitution). For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide’s biological functional activity,
15 certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like properties.

In making such changes, the hydropathic index of amino acids can be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a polypeptide is generally understood in the art. It is known that certain amino acids can be
20 substituted for other amino acids having a similar hydropathic index or score and still result in a polypeptide with similar biological activity. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-
25 1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is believed that the relative hydropathic character of the amino acid determines the secondary structure of the resultant polypeptide, which in turn defines the interaction of the polypeptide with other molecules, such as enzymes, substrates, receptors, antibodies, antigens,
30 and cofactors. It is known in the art that an amino acid can be substituted by another amino acid having a similar hydropathic index and still obtain a functionally equivalent polypeptide. In such

changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

Substitution of like amino acids can also be made on the basis of hydrophilicity, particularly where the biological functional equivalent polypeptide or peptide thereby created is intended for use in immunological embodiments. The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate ($+3.0 \pm 1$); glutamate ($+3.0 \pm 1$); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); proline (-0.5 ± 1); threonine (-0.4); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent polypeptide. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Trp: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include variants having about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to the polypeptide of interest.

The term “percent (%) sequence identity” is defined as the percentage of nucleotides or amino acids in a candidate sequence that are identical with the nucleotides or amino acids in a reference nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for

instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

- 5 For purposes herein, the % sequence identity of a given nucleotide or amino acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given sequence C that has or comprises a certain % sequence identity to, with, or against a given sequence D) is calculated as follows:

100 times the fraction W/Z ,

- 10 where W is the number of nucleotides or amino acids scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides or amino acids in D. It will be appreciated that where the length of sequence C is not equal to the length of sequence D, the % sequence identity of C to D will not equal the % sequence identity of D to C.

- 15 As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

 As used herein, the terms "antigenic determinant" and "epitope" are used interchangeably and refer to the structure recognized by an antibody.

- 20 As used herein, a "conformational epitope" is an epitope that includes discontinuous sections of the antigen's amino acid sequence. Antibodies bind a conformational epitope based on 3-D surface features, shape, or tertiary structure of the antigen.

- As used herein, a "linear epitope" is an epitope that formed by a continuous sequence of amino acids from the antigen. Linear epitopes typically include about 5 to about 10 continuous amino acid residues. Antibodies bind a linear epitope based on the primary sequence of the antigen.

 As used herein, a "paratope," also called an "antigen-binding site," is a part of an antibody which recognizes and binds to an antigen.

- 30 As used herein, "adoptive cell transfer" or ACT is a type of immunotherapy in which a patient's own T cells are collected, expanded ex vivo, and re-infused into the patient. Two types of ACT are chimeric antigen receptor (CAR) and T cell receptor (TCR) T cell therapy. Both

techniques improve the ability of T cell receptors to recognize and attack specific antigens. In CAR T-cell therapy, T cells are engineered to produce receptors on their surface called chimeric antigen receptors. The receptors allow the T cells to recognize and attach to antigens on tumor cells. In TCR-T cell therapy, T cells are collected from a patient, modified to express a TCR
 5 specific to a tumor antigen, expanded *ex vivo*, and re-infused into the patient.

II. T Cell Receptor Compositions

T cell receptor genes are provided that encode a receptor that specifically binds to hAFP, preferably to hAFP₁₅₈ that is expressed on the surface of cancer cells. Nine pairs of murine TCR α and β chain genes specific for the HLA-A2/hAFP₁₅₈ were identified and synthesized. The
 10 amino acid and nucleic acid sequences for the TCR genes are provided below. Vectors that contain one or more of the TCR genes are also provided.

Another embodiment provides hAFP₁₅₈-specific (Tet₁₅₈) mouse CD8 T cells that recognize and kill human HepG2 cells *in vitro* and eradicate large HepG2 tumor xenografts in NSG mice.

15 Still another embodiment provides T cell hybridomas made from the Tet₁₅₈ CD8 T cells. The T cell hybridomas enabled the identification of paired TCR α and β chain genes. One embodiment provides healthy donor CD8 T cells transduced with the murine TCR α and β chain genes specific for the HLA-A2/hAFP₁₅₈. The genetically engineered TCR enables the donor cells to recognize and effectively kill HepG2 tumor cells at very low E/T ratio. These murine
 20 TCR genes specific for the HLA-A2/hAFP₁₅₈ have the potential to modify and redirect the patient's autologous T cells to treat HCC tumors via adoptive cell transfer.

Another embodiment provides soluble T cell receptors. In one embodiment the soluble TCR includes the extracellular domain of the TCR polypeptides.

A. Genetically Engineered T Cell Receptors

25 The disclosed TCR genes are from the recombinant *lv*-primed and peptide-boosted AAD mice. Thus, technically, these TCRs are not "naturally occurring". In addition, after obtaining the V α and V β region of TCRs, full length α and β chains were designed by using the identified V-region of α and β chains, and the constant region (C-region) of α and β chains of HLA-A2 mouse TCR specific for hgp100. Thus, the entire TCRs and their genes are not naturally
 30 occurring.

1. Protein sequences of the mTCR-1, 2, 3, 6, 8, 10, 11, 17 and 38 V α domain

One embodiment provides mTCR-1 V α (TRAV7D-2*01/TRAJ30*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:2):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 5 101 PSDSALYLCA ASITNAYKVI FGKGTHLHVL PN

Another embodiment provides mTCR-2 V α (TRAV7D-2*01/TRAJ30*01) having at 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:3):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 10 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA AS**TV**NAYKVI FGKGTHLHVL PN

Comparing mTCR-1 α chain V-region to mTCR-2 α chain, there is only 2 amino acids difference (bolded and underlined).

15 Another embodiment provides mTCR-3 V α (TRAV7D-2*01/TRAJ12*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:4):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 20 101 PSDSALYLCA AS**MAGGYKVV** FG**SGTRL**LVS **PD**

There are multiple amino acid differences between mTCR-1 α chain and mTCR-3 α chain (bolded and underlined). In fact, the J segment is different (Instead of TRAJ30, the TRAJ12 is used in *mTCR-3 V α*).

25 One embodiment provides mTCR-6 V α (TRAV7D-2*01/TRAJ30*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:5):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASMINAYKVI FGKGTHLHVL PN

30 Another embodiment provides mTCR-8 V α (TRAV7D-2*01/TRAJ12*01) having at 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:6):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 35 101 PSDSALYLCA AS**ISGGYKVV** FG**SGTRL**LVS **PD**

There are multiple amino acid differences between mTCR-6 α chain and mTCR-8 α chain (bolded and underlined). In fact, the J segment is different (Instead of TRAJ30, the TRAJ12 is used in *mTCR-8 V α*).

40 Another embodiment provides mTCR-10 V α (TRAV7D-2*01/TRAJ12*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:7):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV

51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASIVGGYKVV FGSGTRLLVS PD

Comparing mTCR-6 α chain V-region to mTCR-8 α chain, there is only 1 amino acids difference (bolded and underlined).

- 5 Another embodiment provides mTCR-11 V α (TRAV7D-2*01/TRAJ12*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:8):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASKTGGYKVV FGSGTRLLVS PD

10

Comparing mTCR-6 α chain V-region to mTCR-8 α chain, there are only 2 amino acid differences (bolded and underlined).

One embodiment provides mTCR-17 V α (TRAV7D-2*01/TRAJ12*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:9):

- 15 1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASMTGGYKVV FGSGTRLLVS PD

- 20 Another embodiment provides mTCR-38 V α (TRAV7D-2*01/TRAJ12*01) having at least 90%, 95%, 99 or 100% sequence identity to (SEQ ID NO:10):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ATLTGGYKVV FGSGTRLLVS PD

- 25 In certain embodiments, the leading methionine and/or signal sequence is cleaved in the post-translationally modified protein.

2. Protein sequences of the mTCR-1, 2, 3, 6, 8, 10, 11, 17 and 38 V β domains

One embodiment provides mTCR-1, 2, 8, 11, 17 & 38 V β domain (TRBV13-1*01/TRBJ2-4*01) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID No:11):

- 30 1 MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLSR RQTNSHNYMY
 51 WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FFLLELASP
 101 SQTSLYFCAS SDAGTSQNTL YFGAGTRLSV L

Another embodiment provides mTCR-3 V β domain (TRBV13-1*01/TRBJ2-4*01) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:12):

- 35 1 MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLSR RQTNSHNYMY
 51 WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FFLLELASP
 101 SQTSLYFCAS SDAGTAQNTL YFGAGTRLSV L

There is only one amino acid difference (bold and underlined) between mTCR-1 and mTCR-2 with TCR-3 V β .

Another embodiment provides *m*TCR-6 V β domain (TRBV13-1*01/TRBJ2-4*01) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:13):

1 MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLSR RQTNSHNYMY
 51 WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FFLLELASP
 5 SQTSLYFCAS SDAGVSQNTL YFGAGTRLSV L

There is only one amino acid difference from mTCR-1, 2, 8, and 11, a 2 amino acid difference from mTCR-3, and a 3 amino acid difference from mTCR-10.

Another embodiment provides mTCR-10 V β domain (TRBV13-1*01/TRBJ2-4*01) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:14):

1 MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLSR RQTNSHNYMY
 51 WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FFLLELASP
 101 SQTSLYFCAS SD**HGTG**QNTL YFGAGTRLSV L

There are 3 amino acid differences (bold and underlined) between mTCR-6 V β with TCR-10 V β .

15 In certain embodiments, the leading methionine and/or signal sequence is cleaved in the post-translationally modified protein.

3. Protein sequences of the full length mTCR-1, 2, 3, 6, 8, 10, 11, 17 and 38 α chains

Another embodiment provides mTCR-1 α chain (TRAV7D-2*01/TRAJ30*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:15):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASITNAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD
 151 **STLC**LFTDFD SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIWSNQT
 25 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLFQ NLSVMGLRIL
 251 LLKVAGFNLL MTLRLWSS

The constant region (**C α**) (bolded) of mTCR-1, 2, 3, 6, 8, 10, and 11 α chains are the same, and they are identical to the hgp100 specific TCR α chain C-region from HLA-A2 Tg mice.

Another embodiment provides *m*TCR-2 α chain (TRAV7D-2*01/TRAJ30*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:16):

1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 35 101 PSDSALYLCA AS**TV**NAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD
 151 **STLC**LFTDFD SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIWSNQT
 201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLFQ NLSVMGLRIL
 251 LLKVAGFNLL MTLRLWSS

Another embodiment provides mTCR-3 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:17):

5
10
15
20
25

```

1   MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51  DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101 PSDSALYLCA ASMAGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251 LLKVAGFNLL MTLRLWSS

```

Another embodiment provides mTCR-6 α chain (TRAV7D-2*01/TRAJ30*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:18):

15
20
25

```

1   MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51  DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101 PSDSALYLCA ASMINAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD
151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251 LLKVAGFNLL MTLRLWSS

```

Another embodiment provides mTCR-8 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:19):

20
25

```

1   MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51  DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101 PSDSALYLCA ASISGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251 LLKVAGFNLL MTLRLWSS

```

Another embodiment provides mTCR-10 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:20):

30
35

```

1   MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51  DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101 PSDSALYLCA ASIVGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251 LLKVAGFNLL MTLRLWSS

```

Another embodiment provides mTCR-11 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:21):

40

```

1   MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51  DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101 PSDSALYLCA ASKTGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201 SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251 LLKVAGFNLL MTLRLWSS

```

One embodiment provides mTCR-17 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:22):

1 MKSFSISLVV LWLQLNWNVS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASMTGGYKVV FGSGTRLLVS **PDIQNPEPAV YQLKDPRSQD**
 151 **STLCLFTDFD SQINVPKTME SGTFTITDKTV LDMKAMDSKS NGAIAWSNQT**
 5 201 **SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLFQ NLSVMGLRIL**
 251 **LLKVAGFNLL MTLRLWSS**

Another embodiment provides mTCR-38 α chain (TRAV7D-2*01/TRAJ12*01/TRAC) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:23):

10 1 MKSFSISLVV LWLQLNWNVS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ATLTTGGYKVV FGSGTRLLVS **PDIQNPEPAV YQLKDPRSQD**
 151 **STLCLFTDFD SQINVPKTME SGTFTITDKTV LDMKAMDSKS NGAIAWSNQT**
 201 **SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLFQ NLSVMGLRIL**
 15 251 **LLKVAGFNLL MTLRLWSS**

In certain embodiments, the leading methionine and/or signal sequence is cleaved in the post-translationally modified protein.

In one embodiment, based on the nomenclature on IMGT and Uniprot website, the mTCR α chain features are believed to be as follows:

1. M1-S20: Leader Sequences to be removed on maturation of TCR α chain
2. Q21-S268: TCR-1, 2, 3 α chain
3. Q21-N132 (or D132): α chain V-region
4. I133-S268: TCR α chain C-region identical to the HLA-A2 mouse TCR specific for hgp100 (DQ452619)
5. Q21-L242: TCR α chain extracellular domain
6. S243-L263: transmembrane region of mature TCR α chain
7. R264-S268: intracellular region of mature TCR α chain

4. Protein sequences of full length mTCR-1, 2, 3, 6, 8, 10, 11, 17, and 38 β chains

Another embodiment provides mTCR-1, 2, 8, 11, 17, and 38 β chain (TRBV13-1*01/TRBJ2-4*01/TRBC1) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:24):

1 MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLSC RQTNSHNYMY
 51 WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FFLLELASP
 35 101 SQTSLYFCAS SDAGTSQNTL YFGAGTRLSV **LEDLRNVTPP KVSLEPSKA**
 151 **EIANKRKATL VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS**
 201 **YCLSSRLRVS ATFWHNPRNH FRCQVQFHGL SEEDKWPEGS PKPVTQNISA**
 251 **EAWGRADCGI TSASYQQGVL SATILYEILL GKATLYAVLV STLVMAMVK**
 40 301 **RKNS**

The constant region (**Cβ**) (bolded) of mTCR-1, 2, 3, 6, 8, 10, 11, 17, and 38β chains are the same, and they are identical to the hgp100 specific TCR β chain C-region from HLA-A2 Tg mice.

In certain embodiments, the leading methionine and/or signal sequence is cleaved in the post-translationally modified protein.

Another embodiment provides mTCR-3 β chain (TRBV13-1*01/TRBJ2-4*01/TRBC1) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:25)

```

1      MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLS C RQTNSHNYMY
51     WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FLLLELASP
101    SQTSLYFCAS SDAGTQNTL YFGAGTRLSV LEDLRNVTPP KVSLEFESKA
151    EIANKRKATL VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS
201    YCLSSRLRVS ATFWHNPRNH FRCQVQFHGL SEEDKWPEGS PKPVTQNISA
251    EAWGRADCGI TSASYQQGVL SATILYEILL GKATLYAVLV STLVVMAMVK
301    RKNS

```

Another embodiment provides mTCR-6 β chain (TRBV13-1*01/TRBJ2-4*01/TRBC1) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:26)

```

1      MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLS C RQTNSHNYMY
51     WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FLLLELASP
101    SQTSLYFCAS CDAGVSQNTL YFGAGTRLSV LEDLRNVTPP KVSLEFESKA
151    EIANKRKATL VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS
201    YCLSSRLRVS ATFWHNPRNH FRCQVQFHGL SEEDKWPEGS PKPVTQNISA
251    EAWGRADCGI TSASYQQGVL SATILYEILL GKATLYAVLV STLVVMAMVK
301    RKNS

```

Another embodiment provides mTCR-10 β chain (TRBV13-1*01/TRBJ2-4*01/TRBC1) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:27)

```

1      MGSRLFLVLS LLCTKHMEAA VTQSPRNKVT VTGGNVTLS C RQTNSHNYMY
51     WYRQDTGHGL RLIHYSYGAG NLQIGDVPDG YKATRTTQED FLLLELASP
101    SQTSLYFCAS SDHGTGQNTL YFGAGTRLSV LEDLRNVTPP KVSLEFESKA
151    EIANKRKATL VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS
201    YCLSSRLRVS ATFWHNPRNH FRCQVQFHGL SEEDKWPEGS PKPVTQNISA
251    EAWGRADCGI TSASYQQGVL SATILYEILL GKATLYAVLV STLVVMAMVK
301    RKNS

```

In certain embodiments, the leading methionine and/or signal sequence is cleaved in the post-translationally modified protein.

In one embodiment, based on the nomenclature on IMGT and Uniprot website, the mTCR α chain features are as follows:

1. M1-M17: Leader Sequences to be removed on maturation of mTCR β chain
2. E18-S304: mTCR β chain
3. E18-L131: mTCR β chain V-region (TRBV13-1*01/TRBJ2-4*01 with S113 (TCR-1&2) or A113 (TCR-3) -A116 as the D region)

4. E132-S304: mTCR β chain C1-region of HLA-A mTCR specific for hgp100 (DQ452620)
5. E18-Y276: mTCR β chain extracellular domain
6. E277-M298: transmembrane region of mature mTCR β chain
- 5 7. V299-S304: intracellular (topological) region of mature mTCR β chain

5. Protein sequences of CDR3 region of mTCR-1, 2, 3, 6, 8, 10, 11, 17, and 38 α chains

One embodiment provides mTCR-1 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:28):

10 AASITNAYKVIFGKGTHLHVLPNIQNPE

Another embodiment provides mTCR-2 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:29):

AASTVNAYKVIFGKGTHLHVLPNIQNPE

One embodiment provides mTCR-3 α chain CDR3 region having 90%, 95%, 99%, or 15 100% sequence identity to (SEQ ID NO:30):

AASMAGGYKVVFGSGTRLLVSPDIQNPE

One embodiment provides mTCR-6 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:31):

AASMINAYKVIFGKGTHLHVLPNIQNPE

20 Another embodiment provides mTCR-8 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:32):

AASISGGYKVVFGSGTRLLVSPDIQNPE

One embodiment provides mTCR-10 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:33):

25 AASIVGGYKVVFGSGTRLLVSPDIQNPE

One embodiment provides mTCR-11 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:34):

AASKTGGYKVVFGSGTRLLVSPDIQNPE

30 Another embodiment provides mTCR-17 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:35):

AASMTGGYKVVFGSGTRLLVSPDIQNPE

One embodiment provides mTCR-38 α chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:36):

AATLTGGYKVVFGSGTRLLVSPDIQNPE

6. Protein sequences of CDR3 region of mTCR-1, 3, 6, and 10 β chains

One embodiment provides mTCR-1 β chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:37):

ASSDAGTSQNTLYFGAGTRLSVL

One embodiment provides mTCR-3 β chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:38):

ASSDAGTAQNTLYFGAGTRLSVL

Another embodiment provides mTCR-6 β chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:39):

ASSDAGVSQNTLYFGAGTRLSVL

One embodiment provides mTCR-10 β chain CDR3 region having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:40):

ASSDHGTGQNTLYFGAGTRLSVL

7. The extracellular domain of mTCR-1, 2, 3, 6, 8, 10, 11, 17 and 38 α chains (for Soluble TCRs) (underlined are the V α)

The extracellular domain of TCR α and β chains can form soluble TCRs. If they are labeled with detectable labels, for example fluorescent molecules, they can be used to detect the HLA-A2/hAFP₁₅₈ complex presented on tumor cells. The labeled soluble TCRs have the potential of as diagnosis reagents for detecting circulating tumor cells in the blood and to determine the cognate antigen epitope presentation in tumor tissues, which is important for the success of using such TCR-T cells in the setting of adoptive cell transfer therapy.

One embodiment provides the soluble mTCR-1 α chain (Q21-L242) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:41):

QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA ASITNAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD STLCLFTDFD
SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
FETDMNLNFQ NL

Another embodiment provides soluble mTCR-2 α chain (Q21-L242) having at least 90%, 95%, 99%, 100% to (SEQ ID NO:42):

5 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA AS**TV**NAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

Another embodiment provides soluble mTCR-3 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:43):

10 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA AS**MAGGYKVV FGSGTRLLVS**PDIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

15 Another embodiment provides soluble mTCR-6 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:44):

20 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA ASMINAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

Another embodiment provides soluble mTCR-8 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:45):

25 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA ASISGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

Another embodiment provides soluble mTCR-10 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:46):

30 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA ASIVGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

35 Another embodiment provides soluble mTCR-11 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:47):

40 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL
HTSLHIRDSQ PSDSALYLCA ASKTGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD STLCLFTDFD
 SQINVPKTME SGTFITDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV PCDATLTEKS
 FETDMNLNFQ NL

One embodiment provides soluble mTCR-17 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:48):

1 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI
 51 FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ PSDSALYLCA ASMTGGYKVV
 101 FGSSTRLLVS PDIQNPEPAV YQLKDPRSQD STLCLFTDFD SQINVPKTME
 151 SGTFTDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV
 5 201 PCDATLTEKS FETDMNLNFQ NL

Another embodiment provides soluble mTCR-38 α chain (Q21-L242) having 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:49):

10 1 QQKVQQSPES LIVPEGGMAS LNCTSSDRNV DYFWWYRQHS GKSPKMLMSI
 51 FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ PSDSALYLCA ATLTTGGYKVV
 101 FGSSTRLLVS PDIQNPEPAV YQLKDPRSQD STLCLFTDFD SQINVPKTME
 151 SGTFTDKTV LDMKAMDSKS NGAIAWSNQT SFTCQDIFKE TNATYPSSDV
 201 PCDATLTEKS FETDMNLNFQ NL

15 **8. The extracellular domain of TCR-1, 2, 3, 6, 8, 10, and 11 β chains** (underlined are the V β)

One embodiment provides soluble mTCR-1, 2, 8, 11 β chain (E18-Y276) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:50):

EAA VTQSPRNKVT VTGGNVTLSG RQTNSHNYMY WYRQDTGHGL RLIHYSYGAG NLQIGDVDPG YKATRTTQED
 20 FFLLELLEASP SQTSLYFCAS SDAGTSQNTL YFGAGTRLSV LEDLRNVTPP KVSLEFESKA EIANKRKATL
 VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS YCLSSRLRVS ATFWHNP RNH FRCQVQFHGL
 SEEDKWPEGS PKPVTQNISA EAWGRADCGI TSASYQQGVL SATILY

Still another embodiment soluble mTCR-3 β chain (E18-Y276) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:51):

EAA VTQSPRNKVT VTGGNVTLSG RQTNSHNYMY WYRQDTGHGL RLIHYSYGAG NLQIGDVDPG YKATRTTQED
 20 FFLLELLEASP SQTSLYFCAS SDAGT**A**QNTL YFGAGTRLSV LEDLRNVTPP KVSLEFESKA EIANKRKATL
 VCLARGFFPD HVELSWWVNG KEVHSGVSTD PQAYKESNYS YCLSSRLRVS ATFWHNP RNH FRCQVQFHGL
 SEEDKWPEGS PKPVTQNISA EAWGRADCGI TSASYQQGVL SATILYEILL

30

Another embodiment soluble mTCR-6 β chain (E18-Y276) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:52):

EA AVTQSPRN KVTVTGGNVT LSCRQTNSHN YMYWYRQDTG HGLRLIHYSY GAGNLQIGDV PDGYKATRTT
 QEDFFLLLEL ASPSQTSLYF CASCDAGVSQ NTLYFGAGTR LSVLEDLRNV TPPKVSLEFEP SKAEIANKRK
 35 ATLVCLARGF FPDHVELSWW VNGKEVHSGV STDPQAYKES NYSYCLSSRL RVSATFWHNP RNHFRCQVQF
 HGLSEEDKWP EGSPKPVTON ISAEAWGRAD CGITSASYQQ GVLSATILY

Another embodiment soluble mTCR-10 β chain (E18-Y276) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:53):

EA AVTQSPRN KVTVTGGNVT LSCRQTNSHN YMYWYRQDTG HGLRLIHYSY GAGNLQIGDV PDGYKATRTT
 40 QEDFFLLLEL ASPSQTSLYF CASSDHGTGQ NTLYFGAGTR LSVLEDLRNV TPPKVSLEFEP SKAEIANKRK
 ATLVCLARGF FPDHVELSWW VNGKEVHSGV STDPQAYKES NYSYCLSSRL RVSATFWHNP RNHFRCQVQF
 HGLSEEDKWP EGSPKPVTON ISAEAWGRAD CGITSASYQQ GVLSATILY

9. Fusion protein sequences of designed mTCRs

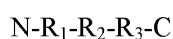
Fusion Proteins

In another embodiment, fusion proteins are provided that contain a first polypeptide domain and a second polypeptide. The fusion proteins can optionally contain a targeting domain
 5 that targets the fusion protein specific cells or tissues, for example the tumor cells or tumor cell-associated neovasculature.

The fusion proteins also optionally contain a peptide or polypeptide linker domain that separates the first polypeptide domain from the antigen-binding domain.

Fusion proteins disclosed herein are of formula I:

10



wherein “N” represents the N-terminus of the fusion protein, “C” represents the C-terminus of the fusion protein, “R₁” is one of the disclosed α mTCR chains or a fragment thereof. “R₂” is a
 15 peptide/polypeptide linker domain, and “R₃” is a β mTCR chain or fragment thereof. In an alternative embodiment, R₁ is a β mTCR chain or fragment thereof and R₃ is an α mTCR chain.

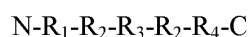
Optionally, the fusion proteins additionally contain a domain that functions to dimerize or multimerize two or more fusion proteins. The domain that functions to dimerize or multimerize the fusion proteins can either be a separate domain, or alternatively can be contained within one
 20 of one of the other domains of the fusion protein.

The fusion proteins can be dimerized or multimerized. Dimerization or multimerization can occur between or among two or more fusion proteins through dimerization or multimerization domains. Alternatively, dimerization or multimerization of fusion proteins can occur by chemical crosslinking. The dimers or multimers that are formed can be
 25 homodimeric/homomultimeric or heterodimeric/heteromultimeric.

The modular nature of the fusion proteins and their ability to dimerize or multimerize in different combinations provides a wealth of options for targeting molecules that function to enhance an immune response to the tumor cell microenvironment.

Another embodiment provides a fusion protein according to formula II,

30



wherein “N” represents the N-terminus of the fusion protein, “C” represents the C-terminus of the fusion protein, “R₁” is one of the disclosed α mTCR chains or a fragment thereof. “R₂” is a peptide/polypeptide linker domain, and “R₃” is a β mTCR chain or fragment thereof and “R₄” is an anti-CD3 single chain antibody. Anti-CD3 single chain antibodies are known in the art and are commercially available.

One embodiment provides fusion protein of designed mTCR-1 (α chain is underlined, P2A is bolded, and β chain is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:54):

```

1      MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51     DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101    PSDSALYLCA ASITNAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD
151    STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
15     201    SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251    LLKVAGFNLL MTLRLWSSGS RAKRSGSGAT NFSLLKQAGD VEENPGPRMG
301    SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
351    RQDTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTQEDFF LLELASPSQ
401    TSLYFCASSD AGTSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
20     451    ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
501    LSSRLRVSAT FWHNPRNHFR COVQFHGLSE EDKWPEGSPK PVTQNISAEA
551    WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVMAMVKRK
601    NS

```

The α chain is underlined, the β chain is double underlined, and the P2A and furin cleavage site (from US2016/0137715 A1) is in bold.

Another embodiment provides designed mTCR-2 (α chain, P2A, and β chain) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:55)

```

30     1      MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51     DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101    PSDSALYLCA ASTVNAYKVI FGKGTHLHVL PNIQNPEPAV YQLKDPRSQD
151    STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201    SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
35     251    LLKVAGFNLL MTLRLWSSGS RAKRSGSGAT NFSLLKQAGD VEENPGPRMG
301    SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
351    RQDTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTQEDFF LLELASPSQ
401    TSLYFCASSD AGTSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
451    ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
40     501    LSSRLRVSAT FWHNPRNHFR COVQFHGLSE EDKWPEGSPK PVTQNISAEA
551    WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVMAMVKRK
601    NS

```

Another embodiment provides designed mTCR-3 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:56):

```

1      MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
5 51     DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101    PSDSALYLCA ASMAGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151    STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201    SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251    LLKVAGFNLL MTLRLWSSGS RAKRSGSGAT NFSLLKQAGD VEENPGPRMG
10 301   SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
351    RQDTGHGLRL IHYSYGAGNL QIGDVDPGYK ATRTTOEDFF LLELASPSQ
401    TSLYFCASSD AGTAQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
451    ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
501    LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQNISAEA
15 551   WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVMAMVKRK
601    NS

```

Another embodiment provides designed mTCR-6 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:57):

```

20      1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51     DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101    PSDSALYLCA ASMINAYKVI FGKGTHLHVL PNIONPEPAV YQLKDPRSQD
151    STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
201    SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
25 251   LLKVAGFNLL MTLRLWSSGS RAKRSGSGAT NFSLLKQAGD VEENPGPRMG
301   SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
351   RQDTGHGLRL IHYSYGAGNL QIGDVDPGYK ATRTTOEDFF LLELASPSQ
401   TSLYFCASSD AGVSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
451   ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
30 501   LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQNISAEA
551   WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVMAMVKRK
601   NS

```

Another embodiment provides designed mTCR-8 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:58):

```

35      1 MKSFSISLVV LWLQLNWVNS QQKVQQSPES LIVPEGGMAS LNCTSSDRNV
51     DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
101    PSDSALYLCA ASISGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
151    STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNQT
40 201    SFTCQDIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
251    LLKVAGFNLL MTLRLWSSGS RAKRSGSGAT NFSLLKQAGD VEENPGPRMG
301   SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
351   RQDTGHGLRL IHYSYGAGNL QIGDVDPGYK ATRTTOEDFF LLELASPSQ
401   TSLYFCASSD AGTSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
45 451   ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
501   LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQNISAEA
551   WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVMAMVKRK
601   NS

```

Another embodiment provides designed mTCR-10 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:59):

1 MKSFSISLVV LWLQLNWNVS QOKVQOSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 5 101 PSDSALYLCA ASIVGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
 151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNOT
 201 SFTCODIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
 251 LLKVAGFNLL MTLRLWSSGS **RAKRSGSGAT NFSLLKQAGD VEENPGPRMG**
 301 SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
 10 351 RQDTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTOEDFF LLELASPSQ
 401 TSLYFCASSD HGTGQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
 451 ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
 501 LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQONISAEA
 551 WGRADCGITS ASYQOGVLSA TILYEILLGK ATLYAVLVST LVVMAMVKRK
 15 601 NS

Another embodiment provides designed mTCR-11 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:60):

1 MKSFSISLVV LWLQLNWNVS QOKVQOSPES LIVPEGGMAS LNCTSSDRNV
 20 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASKTGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
 151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNOT
 201 SFTCODIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
 25 251 LLKVAGFNLL MTLRLWSSGS **RAKRSGSGAT NFSLLKQAGD VEENPGPRMG**
 301 SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
 351 RQDTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTOEDFF LLELASPSQ
 401 TSLYFCASSD AGTSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
 451 ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
 501 LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQONISAEA
 30 551 WGRADCGITS ASYQOGVLSA TILYEILLGK ATLYAVLVST LVVMAMVKRK
 601 NS

One embodiment provides designed mTCR-17 (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:61):

35 1 MKSFSISLVV LWLQLNWNVS QOKVQOSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 101 PSDSALYLCA ASMTGGYKVV FGSGTRLLVS PDIQNPEPAV YQLKDPRSQD
 151 STLCLFTDFD SQINVPKTME SGTFTIDKTV LDMKAMDSKS NGAIAWSNOT
 201 SFTCODIFKE TNATYPSSDV PCDATLTEKS FETDMNLNFQ NLSVMGLRIL
 40 251 LLKVAGFNLL MTLRLWSSGS **RAKRSGSGAT NFSLLKQAGD VEENPGPRMG**
 301 SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRQ TNSHNYMYWY
 351 RQDTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTOEDFF LLELASPSQ
 401 TSLYFCASSD AGTSQNTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
 451 ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPO AYKESNYSYC
 45 501 LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQONISAEA
 551 WGRADCGITS ASYQOGVLSA TILYEILLGK ATLYAVLVST LVVMAMVKRK
 601 NS

One embodiment provides designed **mTCR-38** (α chain, P2A, and β chain) having at least 90%, 95%, 99% or 100% sequence identity to (SEQ ID NO:62):

1 MKSFSISLVV LWLQLNWNVS OOKVQOSPES LIVPEGGMAS LNCTSSDRNV
 51 DYFWWYRQHS GKSPKMLMSI FSNGEKEEGR FTVHLNKASL HTSLHIRDSQ
 5 101 PSDSALYLCA ATLTGGYKVV FGSGTRLLVS PDIONPEPAV YQLKDPRSOD
 151 STLCLFTDFD SQINVPKME SGTFTDKTV LDMKAMDSKS NGAIASNOT
 201 SFTCODIFKE TNATYPSSDV PCDATLTEKS FETDMNLFQ NLSVMGLRL
 251 LLKVAGFNLL MTLRLWSSGS **RAKRSGSGAT NFSLLKQAGD VEENPGPRMG**
 301 SRLFLVLSLL CTKHMEAAVT QSPRNKVTVT GGNVTLSCRO TNSHNYMYWY
 10 351 RODTGHGLRL IHYSYGAGNL QIGDVPDGYK ATRTTOEDFF LLELASPSQ
 401 TSLYFCASSD AGTSONTLYF GAGTRLSVLE DLRNVTPPKV SLFEPKAEI
 451 ANKRKATLVC LARGFFPDHV ELSWWVNGKE VHSGVSTDPQ AYKESNYSYC
 501 LSSRLRVSAT FWHNPRNHFR CQVQFHGLSE EDKWPEGSPK PVTQNISAEA
 551 WGRADCGITS ASYQQGVLSA TILYEILLGK ATLYAVLVST LVVMAMVKRK
 15 601 NS

10. Nucleotide sequences of the designed TCR-1, TCR-2, TCR-3, TCR-6, TCR-8, TCR-10, TCR-11, TCR-17, and TCR-38:

The sequences are based on the above protein sequences and codon optimized for expression in human cells.

One embodiment provides a designed mTCR-1 (α is underlined, P2A is bolded, and β is double underlined) having a nucleic acid sequence that is at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:63):

25 1 ATGAAATCTT TTAGCATCTC CCTGGTCGTC CTGTGGCTGC AGCTGAATTG GGTGAATAGT
 61 CAGCAGAAGG TCCAGCAGTC CCCCAGATCC CTGATCGTGC CTGAGGGCGG CATGGCCTCT
 121 CTGAACTGCA CCAGCTCCGA CCGGAATGTG GATTATTCTT GGTGGTACAG ACAGCACTCT
 181 GGCAAGAGCC CAAAGATGCT GATGTCCATC TTCTCTAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCTAGCCTG CACACCTCCC TGCACATCAG AGACTCCAG
 30 301 CCCTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCTATCA CAAACGCCTA CAAAGTGATC
 361 TTCCGCAAGG GAACCCACCT GCACGTGCTG CCTAACATCC AGAATCCAGA GCCCGCCGTG
 421 TATCAGCTGA AGGACCCACG GAGCCAGGAT AGCACCCTGT GCCTGTTCAC CGACTTTGAT
 481 AGCCAGATCA ATGTGCCTAA GACAATGGAG TCCGGCACCT TTATCACAGA CAAGACCGTG
 541 CTGGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCCTGGTC TAATCAGACA
 35 601 AGCTTCACCT GCCAGGATAT CTTTAAGGAG ACAAACGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCCTGAC AGAGAAGAGC TTCGAGACAG ACATGAACCT GAATTTTCAG
 721 AACCTGTCCG TGATGGGCCT GAGAATCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGC GCCTGTGGTC CTCTGGCTCT **AGGGCAAAGC GGAGCGGCAG CGGAGCAACC**
 841 **AACTTCAGCC TGCTGAAGCA GGCCGGCGAT GTGGAGGAGA ATCCTGGCCC ACGGATGGGC**
 40 901 TCTAGACTGT TTCTGGTGCT GACCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 961 CAGTCCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGAG CTGTAGACAG
 1021 ACCAACTCCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGACACGG CCTGAGACTG
 1081 ATCCACTATA GCTACGGCGC CGGCAATCTG CAGATCGGCG ACGTGCCAGA TGGCTATAAG
 1141 GCCACAAGGA CCACACAGGA GGAATTCTTT CTGCTGCTGG AGCTGGCCTC CCCCTCTCAG
 45 1201 ACCTCTCTGT ATTTCTGCGC CAGCTCCGAT GCCGGCACAA GCCAGAACAC CCTGTACTTT
 1261 GGAGCAGGAA CAAGGCTGTC CGTCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 1321 TCCCTGTTTCG AGCCTTCTAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC AGATCACGTG GAGCTGTCCT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCTG GCGTGAGCAC AGACCCCCAG GCCTACAAGG AGTCCAATA TTCTTACTGC

1501 CTGTCTAGCC GGCTGAGAGT GAGCGCCACC TTTTGGCACA ACCCAGGAA TCACTTCCGC
 1561 TGTCAGGTGC AGTTTCACGG CCTGTCCGAG GAGGATAAGT GGCCTGAGGG CTCTCCCAAG
 1621 CCTGTGACAC AGAACATCAG CGCCGAGGCA TGGGGAAGGG CAGACTGTGG CATCACCAGC
 1681 GCCTCCTATC AGCAGGGCGT GCTGAGCGCC ACAATCCTGT ACGAGATCCT GCTGGGCAAG
 5 1741 GCCACCCTGT ATGCTGTGCT GGTGCAACT CTGGTGGTCA TGGCTATGGT GAAACGGAAA
 1801 AACTCCTAA

Another embodiment provides a designed mTCR-2 (α is under lined, P2A is bolded, and β double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:64):

10 1 ATGAAATCTT TTAGCATCTC CCTGGTCGTC CTGTGGCTGC AGCTGAATTG GGTGAATAGT
 61 CAGCAGAAGG TCCAGCAGTC CCCCGAGTCC CTGATCGTGC CTGAGGGCGG CATGGCCTCT
 121 CTGAACTGCA CCAGCTCCGA CCGGAATGTG GATTATTTCT GGTGGTACAG ACAGCACTCT
 181 GGCAAGAGCC CAAAGATGCT GATGTCCATC TTCTCTAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCTAGCCTG CACACCTCCC TGCACATCAG AGACTCCCAG
 15 301 CCCTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCTACCG TGAACGCCTA CAAAGTGATC
 361 TTGCGCAAGG GAACCCACCT GCACGTGCTG CCTAACATCC AGAATCCAGA GCCCGCCGTG
 421 TATCAGCTGA AGGACCCACG GAGCCAGGAT AGCACCCTGT GCCTGTTCAC CGACTTTGAT
 481 AGCCAGATCA ATGTGCCTAA GACAAATGGAG TCCGGCACCT TTATCACAGA CAAGACCGTG
 541 CTGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCTTGGTC TAATCAGACA
 20 601 AGCTTCACCT GCCAGGATAT CTTTAAGGAG ACAAACGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCCTGAC AGAGAAGAGC TTGAGACAG ACATGAACCT GAATTTTCAG
 721 AACCTGTCCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGC GCCTGTGGTC CTCTGGCTCT **AGGGCAAAGC** **GGAGCGGCAG** **CGGAGCAACC**
 841 **AACTTCAGCT** **TGCTGAAGCA** **GGCCGGCGAT** **GTGGAGGAGA** **ATCCTGGCCC** **ACGGATGGGC**
 25 901 TCTAGACTGT TTCTGGTGCT GAGCCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 961 CAGTCCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGAG CTGTAGACAG
 1021 ACCAACTCCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGACACGG CCTGAGACTG
 1081 ATCCACTATA GCTACGGCGC CGGCAATCTG CAGATCGGCG ACGTGCCAGA TGGCTATAAG
 1141 GCCACAAGGA CCACACAGGA GGACTTCTTT CTGCTGCTGG AGTGGCCTC CCCCTCTCAG
 30 1201 ACCTCTCTGT ATTTCTGCGC CAGCTCCGAT GCCGGCACAA GCCAGAACAC CCTGTACTTT
 1261 GGAGCAGGAA CAAGGCTGTC CGTGCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 1321 TCCCTGTTTC AGCCTTCTAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC AGATCACGTG GAGCTGTCTT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCTG GCGTGAGCAC AGACCCCCAG GCCTACAAGG AGTCCAACCTA TTCTTACTGC
 35 1501 CTGTCTAGCC GGCTGAGAGT GAGCGCCACC TTTTGGCACA ACCCAGGAA TCACTTCCGC
 1561 TGTCAGGTGC AGTTTCACGG CCTGTCCGAG GAGGATAAGT GGCCTGAGGG CTCTCCCAAG
 1621 CCTGTGACAC AGAACATCAG CGCCGAGGCA TGGGGAAGGG CAGACTGTGG CATCACCAGC
 1681 GCCTCCTATC AGCAGGGCGT GCTGAGCGCC ACAATCCTGT ACGAGATCCT GCTGGGCAAG
 1741 GCCACCCTGT ATGCTGTGCT GGTGCAACT CTGGTGGTCA TGGCTATGGT GAAACGGAAA
 40 1801 AACTCCTAA

Another embodiment provides a designed mTCR-3 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:65):

45 1 ATGAAATCTT TTAGCATCTC CCTGGTCGTC CTGTGGCTGC AGCTGAATTG GGTGAATAGT
 61 CAGCAGAAGG TCCAGCAGTC CCCCGAGTCC CTGATCGTGC CTGAGGGCGG CATGGCCTCT
 121 CTGAACTGCA CCAGCTCCGA CCGGAATGTG GATTATTTCT GGTGGTACAG ACAGCACTCT
 181 GGCAAGAGCC CAAAGATGCT GATGTCCATC TTCTCTAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCTAGCCTG CACACCTCCC TGCACATCAG AGACTCCCAG
 50 301 CCCTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCTATGG CCGGCGGCTA CAAAGTGGTG
 361 TTGCGCAGCG GAACCCGGCT GCTGGTGAGC CCTGACATCC AGAATCCAGA GCCCGCCGTG

421 TATCAGCTGA AGGACCCACG GAGCCAGGAT AGCACCTGT GCCTGTTTAC CGACTTTGAT
 481 AGCCAGATCA ATGTGCCTAA GACAATGGAG TCCGGCACCT TTATCACAGA CAAGACCGTG
 541 CTGGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCCTGGTC TAATCAGACA
 601 AGCTTCACCT GCCAGGATAT CTTTAAGGAG ACAAACGCCA CCTACCCATC TAGCGACGTG
 5 661 CCCTGTGATG CCACCTGAC AGAGAAGAGC TTCGAGACAG ACATGAACCT GAATTTTCAG
 721 AACCTGTCCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGC GCCTGTGGTC CTCTGGCTCT **AGGGCAAAGC** **GGAGCGGCAG** **CGGAGCAACC**
 841 **AACTTCAGCC** **TGCTGAAGCA** **GGCCGGCGAT** **GTGGAGGAGA** **ATCCTGGCCC** **ACGGATGGGC**
 901 TCTAGACTGT TTCTGGTGCT GAGCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 10 961 CAGTCCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGAG CTGTAGACAG
 1021 ACCAACTCCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGACACGG CCTGAGACTG
 1081 ATCCACTATA GCTACGGCGC CGGCAATCTG CAGATCGGCG ACGTGCCAGA TGGCTATAAG
 1141 GCCACAAGGA CCACACAGGA GGAATCTTTT CTGCTGCTGG AGCTGGCCTC CCCCTCTCAG
 1201 ACCTCTCTGT ATTTCTGCGC CAGCTCCGAT GCCGGCACAG CCCAGAACAC CCTGTACTTT
 15 1261 GGAGCAGGAA CAAGGCTGTC CGTGCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 1321 TCCCTGTTTC AGCCTTCTAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC AGATCACGTG GAGCTGTCTT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCTG GCGTGAGCAC AGACCCCCAG GCCTACAAGG AGTCCAACTA TTCTTACTGC
 1501 CTGTCTAGCC GGCTGAGAGT GAGCGCCACC TTTTGGCACA ACCCAGGAA TCACTTCCGC
 20 1561 TGTCAAGTGC AGTTTCACGG CCTGTCCGAG GAGGATAAGT GGCCTGAGGG CTCTCCCAG
 1621 CCTGTGACAC AGAACATCAG CGCCGAGGCA TGGGGAAGGG CAGACTGTGG CATCACCAGC
 1681 GCCTCCTATC AGCAGGGCGT GCTGAGCGCC ACAATCCTGT ACGAGATCCT GCTGGGCAAG
 1741 GCCACCCTGT ATGCTGTGCT GGTGCTCACT CTGGTGGTCA TGGCTATGGT GAAACGGAAA
 1801 AACTCCTAA

25 Another embodiment provides a designed mTCR-6 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:66):

1 ATGAAGTCCT TCTCTATCAG CCTGGTGGTG CTGTGGCTGC AGCTGAACTG GGTGAATAGC
 61 CAGCAGAAGG TGCAGCAGTC TCCTGAGAGC CTGATCGTGC CAGAGGGCGG CATGGCCTCC
 30 121 CTGAAGTGCA CCAGCTCCGA CCGGAATGTG GATTATTTTT GGTGGTACAG ACAGCACTCC
 181 GGCAAGTCTC CCAAGATGCT GATGAGCATC TTCTCCAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCCTCTCTG CACACCAGCC TGCACATCAG AGACTCCAG
 301 CCTTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCTATGA TCAATGCCTA CAAAGTGATC
 361 TTCCGGCAAGG GCACACACCT GCACGTGCTG CCCAACATCC AGAATCCAGA GCCCGCCGTG
 35 421 TATCAGCTGA AGGACCTCG GTCTCAGGAT AGCACCTGT GCCTGTTTAC CGACTTTGAT
 481 AGCCAGATCA ATGTGCCAAA GACCATGGAG TCCGGCACCT TTATCACAGA CAAGACCGTG
 541 CTGGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCCTGGTC CAATCAGACA
 601 TCTTTCACCT GCCAGGATAT CTTTAAGGAG ACAAACGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCTGAC AGAGAAGAGC TTCGAGACCG ACATGAACCT GAATTTTCAG
 40 721 AACCTGTCCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGC GCCTGTGGTC CTCTGGCTCT **AGGGCAAAGC** **GGAGCGGCAG** **CGGAGCAACC**
 841 **AACTTCAGCC** **TGCTGAAGCA** **GGCCGGCGAT** **GTGGAGGAGA** **ATCCTGGCCC** **ACGGATGGGC**
 901 TCCAGACTGT TTCTGGTGCT GTCTGTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 961 CAGAGCCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGTC TTGTAGACAG
 45 1021 ACCAACAGCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGACACGG CCTGAGACTG
 1081 ATCCACTATT CCTACGGAGC AGGAAACCTG CAGATCGGCG ACGTGCCTGA TGGCTACAAG
 1141 GCCACAAGAA CCACACAGGA GGAATCTTTT CTGCTGCTGG AGCTGGCCTC CCCATCTCAG
 1201 ACCTCTCTGT ATTTCTGCGC AAGCTCCGAT GCAGGCGTGA GCCAGAACAC ACTGTACTTT
 1261 GGAGCAGGAA CCAGGCTGAG CGTGCTGGAG GACCTGCGCA ATGTGACACC CCCTAAGGTG
 50 1321 AGCCTGTTTC AGCCCTCCAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC TGATCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCCG GCGTGTCTAC AGACCCACAG GCCTATAAGG AGAGCAACTA TTCCTACTGC

1501 CTGTCTAGCC GGCTGAGAGT GTCCGCCACC TTTGGGCACA ACCCAAGGAA TCACTTCCGC
 1561 TGTCAGGTGC AGTTTCACGG CCTGAGCGAG GAGGATAAGT GGCCAGAGGG CTCCCCAAAG
 1621 CCTGTGACCC AGAATATCTC TGCCGAGGCA TGGGGAAGGG CAGACTGTGG AATCACAAGC
 1681 GCCTCTTACC AGCAGGGCGT GCTGTCCGCC ACCATCCTGT ATGAGATCCT GCTGGGCAAG
 5 1741 GCCACACTGT ACGCCGTGCT GGTGTCACC CTGGTGGTCA TGGCCATGGT GAAGCGCAAG
 1801 AACTCTTGA

Another embodiment provides a designed mTCR-8 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID

10 NO:67):

1 ATGAAGTCCT TCTCTATCAG CCTGGTGGTG CTGTGGCTGC AGCTGAACTG GGTGAATTCT
 61 CAGCAGAAGG TGCAGCAGTC CCCTGAGTCT CTGATCGTGC CAGAGGGCGG CATGGCCTCC
 121 CTGAAGTGCA CCAGCTCCGA CCGGAATGTG GATTATTTTT GGTGGTACAG ACAGCACAGC
 181 GGCAAGTCCC CCAAGATGCT GATGTCTATC TTCAGCAACG GCGAGAAGGA GGAGGGCCGG
 15 241 TTTACAGTGC ACCTGAATAA GGCCTCCCTG CACACCTCTC TGCACATCAG AGACAGCCAG
 301 CCTTCCGATT CTGCCCTGTA TCTGTGCGCA GCAAGCATCT CCGGAGGATA CAAGGTGGTG
 361 TTCGGCAGCG GAACAAGGCT GCTGGTGTC CCCGATATCC AGAATCCAGA GCCCGCCGTG
 421 TATCAGCTGA AGGACCCCTG CTCCCAGGAT AGCACCCCTGT GCCTGTTTAC CGACTTTGAT
 481 TCCAGATCA ACGTGCCAAA GACCATGGAG TCTGGCACCT TTATCACAGA CAAGACCGTG
 20 541 CTGGATATGA AGGCCATGGA CTCTAAGAGC AACGGCGCCA TGCCTGGAG CAATCAGACA
 601 TCCTTCACCT GCCAGGATAT CTTTAAGGAG ACAAATGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCCTGAC AGAGAAGTCT TTCGAGACCG ACATGAACCT GAATTTTCAG
 721 AACCTGAGCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGA GGCTGTGGTC **CTCTGGCTCC** **AGGGCAAAGC** **GGAGCGGCTC** **TGGAGCCACC**
 25 841 **AACTTCTCTC** **TGCTGAAGCA** **GGCAGGCGAC** **GTGGAGGAGA** **ATCCTGGACC** **ACGGATGGGC**
 901 TCTAGACTGT TTCTGGTGCT GAGCCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 961 CAGAGCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGTC CTGTAGACAG
 1021 ACCAACTCTC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGCCACGG CCTGAGACTG
 1081 ATCCACTATT CCTACGGAGC AGGAAACCTG CAGATCGGCG ACGTGCCTGA TGGCTACAAG
 30 1141 GCCACAAGGA CCACACAGGA GGACTTCTTT CTGCTGCTGG AGCTGGCCAG CCCATCCCAG
 1201 ACCAGCCTGT ATTTCTGCGC CAGCTCCGAT GCCGGCACAT CCCAGAACAC CCTGTACTTT
 1261 GGAGCAGGAA CAAGGCTGAG CGTGCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 1321 TCTCTGTTTC AGCCCAGCAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC TGATCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 35 1441 GTGCACAGCG GCGTGTCCAC AGACCCACAG GCCTATAAGG AGTCTAACTA TAGCTACTGC
 1501 CTGTCTAGCC GGCTGAGAGT GTCCGCCACC TTTGGGCACA ACCCAAGGAA TCACTTCCGC
 1561 TGTCAGGTGC AGTTTCACGG CCTGTCCGAG GAGGATAAGT GGCCAGAGGG CTCTCCAAG
 1621 CCTGTGACCC AGAATATCAG CGCCGAGGCA TGGGGAAGGG CAGACTGTGG CATCACATCT
 1681 GCCAGCTACC AGCAGGGCGT GCTGTCCGCC ACCATCCTGT ATGAGATCCT GCTGGGCAAG
 40 1741 GCCACACTGT ACGCCGTGCT GGTGAGCACC CTGGTGGTCA TGGCCATGGT GAAGAGAAAG
 1801 AACTCCTGA

Another embodiment provides a designed mTCR-10 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID

NO:68):

45 1 ATGAAGTCCT TCTCTATCAG CCTGGTGGTG CTGTGGCTGC AGCTGAACTG GGTGAATAGC
 61 CAGCAGAAGG TGCAGCAGTC TCCTGAGAGC CTGATCGTGC CAGAGGGCGG CATGGCCTCC
 121 CTGAAGTGCA CCAGCTCCGA CCGGAATGTG GATTATTTTT GGTGGTACAG ACAGCACTCC
 181 GGCAAGTCTC CCAAGATGCT GATGAGCATC TTCTCCAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCCTCTCTG CACACCAGCC TGCACATCAG AGACTCCAG
 50 301 CCTTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCTATCG TGGGCGGCTA CAAGGTGGTG
 361 TTCGGCTCCG GCACAAGGCT GCTGGTGCTT CCCGATATCC AGAATCCAGA GCCCGCCGTG

421 TATCAGCTGA AGGACCCTCG CTCTCAGGAT AGCACCCCTGT GCCTGTTTAC CGACTTTTAT
 481 TCTCAGATCA ACGTGCCAAA GACCATGGAG AGCGGCACCT TTATCACAGA CAAGACCCTG
 541 CTGGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCCTGGTC CAATCAGACA
 5 601 TCTTTCACCT GCCAGGATAT CTTTAAGGAG ACAAATGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCCTGAC AGAGAAGAGC TTCGAGACCG ACATGAACCT GAATTTTCAG
 721 AACCTGTCCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGC GCCTGTGGTC **CTCTGGCTCT** **AGGGCAAAGC** **GGAGCGGCAG** **CGGAGCAACC**
 841 **AACTTCAGCC** **TGCTGAAGCA** **GGCAGGCGAC** **GTGGAGGAGA** **ATCCTGGACC** **ACGGATGGGC**
 901 AGCAGACTGT TTCTGGTGCT GTCCCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 10 961 CAGAGCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGTC TTGTAGACAG
 1021 ACCAACAGCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGCCACGG CCTGAGACTG
 1081 ATCCACTATT CCTACGGAGC AGGAAACCTG CAGATCGGCG ACGTGCCTGA TGGCTACAAG
 1141 GCCACAAGGA CCACACAGGA GGACTTCTTT CTGCTGCTGG AGCTGGCCTC CCCATCTCAG
 1201 ACCAGCCTGT ATTTCTGCGC CAGCTCCGAT CACGGCACAG GCCAGAACAC CCTGTACTTT
 15 1261 GGAGCAGGAA CAAGGCTGTC CGTGCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 1321 AGCCTGTTTC AGCCCTCCAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC TGATCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCCG GCGTGTCTAC AGACCCACAG GCCTATAAGG AGAGCAACTA TTCTACTGTC
 1501 CTGTCTAGCC GGCTGAGAGT GTCCGCCACC TTTGGGCACA ACCCAAGGAA TCACTTCCGC
 20 1561 TGTCAGGTGC AGTTTCACGG CCTGTCTGAG GAGGATAAGT GGCCAGAGGG CAGCCCAAAG
 1621 CCTGTGACCC AGAATATCTC CGCCGAGGCA TGGGGAAGGG CAGACTGTGG AATCACAAGC
 1681 GCCTCCTACC AGCAGGGCGT GCTGAGCGCC ACCATCCTGT ATGAGATCCT GCTGGGCAAG
 1741 GCCACACTGT ACGCCGTGCT GGTGTCACC CTGGTGCTCA TGGCCATGGT GAAGAGAAAG
 1801 AACTCTTGA

25 Another embodiment provides a designed mTCR-11 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:69):

1 ATGAAGTCCT TCTCTATCAG CCTGGTGGTG CTGTGGCTGC AGCTGAACTG GGTGAATAGC
 61 CAGCAGAAGG TGCAGCAGTC TCCTGAGAGC CTGATCGTGC CAGAGGGCGG CATGGCCTCC
 30 121 CTGAACTGCA CCAGCTCCGA CCGGAATGTG GATTATTTTT GGTGGTACAG ACAGCACTCC
 181 GGCAAGTCTC CCAAGATGCT GATGAGCATC TTCTCCAACG GCGAGAAGGA GGAGGGCCGG
 241 TTTACAGTGC ACCTGAATAA GGCCTCTCTG CACACCAGCC TGCACATCAG AGACAGCCAG
 301 CCTTCCGATT CTGCCCTGTA TCTGTGCGCC GCCTCCAAGA CAGGCGGCTA CAAGGTGGTG
 361 TTCGGCTCCG GAACCAGGCT GCTGGTGTCT CCCGATATCC AGAATCCAGA GCCCGCCGTG
 35 421 TATCAGCTGA AGGACCCTCG CTCTCAGGAT AGCACCCCTGT GCCTGTTTAC CGACTTTTAT
 481 TCTCAGATCA ACGTGCCAAA GACAAATGGAG AGCGGCACCT TTATCACAGA CAAGACCCTG
 541 CTGGATATGA AGGCCATGGA CAGCAAGTCC AACGGCGCCA TCGCCTGGTC CAATCAGACA
 601 TCTTTCACCT GCCAGGATAT CTTTAAGGAG ACAAATGCCA CCTACCCATC TAGCGACGTG
 661 CCCTGTGATG CCACCCTGAC AGAGAAGTCT TTCGAGACCG ACATGAACCT GAATTTTCAG
 40 721 AACCTGAGCG TGATGGGCCT GAGAATCCTG CTGCTGAAGG TGGCCGGCTT CAATCTGCTG
 781 ATGACACTGA GGCTGTGGTC CTCTGGCTCC **AGGGCAAAGC** **GGAGCGGCAG** **CGGAGCAACC**
 841 **AACTTCTCTC** **TGCTGAAGCA** **GGCAGGCGAC** **GTGGAGGAGA** **ATCCTGGACC** **ACGGATGGGC**
 901 AGCAGACTGT TTCTGGTGCT GTCCCTGCTG TGCACAAAGC ACATGGAGGC AGCAGTGACC
 961 CAGAGCCCAC GGAACAAGGT GACCGTGACA GGCGGCAATG TGACACTGTC TTGTAGACAG
 45 1021 ACCAACAGCC ACAATTACAT GTATTGGTAC CGGCAGGATA CCGGCCACGG CCTGAGACTG
 1081 ATCCACTATT CCTACGGAGC AGGAAACCTG CAGATCGGCG ACGTGCCTGA TGGCTACAAG
 1141 GCCACAAGGA CCACACAGGA GGACTTCTTT CTGCTGCTGG AGCTGGCCTC CCCATCTCAG
 1201 ACCTCCCTGT ATTTCTGCGC CAGCTCCGAT GCCGGCACAT CTCAGAACAC CCTGTACTTT
 1261 GGAGCAGGAA CAAGGCTGAG CGTGCTGGAG GACCTGCGCA ATGTGACCCC CCCTAAGGTG
 50 1321 AGCCTGTTTC AGCCCTCCAA GGCCGAGATC GCCAACAAGA GGAAGGCCAC CCTGGTGTGC
 1381 CTGGCAAGGG GCTTCTTTCC TGATCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 1441 GTGCACTCCG GCGTGTCTAC AGACCCACAG GCCTATAAGG AGAGCAACTA TTCTACTGTC
 1501 CTGTCTAGCC GGCTGAGAGT GTCCGCCACC TTTGGGCACA ACCCAAGGAA TCACTTCCGC
 1561 TGTCAGGTGC AGTTTCACGG CCTGTCTGAG GAGGATAAGT GGCCAGAGGG CAGCCCAAAG

1621 CCTGTGACCC AGAATATCTC CGCCGAGGCA TGGGGAAGGG CAGACTGTGG AATCACAAGC
 1681 GCCTCCTACC AGCAGGGCGT GCTGAGCGCC ACCATCCTGT ATGAGATCCT GCTGGGCAAG
 1741 GCCACACTGT ACGCCGTGCT GGTGAGCACC CTGGTGGTCA TGGCCATGGT GAAGAGAAAG
 1801 AACTCCTGA

5 One embodiment provides a designed mTCR-17 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:70):

1 ATGAAATCCT TTAGTATTTT CCTAGTGGTC CTGTGGCTTC AGCTAAACTG GGTGAACAGC
 61 CAACAGAAGG TGCAGCAGAG CCCAGAATCC CTCATTGTTC CAGAGGGAGG CATGGCCTCT
 10 121 CTCAACTGCA CTTCCAGTGA TCGTAATGTT GACTACTTCT GGTGGTACAG ACAGCACTCT
 181 GGGAAAAGCC CCAAGATGCT GATGTCTATC TTCTCCAATG GTGAAAAGGA AGAAGGCAGA
 241 TTCACAGTTC ACCTCAATAA AGCCAGCCTG CATACTTCCC TGCACATCAG AGACTCCCAG
 301 CCCAGTGACT CTGCTCTCTA CCTCTGTGCA GCAACCTGA CTGGAGGCTA TAAAGTGGTC
 361 TTTGGAAGTG GGAATCGATT GCTGGTAAGC CCTGACATCC AGAAGCCAGA ACCTGCTGTG
 15 421 TACCAGTTAA AAGATCCTCG GTCTCAGGAC AGCACCCTCT GCCTGTTCAC CGACTTTGAC
 481 TCCCAAATCA ATGTGCCGAA AACCATGGAA TCTGGAACGT TCATCACTGA CAAAACCTGTG
 541 CTGGACATGA AAGCTATGGA TTCCAAGAGC AATGGGGCCA TTGCCTGGAG CAACCAGACA
 601 AGCTTCACCT GCCAAGATAT CTTCAAAGAG ACCAACGCCA CCTACCCAG TTCAGACGTT
 661 CCCTGTGATG CCACGTTGAC TGAGAAAAGC TTTGAAACAG ATATGAACCT AAACCTTCAA
 20 721 AACCTGTCAG TTATGGGACT CCGAATCCTC CTGCTGAAAG TAGCCGGATT TAACCTGCTC
 781 ATGACGCTGA GGCTGTGGTC CAGTGGCAGC AGAGCCAAGA GAAGCGGATC CGGCGCCACC
 841 AACTTCAGCC TGCTGAAGCA GGCCGGCGAC GTGGAGGAAA ACCCTGGCCC TAGGATGGGC
 901 TCCAGGCTCT TTCTGGTCTT GAGCCTCCTG TGTACAAAAC ACATGGAGGC TGCAGTCACC
 961 CAAAGCCCTA GAAACAAGGT GACAGTAACA GGAGGAAACG TGACATTGAG CTGTGCGCCAG
 25 1021 ACTAATAGCC ACAACTACAT GTACTGGTAT CGGCAGGACA CTGGGCATGG GCTGAGGCTG
 1081 ATCCATTACT CATATGGTGC TGGCAACCTT CAAATAGGAG ATGTCCCTGA TGGGTACAAG
 1141 GCCACCAGAA CAACGCAAGA AGACTTCTTC CTCCTGCTGG AATTGGCTTC TCCCTCTCAG
 1201 ACATCTTTGT ACTTCTGTGC CAGCAGTGAT GCAGGACAAA GTCAAAACAC CTTGTACTTT
 1261 GGTGCGGGCA CCCGACTATC GGTGCTAGAG GATCTGAGAA ATGTGACTCC ACCCAAGGTC
 30 1321 TCCTTGTGTTG AGCCATCAAA AGCAGAGATT GCAACAAAC GAAAGGCTAC CCTCGTGTGC
 1381 TTGGCCAGGG GCTTCTTCCC TGACCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 1441 GTCCACAGTG GGGTCAGCAC GGACCCTCAG GCCTACAAGG AGAGCAATTA TAGCTACTGC
 1501 CTGAGCAGCC GCCTGAGGGT CTCTGCTACC TTCTGGCACA ATCCTCGAAA CCACTTCCGC
 1561 TGCCAAGTGC AGTTCCATGG GCTTTCAGAG GAGGACAAGT GGCCAGAGGG CTCACCCAAA
 35 1621 CCTGTACAC AGAACATCAG TGCAGAGGCC TGGGGCCGAG CAGACTGTGG GATTACCTCA
 1681 GCATCCTATC AACAAAGGGT CTTGTCTGCC ACCATCCTCT ATGAGATCCT GTAGGGGAAA
 1741 GCCACCCTGT ATGCTGTGCT TGTCACTACA CTGGTGGTGA TGGCTATGGT CAAAAGAAAG
 1801 AATTCATGA

Another embodiment provides a designed mTCR-38 (α is underlined, P2A is bolded, and β is double underlined) having at least 90%, 95%, 99%, or 100% sequence identity to (SEQ ID NO:71):

1 ATGAAATCCT TTAGTATTTT CCTAGTGGTC CTGTGGCTTC AGCTAAACTG GGTGAACAGC
 61 CAACAGAAGG TGCAGCAGAG CCCAGAATCC CTCATTGTTC CAGAGGGAGG CATGGCCTCT
 121 CTCAACTGCA CTTCCAGTGA TCGTAATGTT GACTACTTCT GGTGGTACAG ACAGCACTCT
 45 181 GGGAAAAGCC CCAAGATGCT GATGTCTATC TTCTCCAATG GTGAAAAGGA AGAAGGCAGA
 241 TTCACAGTTC ACCTCAATAA AGCCAGCCTG CATACTTCCC TGCACATCAG AGACTCCCAG
 301 CCCAGTGACT CTGCTCTCTA CCTCTGTGCA GCAACCTGA CTGGAGGCTA TAAAGTGGTC
 361 TTTGGAAGTG GGAATCGATT GCTGGTAAGC CCTGACATCC AGAAGCCAGA ACCTGCTGTG
 421 TACCAGTTAA AAGATCCTCG GTCTCAGGAC AGCACCCTCT GCCTGTTCAC CGACTTTGAC
 50 481 TCCCAAATCA ATGTGCCGAA AACCATGGAA TCTGGAACGT TCATCACTGA CAAAACCTGTG
 541 CTGGACATGA AAGCTATGGA TTCCAAGAGC AATGGGGCCA TTGCCTGGAG CAACCAGACA

601 AGCTTCACCT GCCAAGATAT CTTCAAAGAG ACCAACGCCA CCTACCCCAG TTCAGACGTT
 661 CCCTGTGATG CCACGTTGAC TGAGAAAAGC TTTGAAACAG ATATGAACCT AACTTTTCAA
 721 AACCTGTCAG TTATGGGACT CCGAATCCTC CTGCTGAAAG TAGCCGGATT TAACCTGCTC
 781 ATGACGCTGA GGCTGTGGTC CAGTGGCAGC AGAGCCAAGA GAAGCGGATC CGGCGCCACC
 5 841 AACTTCAGCC TGCTGAAGCA GGCCGGCGAC GTGGAGGAAA ACCCTGGCCC TAGGATGGGC
 901 TCCAGGCTCT TTCTGGTCTT GAGCCTCCTG TGTACAAAAC ACATGGAGGC TGCAGTCACC
 961 CAAAGCCCTA GAAACAAGGT GACAGTAACA GGAGGAAACG TGACATTGAG CTGTCGCCAG
 1021 ACTAATAGCC ACAACATACAT GTAATGGTAT CGGCAGGACA CTGGGCATGG GCTGAGGCTG
 1081 ATCCATTACT CATATGGTGC TGGCAACCTT CAAATAGGAG ATGTCCCTGA TGGGTACAAG
 10 1141 GCCACCAGAA CAACGCAAGA AGACTTCTTC CTCCTGCTGG AATGGCTTC TCCCTCTCAG
 1201 ACATCTTTGT ACTTCTGTGC CAGCAGTGAT GCTGGGACTA GTCAAAACAC CTTGACTTTT
 1261 GGTGCGGGCA CCCGACTATC GGTGCTAGAG GATCTGAGAA ATGTGACTCC ACCCAAGGTC
 1321 TCCTTGTTTG AGCCATCAAA AGCAGAGATT GCAAACAAAC GAAAGGCTAC CCTCGTGTGC
 1381 TTGGCCAGGG GCTTCTTCCC TGACCACGTG GAGCTGAGCT GGTGGGTGAA TGGCAAGGAG
 15 1441 GTCCACAGTG GGGTGAGCAC GGACCCTCAG GCCTACAAGG AGAGCAATTA TAGCTACTGC
 1501 CTGAGCAGCC GCCTGAGGGT CTCTGCTACC TTCTGGCACA ATCCTCGAAA CCACTTCCGC
 1561 TGCCAAGTGC AGTTCCATGG GCTTTCAGAG GAGGACAAGT GGCCAGAGGG CTCACCCAAA
 1621 CCTGTACACAC AGAACATCAG TGCAGAGGCC TGGGGCCGAG CAGACTGTGG GATTACCTCA
 1681 GCATCCTATC AACAAGGGGT CTTGTCTGCC ACCATCCTCT ATGAGATCCT GCTAGGGAAA
 20 1741 GCCACCCTGT ATGCTGTGCT TGTCAGTACA CTGGTGGTGA TGGCTATGGT CAAAAGAAAG
 1801 AATTCATGA

B. Genetically Engineered T Cells

Another embodiment provides genetically engineered CD8⁺ immune cells that express
 the disclosed mouse TCR genes to produce a TCR that specifically binds to hAFP or a fragment
 thereof expressed on the surface of a tumor cell. Preferably, the immune cells are human T
 25 cells, for example human cytotoxic T cells. The engineered human cytotoxic T cells can be
 autologous human cytotoxic T cells. TCR genes can be codon optimized.

Another embodiment provides CD4⁺ immune cells, preferably human CD4⁺ immune
 cells, genetically engineered to express the disclosed mouse TCR genes. CD4⁺ cells include T
 30 helper cells, monocytes, macrophages, and dendritic cells. The engineered human CD4⁺
 immune cells can be autologous human T helper cells. The TCR genes can be codon optimized.

The 9 hAFP₁₅₈ specific murine TCRs identified in this study are able to render CD4 T
 cells the capability of binding Tet₁₅₈ tetramer, suggesting that they are high affinity and
 independent of CD8 help. The role of CD4 in adoptive cell therapy is not clear. But the
 35 production of IL-2 by CD4 TCR-T cells in response to HepG2 tumor cells stimulation may
 provide the cytokine for maintaining T cell proliferation. In addition, the CD4 TCR-T cells
 demonstrate a low cytotoxicity against HepG2 tumor cells. Thus, although the CD8 TCR-T may
 be the major player for killing hAFP⁺ tumor cells, the CD4 TCR-T can provide the help for T
 cell proliferation, which can be critical for generating antitumor effect *in vivo*.

It is believed that murine TCRs may provide increased expression on the surface of a human host cell as compared to human TCR. Murine TCRs can also replace endogenous TCRs on the surface of the human host cell more effectively than exogenous human TCR. However, humanization of the mTCR may be necessary to avoid anti-TCR responses with repeated use of TCR-T cells. Methods of humanizing mTCRs are known in the arts. See for example US Patent Nos. 5,861,155, and 5,859,205, WO2007/131092, EP0460167 and Davis, et al., *Clin Cancer Res.*, 16:5852–5861 (2010), which are incorporated by reference in their entirety. In one embodiment, the disclosed mTCR genes are humanized before being introduced to a T cell or administered to a human subject.

10 **C. T Cell Hybridomas**

Another embodiment provides T cell hybridomas that express the disclosed mTCR polypeptides. For example, the hybridoma can be a result of the fusion of fusing sorted mouse CD8⁺Tet₁₅₈⁺ cells with BW-Lyt2.4 cells that lack TCR α and β chains and are selected as described (He., Y. et al., *J Immunol*, 174:3808-3817 (2005)). Methods of making the hybridomas are provided in the Examples.

D. Antibodies

One embodiment provides antibodies that specifically bind the disclosed mTCR proteins, for example SEQ ID NO:2-62. Suitable antibodies can be prepared by one of skill in the art. The antibody or antigen binding fragment therefore, can be an agonist or antagonist of mTCR or simply binds specifically to the mTCR or a polypeptide thereof.

In some embodiments, the disclosed antibodies and antigen binding fragments thereof immunospecifically bind to mTCR or a polypeptide thereof (e.g., any one of SEQ ID NO:2-62). In some embodiments, the antibody binds to an extracellular domain of an mTCR (SEQ ID NOs:41-53).

For example, molecules are provided that can immunospecifically bind to the disclosed mTCR polypeptides:

- (I) arrayed on the surface of a cell (especially a live cell); or
- (II) arrayed on the surface of a cell (especially a live cell) at an endogenous concentration;

Compositions are also provided that can immunospecifically bind to soluble endogenous mTCR polypeptides. In some embodiments the molecules reduce or prevent the soluble mTCR polypeptide from binding or otherwise interacting with its ligand.

The antibodies or antigen binding fragments thereof can be prepared using any suitable methods known in the art such as those discussed in more detail below.

1. Human and Humanized Antibodies

The antibodies that specifically bind to the disclosed mTCR polypeptides can be human or humanized. Many non-human antibodies (*e.g.*, those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies in the methods serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

Transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production can be employed. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J(H)) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge.

Optionally, the antibodies are generated in other species and “humanized” for administration in humans. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab’, F(ab’)₂, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient antibody are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also contain residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will contain substantially all of at least one, and typically two,

variable domains, in which all or substantially all, of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will contain at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule. Humanization can be essentially performed by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, a humanized form of a nonhuman antibody (or a fragment thereof) is a chimeric antibody or fragment, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important in order to reduce antigenicity. According to the "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies.

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in

the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

The antibody can be bound to a substrate or labeled with a detectable moiety or both bound and labeled. The detectable moieties contemplated with the present compositions include fluorescent, enzymatic and radioactive markers.

2. Single-Chain Antibodies

The antibodies that specifically bind the disclosed mTCR polypeptides can be single-chain antibodies. Methods for the production of single-chain antibodies are well known to those of skill in the art. A single chain antibody is created by fusing together the variable domains of the heavy and light chains using a short peptide linker, thereby reconstituting an antigen binding site on a single molecule. Single-chain antibody variable fragments (scFvs) in which the C-terminus of one variable domain is tethered to the N-terminus of the other variable domain via a 15 to 25 amino acid peptide or linker have been developed without significantly disrupting antigen binding or specificity of the binding. The linker is chosen to permit the heavy chain and light chain to bind together in their proper conformational orientation. These Fvs lack the constant regions (Fc) present in the heavy and light chains of the native antibody.

3. Monovalent Antibodies

The antibodies that specifically bind to the disclosed mTCR polypeptides can be monovalent antibodies. *In vitro* methods can be used for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment, called the F(ab')₂ fragment, that has two antigen combining sites and is still capable of cross-linking antigen.

The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain domain including one or more cysteines from the antibody hinge region. The F(ab')₂ fragment is a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. Antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

4. Hybrid Antibodies

The antibodies that specifically bind the disclosed mTCR polypeptides can be hybrid antibodies. In hybrid antibodies, one heavy and light chain pair is homologous to that found in an antibody raised against one epitope, while the other heavy and light chain pair is homologous to a pair found in an antibody raised against another epitope. This results in the property of multi-functional valency, *i.e.*, ability to bind at least two different epitopes simultaneously. Such hybrids can be formed by fusion of hybridomas producing the respective component antibodies, or by recombinant techniques. Such hybrids may, of course, also be formed using chimeric chains.

5. Conjugates or Fusions of Antibody Fragments

The targeting function of the antibody can be used therapeutically by coupling the antibody or a fragment thereof with a therapeutic agent. Such coupling of the antibody or fragment (*e.g.*, at least a portion of an immunoglobulin constant region (Fc)) with the therapeutic agent can be achieved by making an immunoconjugate or by making a fusion protein, comprising the antibody or antibody fragment and the therapeutic agent.

Such coupling of the antibody or fragment with the therapeutic agent can be achieved by making an immunoconjugate or by making a fusion protein, or by linking the antibody or fragment to a nucleic acid such as an siRNA, comprising the antibody or antibody fragment and the therapeutic agent.

In some embodiments, the antibody is modified to alter its half-life. In some embodiments, it is desirable to increase the half-life of the antibody so that it is present in the circulation or at the site of treatment for longer periods of time. For example, it may be desirable

to maintain titers of the antibody in the circulation or in the location to be treated for extended periods of time. Antibodies can be engineered with Fc variants that extend half-life, *e.g.*, using Xtend™ antibody half-life prolongation technology (Xencor, Monrovia, CA). In other embodiments, the half-life of the anti-DNA antibody is decreased to reduce potential side effects.

5 The conjugates disclosed can be used for modifying a given biological response. The drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin.

10 The disclosed antibodies and mTCR polypeptides can be conjugated or linked to one or more detectable labels. The disclosed antibodies and mTCR polypeptides can be linked to at least one agent to form a detection conjugate. In order to increase the efficacy of the molecules as diagnostic it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one reporter
15 molecule. A reporter molecule is defined as any moiety that may be detected using an assay. Non-limiting examples of reporter molecules that have been conjugated to antibodies or polypeptides include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, luminescent molecules, photoaffinity molecules, colored particles and/or ligands, such as biotin.

20 **E. Formulations**

The disclosed antibodies, fusion proteins, and mTCR polypeptides can be formulated into pharmaceutical compositions. Pharmaceutical compositions containing the antibody, fusion protein, or mTCR polypeptides can be for administration by parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), transdermal (either passively or
25 using iontophoresis or electroporation), or transmucosal (nasal, vaginal, rectal, or sublingual) routes of administration or using bioerodible inserts and can be formulated in dosage forms appropriate for each route of administration.

In some *in vivo* approaches, the compositions disclosed herein are administered to a subject in a therapeutically effective amount. As used herein the term “effective amount” or
30 “therapeutically effective amount” means a dosage sufficient to treat, inhibit, or alleviate one or more symptoms of the disorder being treated or to otherwise provide a desired pharmacologic

and/or physiologic effect. The precise dosage will vary according to a variety of factors such as subject-dependent variables (e.g., age, immune system health, etc.), the disease, and the treatment being effected.

For the disclosed compositions, as further studies are conducted, information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age, and general health of the recipient, will be able to ascertain proper dosing. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment desired. For the disclosed immunomodulatory agents, generally dosage levels of 0.001 to 20 mg/kg of body weight daily are administered to mammals. Generally, for intravenous injection or infusion, dosage may be lower.

In certain embodiments, the disclosed compositions are administered locally, for example by injection directly into a site to be treated. Typically, the injection causes an increased localized concentration of the composition which is greater than that which can be achieved by systemic administration. The compositions can be combined with a matrix as described above to assist in creating an increased localized concentration of the polypeptide compositions by reducing the passive diffusion of the polypeptides out of the site to be treated.

1. Formulations for Parenteral Administration

In some embodiments, compositions disclosed herein, including those containing peptides and polypeptides, are administered in an aqueous solution, by parenteral injection. The formulation may also be in the form of a suspension or emulsion. In general, pharmaceutical compositions are provided including effective amounts of a peptide or polypeptide, and optionally include pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions optionally include one or more for the following: diluents, sterile water, buffered saline of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; and additives such as detergents and solubilizing agents (e.g., TWEEN 20 (polysorbate-20), TWEEN 80 (polysorbate-80)), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), and preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. The formulations may be lyophilized and

redissolved/resuspended immediately before use. The formulation may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions.

2. Formulations for Oral Administration

5 In embodiments the compositions are formulated for oral delivery. Oral solid dosage forms are described generally in Remington's Pharmaceutical Sciences, 18th Ed. 1990 (Mack Publishing Co. Easton Pa. 18042) at Chapter 89. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets, pellets, powders, or granules or incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the disclosed. See, e.g., Remington's
10 Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder (e.g., lyophilized) form. Liposomal or proteinoid encapsulation may be used to formulate the compositions. Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). See also Marshall, K. In: Modern Pharmaceutics Edited by G. S. Banker and C. T. Rhodes Chapter 10, 1979. In general, the formulation will include the peptide (or chemically modified forms thereof) and inert ingredients which protect peptide in the stomach environment, and release of
15 the biologically active material in the intestine.

The agents can be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the component molecule itself, where the moiety permits uptake into the blood stream from the stomach or intestine, or uptake directly into the intestinal mucosa. Also desired is the
25 increase in overall stability of the component or components and increase in circulation time in the body. PEGylation is an exemplary chemical modification for pharmaceutical usage. Other moieties that may be used include: propylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, polyproline, poly-1,3-dioxolane and poly-1,3,6-tioxocane [see, e.g., Abuchowski and Davis
30 (1981) "Soluble Polymer-Enzyme Adducts," in Enzymes as Drugs. Hocenberg and Roberts, eds.

(Wiley-Interscience: New York, N.Y.) pp. 367-383; and Newmark, et al. (1982) J. Appl. Biochem. 4:185-189].

Another embodiment provides liquid dosage forms for oral administration, including pharmaceutically acceptable emulsions, solutions, suspensions, and syrups, which may contain
5 other components including inert diluents; adjuvants such as wetting agents, emulsifying and suspending agents; and sweetening, flavoring, and perfuming agents.

Controlled release oral formulations may be desirable. The agent can be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms, e.g., gums. Slowly degenerating matrices may also be incorporated into the formulation. Another
10 form of a controlled release is based on the Oros therapeutic system (Alza Corp.), i.e., the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects.

For oral formulations, the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. In some embodiments, the release
15 will avoid the deleterious effects of the stomach environment, either by protection of the agent (or derivative) or by release of the agent (or derivative) beyond the stomach environment, such as in the intestine. To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP),
20 HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D™, Aquateric™, cellulose acetate phthalate (CAP), Eudragit L™, Eudragit S™, and Shellac™. These coatings may be used as mixed films.

3. Formulations for Topical Administration

The disclosed immunomodulatory agents can be applied topically. Topical
25 administration does not work well for most peptide formulations, although it can be effective especially if applied to the lungs, nasal, oral (sublingual, buccal), vaginal, or rectal mucosa.

Compositions can be delivered to the lungs while inhaling and traverse across the lung epithelial lining to the blood stream when delivered either as an aerosol or spray dried particles having an aerodynamic diameter of less than about 5 microns.

30 A wide range of mechanical devices designed for pulmonary delivery of therapeutic products can be used, including but not limited to nebulizers, metered dose inhalers, and powder

inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). Nektar, Alkermes and Mannkind all have inhalable insulin powder preparations approved or in clinical trials where the technology could be applied to the formulations described herein.

Formulations for administration to the mucosa will typically be spray dried drug particles, which may be incorporated into a tablet, gel, capsule, suspension or emulsion.

Standard pharmaceutical excipients are available from any formulator.

Transdermal formulations may also be prepared. These will typically be ointments, lotions, sprays, or patches, all of which can be prepared using standard technology. Transdermal formulations may require the inclusion of penetration enhancers.

4. Controlled Delivery Polymeric Matrices

The fusion proteins, mTCR polypeptides, and antibodies disclosed herein can also be administered in controlled release formulations. Controlled release polymeric devices can be made for long term release systemically following implantation of a polymeric device (rod, cylinder, film, disk) or injection (microparticles). The matrix can be in the form of microparticles such as microspheres, where the agent is dispersed within a solid polymeric matrix or microcapsules, where the core is of a different material than the polymeric shell, and the peptide is dispersed or suspended in the core, which may be liquid or solid in nature. Unless specifically defined herein, microparticles, microspheres, and microcapsules are used interchangeably. Alternatively, the polymer may be cast as a thin slab or film, ranging from nanometers to four centimeters, a powder produced by grinding or other standard techniques, or even a gel such as a hydrogel.

Either non-biodegradable or biodegradable matrices can be used for delivery of fusion polypeptides, antibodies, or mTCR polypeptides or nucleic acids encoding them, although in some embodiments biodegradable matrices are preferred. These may be natural or synthetic polymers, although synthetic polymers are preferred in some embodiments due to the better characterization of degradation and release profiles. The polymer is selected based on the period over which release is desired. In some cases linear release may be most useful, although in

others a pulse release or “bulk release” may provide more effective results. The polymer may be in the form of a hydrogel (typically in absorbing up to about 90% by weight of water), and can optionally be crosslinked with multivalent ions or polymers.

The matrices can be formed by solvent evaporation, spray drying, solvent extraction and other methods known to those skilled in the art. Bioerodible microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, *J. Controlled Release*, 5:13-22 (1987); Mathiowitz, et al., *Reactive Polymers*, 6:275-283 (1987); and Mathiowitz, et al., *J. Appl. Polymer Sci.*, 35:755-774 (1988).

The devices can be formulated for local release to treat the area of implantation or injection – which will typically deliver a dosage that is much less than the dosage for treatment of an entire body – or systemic delivery. These can be implanted or injected subcutaneously, into the muscle, fat, or swallowed.

III. Methods of Use

In certain embodiments, the disclosed antibodies, mTCR polypeptides and engineered immune cells can be used to detect or treat cancer. A preferred cancer to be detected, diagnosed, or treated includes hepatocellular carcinoma or any other cancer that expresses hAFP.

A. Adoptive Transfer

In one embodiment, immune cells are engineered to express the disclosed mTCR. The immune cells are T cells, preferably human T cells. The cells can be autologous or heterologous. The cells are typically transduced in vitro. The transduced cells can optionally be expanded in vitro to obtain a large population of transduced cells that can be administered to a subject in need thereof. Such subjects typically have or are believed to have a cancer or tumor that expresses hAFP. The T cells can be CD8⁺ or CD4⁺. The transduced cells can be administered in one or more doses to the subject.

The adoptive transfer can be combined with other therapies for the treatment of cancer. Such additional therapies include chemotherapy, radiation therapy, and surgery or a combination thereof. The adoptive transfer of TCR-T can be combined with cancer vaccines so that the TCR-T will be further expanded in vivo to reduce the number of transferred TCR-T cells and the associated toxicity.

One embodiment provides a method for reducing tumor burden in a subject in need thereof by administering engineered T cells that express one of the disclosed mTCRs, wherein the engineered mTCR cells inhibit or reduce tumors that express hAFP. The subject can optionally be treated with a cotherapy for cancer. A preferred cancer to be treated is
5 heptacellular carcinoma.

B. Detection or Diagnosis

The disclosed antibodies or mTCR polypeptides can be labeled as discussed above. The labeled polypeptides can be applied to a sample of cancer cells wherein the specific binding of the labeled polypeptides to the sample of cancer cells is indicative of a hAFP specific cancer
10 including heptacellular carcinoma.

The detection of the label can optionally be quantified to determine the number of cancer cells in sample. The sample is typically a liver tissue sample but can include a blood or serum sample.

IV. Compositions and Kits

Also provided are compositions and kits. Such kits can include containers, each with one or more of the various reagents (typically in concentrated form) utilized in the methods, including, for example, one or more binding agents (antibodies), already attached to a marker or optionally with reagents for coupling a binding agent to an antibody (as well as the marker itself), buffers, and/or reagents and instrumentation for the isolation (optionally by
20 microdissection) to support the practice of the invention. A label or indicator describing, or a set of instructions for use of, kit components in a ligand detection method of the present invention, will also be typically included, where the instructions may be associated with a package insert and/or the packaging of the kit or the components thereof.

Additional embodiments provide immunodetection kits for use with the immunodetection
25 methods described herein. As the antibodies or mTCR polypeptides are generally used to detect hAFP, the antibodies and mTCR polypeptides will generally be included in the kit. The immunodetection kits will thus comprise, in suitable container means, a first antibody that binds to an mTCR polypeptide and/or optionally, an immunodetection reagent.

The immunodetection reagents of the kit may take any one of a variety of forms,
30 including those detectable labels that are associated with and/or linked to the given antibody. Detectable labels that are associated with and/or attached to a secondary binding ligand are also

contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody.

Further suitable immunodetection reagents for use in the present kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first antibody, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label. As noted herein, a number of exemplary labels are known in the art and/or all such labels may be suitably employed in connection with the present invention.

The kits may further comprise a therapeutic agent for the treatment of cancer, such as an an engineered immune cell expressing the disclosed mTCR polypeptides.

Examples

Example 1: Immunization of AAD mice with lv-prime and peptide-boost elicits high level of hAFP158- specific CD8 T cells that can recognize and kill human HepG2 tumor cells.

Materials and Methods

Mice: HLA-A2 transgenic AAD mice (Pichard, V., et al, *J Immunother*, 31:246-253 (2008)) and NSG mice were purchased from Jackson Laboratory and housed in specific pathogen-free animal facility. Animal protocols were approved by the Institutional Animal Care and Use Committee of Augusta University.

Immunization: Mice were immunized with recombinant lentivector (lv) expressing hAFP or influenza virus M1 protein subcutaneously as described (Morgan, RA. et al., *J Immunother*, 36:133-151 (2013)). Twelve days later, mice were boosted with hAFP₁₅₈ (FMNKFIYEI) (SEQ ID NO:1) or M₁₅₈ (GILGFVFTL)(SEQ ID NO:72) peptide together with PolyI:C and anti-CD40 antibody as described (Linette, G.P., et al., *Blood*, 122:863-871 (2013)).

Results

To induce mouse CD8 T cells capable of recognizing human HLA-A2 presented hAFP epitopes, the HLA-A2 transgenic AAD mice were immunized with recombinant hAFP-lv. The AAD mice express a chimeric MHC I molecule of human HLA-A2 α 1- α 2 domain and the mouse H-2d α 3 domain that bind mouse CD8 molecule to help immune priming (Pichard, V., et al, *J Immunother*, 31:246-253 (2008)). It was found that hAFP-lv immunization reproducibly induced a modest level of hAFP₁₅₈ epitope-specific CD8 responses in AAD mice (Fig.1A). However, detect CD8 responses were not detected against other 3 hAFP epitopes (hAFP₁₃₇, hAFP₃₂₅, and

hAFP₅₄₂) previously identified in human (Vora, S.R., et al., *Oncologist*, 14:717-725 (2009)) (data not shown). Immunization with hAFP158 peptide alone did not induce measurable CD8 responses. But boost immunization with hAFP₁₅₈ epitope markedly increased the magnitude of hAFP₁₅₈-specific CD8 responses in the hAFP-lv primed, but not in the hAFP158 peptide primed, mice (Fig. 1A). Critically, the mouse CD8 T cells from the immunized mice could produce IFN γ after co-culture with the hAFP+, but not the hAFP-, HepG2 tumor cells (Fig. 1B). This data suggest that the hAFP vaccine-activated mouse CD8 T cells could recognize and respond to hAFP+ HepG2 tumor cells. In addition, after overnight co-culture with splenocytes from the immunized mice, the hAFP+ HepG2 tumor cells were killed in a dose dependent manner (Fig. 1C). Together these data suggest that immunization of AAD mice with hAFP-lv prime and hAFP₁₅₈ peptide boost can elicit high level of hAFP₁₅₈-specific CD8 T cells that can recognize and kill hAFP+ human HepG2 tumor cells.

Example 2: Adoptive transfer of splenocytes of the AAD mice immunized with hAFP-lv and hAFP158 not only prevents, but also eradicates large HepG2 tumor xenografts in NSG mice.

Materials and Methods

Fifteen million total splenocytes (1.5 million of hAFP₁₅₈-specific CD8 T cells) of the naïve or immunized mice were injected into NSG mice, which were then challenged with HepG2 tumor cells. In the therapeutic model, NSG mice were injected with 15 million total splenocytes of the immunized mice when tumor size reaches 2cm in diameter. The experiment was done twice with similar data.

Results

In this adoptive transfer experiment, it was found that the splenocytes from AAD mice immunized with hAFP-lv and hAFP₁₅₈ peptide, but not that from naïve mice, could completely prevent HepG2 tumor cell challenge in NSG mice (Fig. 2A and 2B). Approximately 10% of the immunized mouse splenocytes produced IFN γ in response to hAFP₁₅₈ peptide. Strikingly adoptive transfer of the splenocytes from the immunized AAD mice could completely eradicate HepG2 tumors as large as 20mm in diameter (Fig. 2C).

Example 3: Adoptive transfer of the hAFP₁₅₈-specific mouse CD8 T cells eradicates human HepG2 tumors in NSG mice.

Materials and Methods

To further identify the immune cells responsible for eradicating the established HepG2 tumor, CD8 T cells from the AAD mice immunized with hAFP-lv and hAFP₁₅₈ peptide were isolated using magnetic CD8 beads (Fig3A). The CD8 T cells from the AAD mice immunized with lv
 5 expressing influenza virus M1 antigen and M₁₅₈ peptide were also isolated and used as control.

Cell lines: HEK293T cells and human liver cancer cell line HepG2 was purchased from ATCC. The HCC cell line of Huh7 (HLA-A11) was a gift of Dr. Ande of Georgia Cancer Center, and the AFP- HepG2 cell was provided by Dr. Jingxiong She of Augusta University. The hAFP expression and HLA type were verified by western blot and immunological staining. Cells were
 10 cultured in DMEM media with 10% of FBS.

Immunization: Mice were immunized with recombinant lentivector (lv) expressing hAFP or influenza virus M1 protein subcutaneously as described (Morgan, RA. et al., *J Immunother*, 36:133-151 (2013)). Twelve days later, mice were boosted with hAFP₁₅₈ (FMNKFYIEI)(SEQ ID NO:1) or M₁₅₈ (GILGFVFTL)(SEQ ID NO:72) peptide together with PolyI:C and anti-CD40
 15 antibody as described (Linette, G.P., et al., *Blood*, 122:863-871 (2013)).

Tumor inoculation: Five millions of HepG2 tumor cells were inoculated into the flank of NSG mice. Tumor growth was monitored every other day.

Results

It was found that adoptive transfer of CD8 T cells from the hAFP immunized mice could
 20 eradicate HepG2 tumors in NSG mice (Fig.3B). In contrast, the HepG2 tumors continued growing in the NSG mice received the CD8 T cells from the influenza virus M1 antigen immunized mice. Next, the hAFP₁₅₈.specific CD8 T cells were further isolated by Tet₁₅₈ tetramer staining and cell sorting (Fig.3C). After adoptive transfer, while the Tet₁₅₈.CD8 T cells did not inhibit HepG2 tumor growth, the Tet₁₅₈₊ CD8 T cells could eradicate HepG2 tumors in NSG
 25 mice (Fig.3D). Together, the *in vitro* and *in vivo* data verify that the mouse CD8 T cells specific for HLA-A2 presented hAFP₁₅₈ epitope can recognize and kill human HCC tumor cells.

Example 4: The T cell hybridomas created from the Tet₁₅₈₊ CD8 T cells bind to HLA-A2/hAFP₁₅₈ tetramer and some produce IL-2 response to hAFP+ HepG2 tumor cells.

Materials and Methods

T cell hybridoma: T cell hybridomas were created by fusing the sorted mouse CD8⁺Tet₁₅₈⁺ cells with BW-Lyt2.4 cells that is lack of TCR α and β chains and selected as described (He, Y., et al., *J Immunol*, 174:3808-3817 (2005)).

Immunological and tetramer staining: Splenocytes were stained with surface marker and anti-V β antibody panel (BD Biosciences, San Diego, CA). HLA-A2/hAFP₁₅₈ tetramers (Tet₁₅₈) were kindly provided by NIH Tetramer Core Facility at Emory University.

Results

To identify and clone paired TCR α and β chain gene from a single T cells, T cell hybridoma cell lines were established. Prior to generating T cell hybridomas, the TCR V β chains were characterized using the anti-V β panel antibodies. The data showed that >90% of the Tet₁₅₈⁺ CD8 T cells could be stained with anti-V β 8.3 antibody (Fig. 11A-B). The other less than 10% of the Tet₁₅₈⁺ CD8 T cells could be stained with antibodies against V β 2, V β 5.1/5.2, V β 4, and V β 11. Next, a total of 39 T cell hybridoma clones that could be stained by Tet₁₅₈ tetramer were obtained from two cell fusion experiments using sorted Tet₁₅₈⁺ CD8 T cells (data not shown). Among them, 5 clones could respond to the stimulation of hAFP⁺ HepG2 tumor cells and produce IL-2 (Fig.4A), while other 34 hybridomas produced no or very low level of IL-2 (data not shown). All 5 hybridomas could be stained by anti-V β 8.3 antibody (Fig.4B). Further analysis showed that the 5 T cell hybridomas could bind to Tet₁₅₈ tetramer, but with different mean fluorescent intensity (Fig. 4C), suggesting that their TCR may have different affinity for HLA-A2/hAFP₁₅₈ complex. On the other hand, 4 of the remaining 34 T cell hybridomas consist of V β chains that could not be stained with anti-V β 8.3 antibody (data not shown).

Example 5: Nine sets of paired TCR α and β chain genes are identified from 14 T cell hybridomas.

Materials and Methods

Identification of the TCR α and β chain genes: Total RNA was isolated from T cell hybridomas. 5'- RACE technique (Hong, Y., et al., *Hepatology*, 59:1448-1458 (2014)) was conducted to amplify the TCR α and β chain genes. The cDNA was made with oligo dT primer and then the PolyC was added to the 5' end of cDNA. PCR was conducted by using the 5' pGI primer (CACCGGGIIGGGIIGGGIIGG)(SEQ ID NO:73) and the 3' primers corresponding to the constant (C) region of α chain (GGCATCACAGGGAACG) (SEQ ID NO:74) or β chain

(CCAGAAGGTAGCAGAGACCC) (SEQ ID NO:75). Based on the obtained partial sequence of the TCR α and β variable (V) regions, specific primer corresponding to the V region of α (ATGAAATCCTTTAGTATTTC) (SEQ ID NO:76) or β (ATGGGCTCCAGGCTCTTCTG) (SEQ ID NO:77) chains were used together with the internal primer of α C-region (GCACATTGATTGGGAGTC) (SEQ ID NO:78) or β C-region: (GGGTAGCCTTTTGTGTTTGTG) (SEQ ID NO:79) to amplify and verify the V region. Finally, sequences of the TCR α and β chains were obtained and verified.

Results

In this experiment, the paired TCR α and β chain genes from the 14 hybridomas capable of producing IL-2 after co-culture with HepG2 tumor cells were amplified and sequenced. The result was summarized in Table 1. Out of 14 hybridomas, 9 unique sets of TCR genes were identified. The sequences of TCR2 (Clone 1G8) and TCR4 (Clone 2F6) are identical, so are the TCR3 (Clone 10A3) and TCR5 (Clone 6D9). TCR6 (Clone 5B3), TCR7 (Clone 5B10), and TCR13 (Clone 11B10) are identical, so are TCR38 (Clone 2C3) and TCR39 (Clone 3E4). The amino acid sequences of α and β chains of TCR1-3, 6, 8, 10, 11, 17, and 38 were then compared to the NCBI and IMGT databank. The sequence data showed that all nine TCR α chains had the same variable region (TRAV7D-2), and that the TCR β chains also shared the same variable region (TRBV13-1), which is consistent with the data from anti-V β 8.3 antibody staining (Fig. 16). Detail analysis reveals that the sequence of TCR1 and TCR2 is very similar. The β chain of TCR1 and TCR2 are identical and the α -chain of TCR1 and TCR2 had two amino acid differences in the V-J junction. On the other hand, the TCR3 is more divergent from TCR1 and TCR2. Although the β chain of TCR3 is only one amino acid difference from TCR1 and TCR2, the J region of α chain is encoded by a different J gene. While the J gene of TCR1 and TCR2 α chain is TRAJ30, the TCR3 α chain J gene is TRAJ12.

All of the amino acid differences in the 9 TCR genes identified herein are found in the CDR3 region of the α (Fig. 17A) and β (Fig. 17B) chains.

Table 1. Summary of the TCR Gene Clones

Hybridoma #	TCR Clone #	Chains	V	D	J	C
8D11	TCR1	TCRA-1	Trav7d-2		Traj30	C
		TCRB-1	Trbv13-1	9nts	Trabj2-4	C2
1G8	TCR2	TCRA-2	Trav7d-2		Traj30	C
		TCRB-2	Trbv13-1	9nts	Trbj2-4	C2
10A3	TCR3	TCRA-3	Trav7d-2		Traj12	C
		TCRB-3	Trbv13-1	9nts	Trbj2-4	C2
2F6	TCR4	Identical to TCR-2				
6D9	TCR5	Identical to TCR-3				
5B3	TCR6	TCRA-1	Trav7d-2		Traj30	C
		TCRB-1	Trbv13-1		Trabj2-4	C2
5B10	TCR7	Identical to TCR-6				
5G3	TCR8	TCRA-8	Trav7d-2		Traj12	C
		TCRB-8	Trbv13-1		Trabj2-4	C2
7H9	TCR10	TCRA-10	Trav7d-2		Traj12	C
		TCRB-10	Trbv13-1		Trbj2-4	C2
8E3	TCR11	TCRA-11	Trav7d-2		Traj12	C
		TCRB-11	Trbv13-1		Trabj2-4	C2
11b10	TCR13	Identical to TCR-6				
4D2	TCR17	TCRA-17	Trav7d-2		Traj12	C
		TCRB-17	Trbv13-1		Trabj2-4	C2
2C3	TCR38	TCRA-38	Trav7d-2		Traj12	C
		TCRB-38	Trbv13-1		Trbj2-4	C2
3E4	TCR39	Identical to TCR-17				

Table 1. The 14 paired TCR alpha and beta chains are summarized. TCR4 and TCR2 are identical. TCR5 and TCR3 are identical. TCR6, TCR7 and TCR13 are identical. TCR38 and TCR39 are identical.

1. Protein sequences of TCRA-1 and TCRA-2 have 2AA difference between V-J junction
2. The TCRA-3 is significantly different from TCRA-1 and TCRA-2. In fact, the J region of TCRA-3 is totally different from TCRA-1 and TCRA-2.
3. Protein sequences of TCRB-1 and TCRB-2 are identical.
4. Protein sequences of TCRB-3 and TCRB-1 are identical except one AA from Serine (116S) to Alanine (116A) between V-D Junction.
5. The D region in TCRB between V-J is 9nt without obvious homology to any sequence in the databank.

Example 6: Expression of the paired TCR α and β chain genes in primary human T cells forms functional TCRs that bind Tet158 tetramer.

Materials and Methods

TCR genes and recombinant lv: TCR α and β chains gene were designed and synthesized by using the above identified V-D-J region and C-region. A P2A sequence (Cho, H.I., et al., *Cancer Res*, 69:9012-9019 (2009)) is inserted between α and β chain to separate α and β chains. The entire genes were codon optimized and cloned into lv.

Transduction of human T cells: Human T cells were isolated from buffy coat by negative selection. CD3/CD28 Tetrameric antibody complex (Stemcell Technologies, Vancouver) was then added to activate the cells for 2 days before they were transduced with recombinant lv.

Results

In this experiment, whether the hAFP-specific TCR genes could engineer human T cells to create TCR redirected T cells (TCR-T) and whether such TCR-T cells would acquire the capability of specifically recognizing and killing hAFP+ tumor cells were investigated. To this end, three TCR genes (TCR1, TCR2, and TCR3) were synthesized and cloned into lv. The P2A sequence was inserted between the TCR α and β genes to generate equal number of TCR α and β chains in target T cells (Fig.5A). To study whether the TCR α and β chains can form functional TCRs after expression, Jurkat cells were transduced with the TCR-lv and it was found that gene transfer of any of the three sets of TCR genes could effectively render Jurkat cells the capability of binding Tet₁₅₈ tetramer, indicating the TCR gene expression in Jurkat cells can form functional TCR (Fig.5B). Next, whether these TCR genes could engineer primary human T cells to redirect them to recognize HLA-A2/hAFP158 complex was studied. First, it was found that human T cells of healthy donors could be effectively transduced by recombinant lv after CD3/CD28 activation. Using GFP as reporter protein, at MOI of 10, approximately 60% of human T cells can be transduced by GFP-lv (Figure 12). Next, transduction of primary human T cells with the TCR genes revealed that approximately 20-30% of them could effectively bind Tet₁₅₈ tetramer (Fig.5C). Although the T cells from different donors may be transduced with different efficacy, there was no significant transduction difference among the 3 sets of TCR genes. However, the mean fluorescent intensity (MFI) of Tet₁₅₈ tetramer was consistently higher on the TCR3 gene transduced CD8 and CD4 T cells than TCR1 and TCR2 transduced cells, suggesting that TCR3 may have higher affinity for the HLA-A2/hAFP₁₅₈, which is in agreement

with the data of Tet₁₅₈ tetramer staining on hybridoma cells (Fig.4C). Both CD8 and CD4 TCR-T cells could bind Tet₁₅₈ Tetramer, indicating that the TCR binding to HLA-A2/hAFP₁₅₈ is independent of CD8. However, the MFI of Tet₁₅₈ staining on CD8 TCR-T is always higher than that on CD4 TCR-T cells (Fig. 5D), suggesting that CD8 help enhances the binding of the TCR to HLA-A2/hAFP₁₅₈ complex. In addition, the TCR-T cells were stained with anti-TCR V β 8.3 or Tet₁₅₈ tetramer. The percent of Tet₁₅₈ Tetramer⁺ cells is nearly identical to the percent of anti-TCR V β 8.3⁺ cells (Fig. 13).

Example 7: Human TCR-T cells specifically activated by HLA-A2 presented hAFP₁₅₈ peptide.

10 Materials and Methods

IFN γ was detected by ELISA after the TCR gene transduced human T cells were co-cultured overnight with HLA-A2+hAFP⁺ HepG2 cells, HLA-A2+hAFP⁻ HepG2 cells, or HLA-A2-hAFP⁺ tumor cells.

Results

15 To study whether the human TCR-T cells could be specifically activated by HLA-A2 presented hAFP₁₅₈ peptide, cytokine production of human TCR-T was measured after stimulation with HLA-A2 mouse splenocytes pulsed with hAFP₁₅₈ peptides. The data showed that human TCR-T cells could be specifically stimulated to produce IFN γ by HLA-A2 cells pulsed with hAFP₁₅₈ peptide. In contrast, no IFN γ was detected in the human TCR-T culture after stimulation
20 with influenza virus M1 peptide. The mock transduced human T cells do not respond to hAFP₁₅₈ peptide stimulation (Fig. 14A). The IFN γ production was mainly from CD8 T cells (Fig. 14B), while the IL2 was also produced by CD4 T cells (FIG. 14C). Next, we found that human TCR-T cells could be activated by HLA-A2+ hAFP⁺ HepG2 tumor cells to produce IFN γ . In contrast, HLA-A2+ hAFP⁻ or HLA-A2- hAFP⁺ human tumor cells could not activate TCR-T cells
25 (Fig.6A). Again, the IFN γ production was mainly produced in the CD8 TCR-T cells (Fig.6B). But both the CD4 (Fig. 6C) and CD8 (Fig.6B) TCR-T cells are able to produce IL-2. Again, there is no significant difference among the three TCRs. Furthermore, the induction of TCR- T proliferation by HepG2 tumor cells was studied. We found that, after co-culture with the HepG2 tumor cells, the human TCR-T cells, especially the CD8 T cells, underwent significant
30 proliferation (Fig.6D).

Example 8: Human CD8 TCR-T cells have strong and specific cytotoxicity against HLA-A2+ hAFP+ tumor cells.

Materials and Methods

ELISA of cytokines: ELISA of IFN γ and IL-2 was conducted as instructed by manufacturer (Biolegend, San Diego, CA).

Cytotoxic assay: TCR-T cells were added to growing HepG2 tumor cells. Cytotoxicity assay was conducted using LDH assay as instructed (Promega, Madison, WI).

Prodium iodide (PI) staining: The dead cells were stained with PI as instructed (BD Biosciences, San Diego, CA).

Results

In this experiment, the cytotoxic activity of human TCR-T cells was studied. Using LDH assay, we first showed that the human TCR-T cells could effectively kill 80% of HepG2 tumor cells within 24 hours of co-culture at E/T ratio of 5. In contrast, the TCR-T cells did not generate significant cytotoxic effect on the AFP- or HLA-A2- tumor cells (Fig.7A). The cytotoxicity against HepG2 tumor cells was dose dependent (Fig.7B). Even at E/T ratio of 0.5 (the Tet₁₅₈+ cells is ~30%, thus the ratio of Tet₁₅₈ Tetramer+ T cells to target cells was only 0.15), there was a >50% of killing after 24 hours co-culture. By using purified CD8 and CD4 T cells to conduct cytotoxicity assay, we showed that HepG2 tumor cell killing activity was mainly from the CD8 T cells (Fig.7C- E). However, the CD4 TCR-T cells also had a lower level of cytotoxicity, resulting in 15% of specific killing of HepG2 tumor cells at ratio of 1.5/1 (Fig.7E). After 24 hours of co-culture, the CD8 TCR-T cells encircled (may be attracted to) the HepG2 tumor cells to form clusters (Fig. 7D). On the other hand, the CD4 TCR-T and mock-transduced T cells did not form obvious clusters. In addition, the PI staining revealed that most of the tumor cells in the cluster are dead cells (Fig.7F). Consistent with LDH assay, most of the cytotoxic activity was from the CD8 TCR-T cells. The PI staining showed that CD4 TCR-T cells had a lower activity (15%) of killing hepG2 tumor cells, which is in agreement with LDH assay. The mock-transduced human T cells did not induce cell death of HepG2 tumor cells.

Example 9: Adoptive transfer of human TCR-T generates antitumor effect against HepG2 tumors in NSG mice.

Materials and methods:

In order to study the *in vivo* antitumor effect of human TCR-T, NSG mice bearing 4 day
5 HepG2 tumors were adoptively transferred with 20 million human TCR-T cells. Tumor growth was monitored by measuring the length, width, and height. Tumor volume was calculated as $\frac{1}{2}(\text{length} \times \text{width} \times \text{height})$.

Results:

In this experiment, the *in vivo* antitumor effect of human TCR-T cells was studied.
10 Primary human T cells were transduced with lv and approximately 40% of the total T cells (25% of CD8 T cells and 54% of CD4 T cells) were Tet158+ TCR-T cells before adoptive transfer (Fig. 11A). We found that adoptive transfer of human TCR-T cells could inhibit HpeG2 tumor outgrowth in NSG mice (Fig. 11B). One of the TCR-T treated-mice developed a tumor, but the tumor was the eradicated by 3 weeks after adoptive transfer (Fig. 11C-D). The kinetics of the
15 transferred human T cells showed that both mock-T and TCR-T cells survived approximately 3-4 weeks in the absence of human IL-2 (Fig. 11E). But compared to mock-T cells, there ws a significant increase of the percentage of Tet₁₅₈+ cells among the TCR-T, especially the CD4 TCR-T cells on day 8 after transfer (Fig. 11F), suggesting that there might be an antigen-induced TCR-T expansion.