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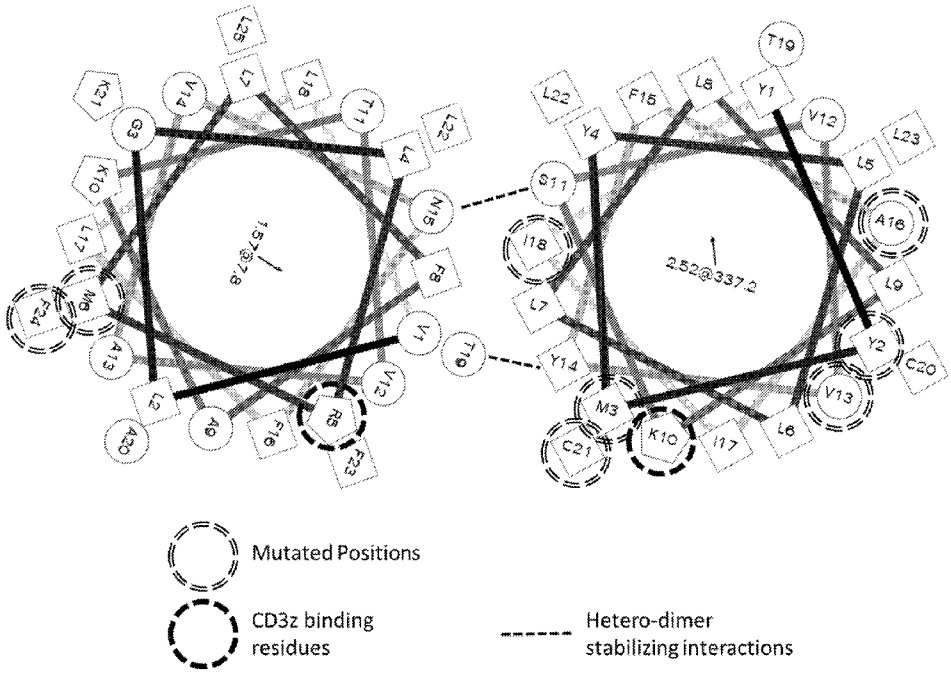
(54) Title: CHIMERIC ANTIBODY/T-CELL RECEPTOR CONSTRUCTS AND USES THEREOF

FIG. 3



(57) Abstract: The present application provides chimeric antibody-TCR constructs comprising an antigen-binding module that specifically binds to a target antigen and a T cell receptor module capable of recruiting at least one TCR-associated signaling molecule. Also provided are methods of making and using these constructs.

[Continued on next page]

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CHIMERIC ANTIBODY/T-CELL RECEPTOR CONSTRUCTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application No. 62/490,576, filed on April 26, 2017, U.S. Provisional Application No. 62/490,578, filed on April 26, 2017, and U.S. Provisional Application No. 62/490,580, filed on April 26, 2017, the contents of which are hereby incorporated by reference in their entireties.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 750042000640SEQLIST.txt, date recorded: April 24, 2018, size: 168 KB).

FIELD OF THE INVENTION

[0003] The invention relates to chimeric antibody-TCR constructs comprising an antigen-binding module that specifically binds to one or more target antigens and a T cell receptor module capable of recruiting at least one TCR-associated signaling molecule.

BACKGROUND OF THE INVENTION

[0004] T-cell mediated immunity is an adaptive process of developing antigen (Ag) – specific T lymphocytes to eliminate viruses, bacterial, parasitic infections or malignant cells. It can also involve aberrant recognition of self-antigen, leading to autoimmune inflammatory diseases. The Ag specificity of T lymphocytes is based on recognition through the T Cell Receptor (TCR) of unique antigenic peptides presented by Major Histocompatibility Complex (MHC) molecules on Ag-presenting cells (APC) (Broere, *et al.*, *Principles of Immunopharmacology*, 2011). Each T lymphocyte expresses a unique TCR on the cell surface as the result of developmental selection upon maturation in the thymus. The TCR occurs in two forms as either an αβ heterodimer or as a γδ heterodimer. T cells express either the αβ form or the γδ form TCR on the cell surface. The four chains, α/β/γ/δ, all have a characteristic extracellular structure consisting of a highly polymorphic "immunoglobulin variable region"-like N-terminal domain and an "immunoglobulin constant region"-like second domain. Each of these domains has a characteristic intra-domain disulfide bridge. The constant region is proximal to the cell membrane, followed by a connecting peptide, a transmembrane region and a short cytoplasmic tail. The covalent linkage between the 2 chains of the heterodimeric TCR is formed by the

cysteine residue located within the short connecting peptide sequence bridging the extracellular constant domain and the transmembrane region which forms a disulfide bond with the paired TCR chain cysteine residue at the corresponding position (The T cell Receptor Factsbook, 2001).

[0005] The αβ and γδ TCRs are associated with the non-polymorphic membrane-bound CD3 proteins to form the functional octameric TCR-CD3 complex, consisting of the TCR heterodimer and three dimeric signaling molecules, CD3δ/ε, CD3γ/ε and CD3ζ/ζ or ζ/η. Ionizable residues in the transmembrane domain of each subunit form a polar network of interactions that hold the complex together. For T cell activation, the TCR N-terminal variable regions recognize the peptide/MHC complex presented on the surface of target cell, whereas the CD3 proteins participate in signal transduction (Call *et al.*, *Cell.* 111(7):967-79, 2002; The T cell Receptor Factsbook, 2001).

[0006] αβ TCR, also called conventional TCR, is expressed on most lymphocytes and consists of the glycosylated polymorphic α and β chains. Different αβ TCRs can discriminate among different peptides embedded in the surfaces of MHC II (mostly expressed on APC cell surfaces) and MHC I (expressed on all nucleated cells) molecules, whose dimensions and shapes are relatively constant. The γδ TCR, though structurally similar to the αβ TCR, recognizes carbohydrate-, nucleotide-, or phosphor-carrying antigens in a fashion independent of MHC presentation (The T cell Receptor Factsbook, 2001; Girardi *et al.*, *J. Invest. Dermatol.* 126(1):25-31, 2006; Hayes *et al.*, *Immunity*. 16(6):827-38, 2002).

[0007] In the past two decades, fundamental advances in immunology and tumor biology, combined with the identification of a large number of tumor antigens, have led to significant progress in the field of cell-based immunotherapy. T cell therapy occupies a large space in the field of cell-based immunotherapy, with the goal of treating cancer by transferring autologous and *ex vivo* expanded T cells to patients, and has resulted in some notable antitumor responses (Blattman *et al.*, *Science*. 305(5681):200-5, 2004). For example, the administration of naturally occurring tumor infiltrating lymphocytes (TILs) expanded *ex vivo* mediated an objective response rate ranging from 50-70% in melanoma patients, including bulky invasive tumors at multiple sites involving liver, lung, soft tissue and brain (Rosenberg *et al.*, *Nat. Rev. Cancer.* 8(4):299-308, 2008; Dudley ME *et al.*, *J. Clin. Oncol.* 23(10):2346-57, 2005).

[0008] A major limitation to the widespread application of TIL therapy is the difficulty in generating human T cells with antitumor potential. As an alternative approach, exogenous high-affinity TCRs can be introduced into normal autologous T cells of the patients through T cell

engineering. The adoptive transfer of these cells into lympho-depleted patients has been shown to mediate cancer regression in cancers such as melanoma, colorectal carcinoma, and synovial sarcoma (Kunert R *et al.*, *Front. Immunol.* 4:363, 2013). A recent phase I clinical trial using anti NY-ESO-1 TCRs against synovial sarcoma reported an overall response rate of 66% and complete response was achieved in one of the patients receiving the T cell therapy (Robbins PF *et al.*, *Clin. Cancer Res.* 21(5):1019-27, 2015).

[0009] One of the advantages of TCR-engineered T cell therapy is that it can target the entire array of potential intracellular tumor-specific proteins, which are processed and delivered to the cell surface through MHC presentation. Furthermore, the TCR is highly sensitive and can be activated by just a few antigenic peptide/MHC molecules, which in turn can trigger a cytolytic T cell response, including cytokine secretion, T cell proliferation and cytolysis of defined target cells. Therefore, compared with antibody or small molecule therapies, TCR-engineered T cells are particularly valuable for their ability to kill target cells with very few copies of target intracellular antigens (Kunert R *et al.*, *Front. Immunol.* 4:363, 2013).

[0010] However, unlike therapeutic antibodies, which are mostly discovered through hybridoma or display technologies, identification of target-specific TCRs requires the establishment of target peptide/MHC specific TCR clones from patient T cells and screening for the right α-β chain combination that has the optimal target antigen-binding affinity. Very often, phage/yeast display is employed after cloning of the TCR from patient T cells to further enhance the target binding affinity of the TCR. The whole process requires expertise in many areas and is time-consuming (Kobayashi E *et al.*, *Oncoimmunology*. 3(1):e27258, 2014). The difficulties in the TCR discovery process have largely impeded the widespread application of TCR-engineered T cell therapy. It has also been hampered by treatment-related toxicity, in particularly with TCRs against antigens that are over-expressed on tumor cells but also expressed on healthy cells, or with TCRs recognizing off-target peptide/MHC complexes (Rosenberg SA *et al.*, *Science*. 348(6230):62-8, 2015).

[0011] A different approach has been developed in recent years to engage T cells for targeted cancer immunotherapy. This new approach is called Chimeric Antigen Receptor T cell Therapy (CAR-T). It merges the exquisite targeting specificity of monoclonal antibodies with the potent cytotoxicity and long-term persistence provided by cytotoxic T cells. A CAR is composed of an extracellular domain that recognizes a cell surface antigen, a transmembrane region, and an intracellular signaling domain. The extracellular domain consists of the antigen-binding variable regions from the heavy and light chains of a monoclonal antibody that are fused into a single-

chain variable fragment (scFv). The intracellular signaling domain contains an immunoreceptor tyrosine-based activation motif (ITAM), such as those from CD3ζ or FcRγ, and one or more co-stimulatory signaling domains, such as those from CD28, 4-1BB or OX40 (Barrett DM *et al.*, *Annu. Rev. Med.* 65:333-47, 2014; Davila ML *et al.*, *Oncoimmunology.* 1(9):1577-1583, 2012). Binding of target antigens by CARs grafted onto a T cell surface can trigger T cell effector functions independent of TCR-peptide/MHC complex interaction. Thus, T cells equipped with CARs can be redirected to attack a broad variety of cells, including those that do not match the MHC type of the TCRs on the T cells but express the target cell-surface antigens. This approach overcomes the constraints of MHC-restricted TCR recognition and avoids tumor escape through impairments in antigen presentation or MHC molecule expression. Clinical trials have shown clinically significant antitumor activity of CAR-T therapy in neuroblastoma (Louis CU *et al.*, *Blood.* 118(23):6050-6056, 2011), B-ALL (Maude, SL, *et al.*, *New England Journal of Medicine* 371:16:1507-1517, 2014), CLL (Brentjens, RJ, *et al. Blood* 118:18:4817-4828, 2011), and B cell lymphoma (Kochenderfer, JN, *et al. Blood* 116:20:4099-4102, 2010). In one study, a 90% complete remission rate in 30 patients with B-ALL treated with CD19-CAR T therapy was reported (Maude, SL, *et al.*, *supra*).

[0012] Most, if not all, CARs studied so far have been directed to tumor antigens with high cell surface expression. To target low-copy number cell-surface tumor antigens and intracellular tumor antigens, which represent 95% of all known tumor-specific antigens, there is a need to develop more potent and effective engineered cell therapies (Cheever, *et al.*, *Clin. Cancer Res.* 15(17):5323-37, 2009).

[0013] Several attempts have been made to engineer chimeric receptor molecules having antibody specificity with T cell receptor effector functions. *See*, for example, Kuwana, Y, *et al.*, *Biochem. Biophys. Res. Commun.* 149(3):960-968, 1987; Gross, G, *et al.*, *Proc. Natl. Acad. Sci. USA.* 86:10024-10028, 1989; Gross, G & Eshhar, Z, *FASEB J.* 6(15):3370-3378, 1992; and US Patent No. 7,741,465. To this date, none of these chimeric receptors have been adopted for clinical use, and novel designs for antibody-TCR chimeric receptors with improved expression and functionality in human T cells are needed.

[0014] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety. PCT Application Number PCT/US2016/058305 is hereby incorporated herein by reference in its entirety.

BRIEF SUMMARY OF THE INVENTION

[0015] The present application in one aspect provides a construct (such as an isolated construct) comprising an antigen-binding module (such as an antibody moiety) fused to a T cell receptor module (said construct also referred to herein as a "chimeric antibody-TCR molecule," or "caTCR"). In some embodiments, the caTCR comprises an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) capable of recruiting at least one TCR-associated signaling molecule. In some embodiments, the target antigen is a complex comprising a peptide and an MHC protein (such as an MHC class I protein or an MHC class II protein). In some embodiments, the target antigen is a cell-surface antigen.

[0016] In some embodiments, there is provided a chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein at least one of the TCR-TMs is non-naturally occurring, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule, and wherein the antigen-binding module is not a naturally occurring T cell receptor antigen-binding moiety.

[0017] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first antigen-binding domain that specifically binds to a first target antigen and a second antigen-binding domain that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first TCR-TM and the second TCR-TM are naturally occurring.

[0018] In some embodiments, there is provided one or more nucleic acids encoding a caTCR described herein. In some embodiments, there is provided one or more vectors comprising the one or more nucleic acids. In some embodiments, there is provided a composition comprising the one or more nucleic acids or the one or more vectors. In some embodiments, there is provided an effector cell comprising the one or more nucleic acids or the one or more vectors.

[0019] In some embodiments, there is provided a complex comprising caTCR as described herein and at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ.

[0020] In some embodiments, there is provided a composition, such as a pharmaceutical composition, comprising an effector cell described herein.

[0021] In some embodiments, there is provided a method of killing a target cell presenting a target antigen, comprising contacting the target cell with an effector cell described herein.

[0022] In some embodiments, there is provided a method of treating a target antigen-associated disease in an individual in need thereof, comprising administering to the individual an effective amount of a pharmaceutical composition described herein.

[0023] In some embodiments, there is provided a method of enriching a heterogeneous cell population for an effector cell described herein, comprising: a) contacting the heterogeneous cell population with a ligand comprising the target antigen or one or more epitopes contained therein to form complexes of the effector cell bound to the ligand; and b) separating the complexes from the heterogeneous cell population, thereby generating a cell population enriched for the effector cell.

[0024] In some embodiments, there is provided a nucleic acid library comprising sequences encoding a plurality of caTCRs as described herein. In some embodiments, there is provided a method of screening a library described herein for caTCRs having high affinity for a target antigen, comprising: a) introducing the nucleic acid library into a plurality of cells, such that the caTCRs are expressed on the surface of the plurality of cells; b) incubating the plurality of cells with a ligand comprising the target antigen or one or more epitopes contained therein; c) collecting cells bound to the ligand; and d) isolating sequences encoding the caTCRs from cells collected in step c), thereby identifying caTCRs specific for the target antigen.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. 1 shows a schematic representation of several caTCR molecules having different antigen-binding modules.

[0026] FIG. 2 shows a schematic representation of the caTCR-1-0 construct, highlighting its transmembrane region that was mutated to create the TM mutants.

[0027] FIG. 3 shows a helical wheel projection of the caTCR transmembrane region. Amino acid positions mutated to make transmembrane mutants are highlighted.

[0028] FIG. 4 shows the expression of the caTCR transmembrane mutants and CD3 on JRT3.1 cells as a percentage of the respective MFI expression on cells transduced with caTCR-1-0 with an anti-AFP158/HLA- A*2:01 binding moiety. Flow cytometry analysis of surface CD3ε or caTCR receptor was used to calculate the MFI of each respective receptor.

[0029] FIG. 5 shows Western blot analysis used to interrogate the binding affinity between endogenous CD3 complex and caTCR-1-0 or the caTCR-1-TM mutants. Lysates or anti-FLAG immunoprecipitates from JRT3-T3.5 cells transduced with caTCR-1-0 or caTCR-1 transmembrane mutants (-TM6, - TM10) were blotted with anti-FLAG and anti-CD3ζ antibodies.

[0030] FIG. 6 shows percent specific lysis from the killing of cancer cell lines SK-HEP- 1 and SK-HEP- 1-AFP- MG, mediated by mock transduced T cells or T cells transduced caTCR-1-0 or T cells transduced with caTCR-1 transmembrane mutants with an anti-AFP158/HLA-A*2:01 binding moiety.

[0031] FIG. 7 shows the concentration of cytokines found in the supernatant after in vitro killing of cancer cell lines SK-HEP- 1-AFP- MG by T cells transduced with caTCR-1-0 or caTCR-1 transmembrane mutants with an anti-AFP158/HLA- A*2:01 binding moiety.

[0032] FIG. 8 shows percent specific lysis from the killing of cancer cell lines SK-HEP- 1 and SK-HEP- 1-AFP- MG, mediated by mock transduced T cells or T cells transduced caTCR-1-0 or T cells transduced with caTCR-1 transmembrane mutants with an anti-AFP158/HLA-A*2:01 binding moiety.

[0033] FIG. 9 shows the concentration of cytokines found in the supernatant after in vitro killing of cancer cell lines SK-HEP- 1-AFP- MG by T cells transduced with caTCR-1-0 or caTCR-1 transmembrane mutants with an anti-AFP158/HLA- A*2:01 binding moiety.

[0034] FIG. 10 shows percent specific lysis from the killing of cancer cell lines NALM6, mediated by T cells transduced caTCR-1-0 or T cells transduced with caTCR-TM5 transmembrane mutant with an anti-CD19 binding moiety.

[0035] FIG. 11 shows the concentration of cytokines found in the supernatant after in vitro killing of cancer cell line NALM6 by mock transduced T cells or T cells transduced caTCR-1-0 or T cells transduced with caTCR-1-TM5 transmembrane mutant with an anti-CD19 binding moiety.

[0036] FIGs. 12A and 12B show the amount of CFSE within T cells transduced caTCR-1-0 or T cells transduced with caTCR-1-TM5 as a demonstration of proliferation rate in response to stimulation by NALM6 tumor cells. The number of live T-cells after tumor cell lysis is listed in Table 5.

[0037] FIGs. 13A-13E show schematic structures of exemplary bispecific caTCR molecules.

[0038] FIG. 14 shows the concentration of IFNγ found in the supernatant after in vitro incubation of cancer cell lines K562, K562-CD19 ($CD19^+$), K562-CD22 ($CD22^+$), Raji and Raji CD19 K/O with T cells mock-transduced or transduced with a vector encoding a bispecific caTCR construct, anti-CD19 anti-CD22 caTCR-7-2a.

[0039] FIG. 15 shows percent specific lysis from the killing of cancer cell line K562, K562-CD19 ($CD19^+$), K562-CD22 ($CD22^+$) and K562-CD19/CD22 ($CD19^+CD22^+$) mediated by T cells transduced with a vector encoding anti-CD19 caTCR-1 or one of four different anti-CD19 anti-CD22 caTCR constructs.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The present application in one aspect provides an isolated chimeric antibody/T cell receptor construct (referred to herein as "caTCR") that comprises an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) comprising one or more non-naturally occurring TCR-derived transmembrane domains, wherein the TCRM is capable of recruiting at least one TCR-associated signaling molecule. In another aspect, the present application provides a caTCR comprising a multispecific (such as bispecific) antigen-binding module that specifically binds to two or more different target antigens or epitopes and a TCRM comprising naturally occurring or non-naturally occurring TCR-derived transmembrane domains. The caTCRs described herein specifically redirected human T cells to kill diseased cells expressing the target antigen(s). The target antigen(s) can be a protein expressed on the cell surface or a complex comprising a peptide and an MHC protein (referred to herein as a "pMHC complex," or "pMHC"), such as an MHC-presented disease-associated antigen peptide on the surface of a diseased cell. caTCRs are regulated by the naturally occurring machinery that controls T cell receptor activation, but unlike naturally-occurring T cell receptors, they include modifications that enhance surface expression and/or function.

[0041] We have developed a series of novel and synthetic chimeric antibody-TCR constructs that include non-naturally occurring variants of TCR sequences that improve surface expression and/or function compared to similar constructs having only naturally-occurring TCR sequences. We have also developed multispecific chimeric antibody-TCR constructs that specifically recognize multiple target antigens to reduce antigen escape. The caTCRs can be efficiently expressed on the surface of T cells and have been demonstrated to form stable TCR-like signaling complexes in association with endogenous CD3 molecules. When engineered into T cells, the caTCRs endowed the T cells with potent cytotoxicity against target-bearing tumor cells

both *in vitro* and *in vivo,* in both MHC-dependent (peptide/MHC antigen) and MHC-independent (cell-surface antigen) configurations.

[0042] Accordingly, one aspect of the present application provides a caTCR (such as an isolated caTCR) comprising an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) comprising one or more non-naturally occurring TCR-derived transmembrane domains, wherein the TCRM is capable of recruiting at least one TCR-associated signaling molecule. The caTCR can take any of a number of formats with variations in the antigen-binding module and/or TCRM. For example, the caTCR can have an antigen-binding module comprising a moiety selected from the group consisting of a Fab, a Fab', a $(Fab')_2$, an Fv, and an scFv, and a TCRM comprising one or more non-naturally occurring sequences derived from an α/β and/or γ/δ TCR, including variants in one or more of the transmembrane domain, connecting peptide, and intracellular domain. *See* FIG. 1 for exemplary caTCR construct designs.

[0043] Another aspect of the present application provides a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first antigen-binding domain that specifically binds to a first target antigen and a second antigen-binding domain that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first target antigen is CD19 and the second target antigen is CD22, or the first target antigen is CD22 and the second target antigen is CD19. In some embodiments, the first target antigen is CD19 and the second target antigen is CD20, or the first target antigen is CD20 and the second target antigen is CD22. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR-derived transmembrane domains. *See*, FIGs. 13A-13E for exemplary multispecific caTCR construct designs.

[0044] In another aspect, there is provided one or more nucleic acids encoding a caTCR.

[0045] In yet another aspect, there is provided a complex (referred to herein as a "caTCR-CD3 complex") comprising a caTCR and at least one TCR-associated signaling molecule. The complex may be an octamer comprising the four dimers caTCR, CD3δε, CD3γε, and ζζ. Also provided is an effector cell, such as a T cell, expressing or associated with a caTCR or caTCR-CD3 complex.

[0046] In yet another aspect, there is provided a composition comprising a caTCR. The composition can be a pharmaceutical composition comprising a caTCR or an effector cell expressing or associated with the caTCR (for example a T cell expressing a caTCR).

[0047] Also provided are methods of making and using a caTCR (or cells expressing or associated with a caTCR) for treatment purposes, as well as kits and articles of manufacture useful for such methods. Further provided are methods of treating a disease using a caTCR (or cells expressing or associated with a caTCR).

Definitions

[0048] The term “antibody” or "antibody moiety" includes full-length antibodies and antigen-binding fragments thereof. A full-length antibody comprises two heavy chains and two light chains. The variable regions of the light and heavy chains are responsible for antigen-binding. The variables region in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1, LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1, HC-CDR2, and HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs of the heavy or light chains are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as lgG1 (γ1 heavy chain), lgG2 (γ2 heavy chain), lgG3 (γ3 heavy chain), lgG4 (γ4 heavy chain), lgA1 (α1 heavy chain), or lgA2 (α2 heavy chain).

[0049] The term "antigen-binding fragment" as used herein refers to an antibody fragment including, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete

antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment (*e.g.*, a parent scFv) binds. In some embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

[0050] As used herein, a first antibody moiety "competes" for binding to a target antigen with a second antibody moiety when the first antibody moiety inhibits target antigen-binding of the second antibody moiety by at least about 50% (such as at least about any of 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) in the presence of an equimolar concentration of the first antibody moiety, or vice versa. A high throughput process for “binning” antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

[0051] As use herein, the term "specifically binds" or "is specific for" refers to measurable and reproducible interactions, such as binding between a target and an antibody or antibody moiety, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody moiety that specifically binds to a target (which can be an epitope) is an antibody moiety that binds the target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, an antibody moiety that specifically binds to an antigen reacts with one or more antigenic determinants of the antigen (for example a cell surface antigen or a peptide/MHC protein complex) with a binding affinity that is at least about 10 times its binding affinity for other targets.

[0052] The term “T cell receptor,” or “TCR,” refers to a heterodimeric receptor composed of αβ or γδ chains that pair on the surface of a T cell. Each α, β, γ, and δ chain is composed of two Ig-like domains: a variable domain (V) that confers antigen recognition through the complementarity determining regions (CDR), followed by a constant domain (C) that is anchored to cell membrane by a connecting peptide and a transmembrane (TM) region. The TM region associates with the invariant subunits of the CD3 signaling apparatus. Each of the V domains has three CDRs. These CDRs interact with a complex between an antigenic peptide bound to a protein encoded by the major histocompatibility complex (pMHC) (Davis and Bjorkman (1988) Nature, 334, 395-402; Davis et al. (1998) Annu Rev Immunol, 16, 523-544; Murphy (2012), xix, 868 p.).

[0053] The term “TCR-associated signaling molecule” refers to a molecule having a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) that is part of the TCR-

CD3 complex. TCR-associated signaling molecules include CD3γε, CD3δε, and ζζ (also known as CD3ζ or CD3ζζ).

[0054] "Activation", as used herein in relation to a cell expressing CD3, refers to the state of the cell that has been sufficiently stimulated to induce a detectable increase in downstream effector functions of the CD3 signaling pathway, including, without limitation, cellular proliferation and cytokine production.

[0055] The term "module" when referring to a protein or portion of a protein is meant to include one or more polypeptide chains (*e.g.*, a dimeric protein or portion of a dimeric protein). The one or more chains may be linked, such as by a linker (*e.g.*, a peptide linker) or chemical linkage (*e.g.*, a peptide linkage). A "module" is also meant to include structurally and/or functionally related portions of one or more polypeptides which make up a protein. For example, a transmembrane module of a dimeric receptor may refer to the portions of each polypeptide chain of the receptor that span the membrane. A module may also refer to related portions of a single polypeptide chain. For example, a transmembrane module of a monomeric receptor may refer to portions of the single polypeptide chain of the receptor that span the membrane. A module may also include only a single portion of a polypeptide.

[0056] An "isolated" construct (such as a caTCR) as used herein refers to a construct that (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, (3) is expressed by a cell from a different species, or, (4) does not occur in nature.

[0057] The term "isolated nucleic acid" as used herein is intended to mean a nucleic acid of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated nucleic acid" (1) is not associated with all or a portion of a polynucleotide in which the "isolated nucleic acid" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[0058] As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); Al-Lazikani B. *et al.*, *J. Mol. Biol.*, 273: 927-948 (1997); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996); Abhinandan and Martin, *Mol. Immunol.*, 45: 3832-3839 (2008); Lefranc M.P. *et al.*, *Dev. Comp. Immunol.*, 27: 55-77 (2003); and Honegger and Plückthun, *J. Mol. Biol.*, 309:657-670 (2001), where the definitions include overlapping or subsets of amino

acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. CDR prediction algorithms and interfaces are known in the art, including, for example, Abhinandan and Martin, *Mol. Immunol.,* 45: 3832-3839 (2008); Ehrenmann F. *et al.*, *Nucleic Acids Res.*, 38: D301-D307 (2010); and Adolf-Bryfogle J. *et al.*, *Nucleic Acids Res.*, 43: D432-D438 (2015). The contents of the references cited in this paragraph are incorporated herein by reference in their entireties for use in the present invention and for possible inclusion in one or more claims herein.

TABLE 1: CDR DEFINITIONS

	Kabat[1]	**Chothia[2]**	**MacCallum[3]**	**IMGT[4]**	**AHo[5]**
V_H CDR1	31-35	26-32	30-35	27-38	25-40
V_H CDR2	50-65	53-55	47-58	56-65	58-77
V_H CDR3	95-102	96-101	93-101	105-117	109-137
V_L CDR1	24-34	26-32	30-36	27-38	25-40
V_L CDR2	50-56	50-52	46-55	56-65	58-77
V_L CDR3	89-97	91-96	89-96	105-117	109-137

[1]Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

[2]Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

[3]Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*

[4]Residue numbering follows the nomenclature of Lefranc *et al.*, *supra*

[5]Residue numbering follows the nomenclature of Honegger and Plückthun, *supra*

[0059] The term "chimeric antibodies" refer to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit a biological activity of this invention (see U.S. Patent No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

[0060] The term "semi-synthetic" in reference to an antibody or antibody moiety means that the antibody or antibody moiety has one or more naturally occurring sequences and one or more non-naturally occurring (*i.e.*, synthetic) sequences.

[0061] The term "fully synthetic" in reference to an antibody or antibody moiety means that the antibody or antibody moiety has fixed, mostly or all naturally occurring V_H/V_L framework pairings, but non-naturally occurring (*i.e.*, synthetic) sequences of all 6 CDRs of both heavy and light chains. Non-naturally occurring CDRs include those comprising modified human CDR sequences, such as CDR sequences modified by conservative amino acid substitutions or introduced cysteine residues.

[0062] "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[0063] "Homology" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are "homologous" at that position. The "percent of homology" or "percent sequence identity" between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared times 100, considering any

conservative substitutions as part of the sequence identity. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology. Alignment for purposes of determining percent amino acid 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, Megalign (DNASTAR), or MUSCLE software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program MUSCLE (Edgar, R.C., *Nucleic Acids Research* 32(5):1792-1797, 2004; Edgar, R.C., *BMC Bioinformatics* 5(1):113, 2004).

[0064] The "C_H1 domain" of a human IgG (also referred to as "C1" of "H1" domain) usually extends from about amino acid 118 to about amino acid 215 (EU numbering system).

[0065] Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

[0066] The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0067] The term "inducible promoter" refers to a promoter whose activity can be regulated by adding or removing one or more specific signals. For example, an inducible promoter may activate transcription of an operably linked nucleic acid under a specific set of conditions, *e.g.*, in the presence of an inducing agent that activates the promoter and/or relieves repression of the promoter.

[0068] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results, including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing or improving the quality of life, increasing weight gain, and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of the disease (such as, for example, tumor volume in cancer). The methods of the invention contemplate any one or more of these aspects of treatment.

[0069] The terms "recurrence," "relapse" or "relapsed" refers to the return of a cancer or disease after clinical assessment of the disappearance of disease. A diagnosis of distant metastasis or local recurrence can be considered a relapse.

[0070] The term "refractory" or "resistant" refers to a cancer or disease that has not responded to treatment.

[0071] An "effective amount" of a caTCR or composition comprising a caTCR as disclosed herein is an amount sufficient to carry out a specifically stated purpose. An "effective amount" can be determined empirically and by known methods relating to the stated purpose.

[0072] The term "therapeutically effective amount" refers to an amount of a caTCR or composition comprising a caTCR as disclosed herein, effective to "treat" a disease or disorder in an individual. In the case of cancer, the therapeutically effective amount of a caTCR or composition comprising a caTCR as disclosed herein can reduce the number of cancer cells; reduce the tumor size or weight; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent a caTCR or composition comprising a caTCR as disclosed herein can prevent growth and/or kill existing cancer cells, it can be cytostatic and/or cytotoxic. In some embodiments, the therapeutically effective amount is a growth inhibitory amount. In some embodiments, the therapeutically effective amount is an amount that improves progression free survival of a patient. In the case of infectious disease,

such as viral infection, the therapeutically effective amount of a caTCR or composition comprising a caTCR as disclosed herein can reduce the number of cells infected by the pathogen; reduce the production or release of pathogen-derived antigens; inhibit (*i.e.*, slow to some extent and preferably stop) spread of the pathogen to uninfected cells; and/or relieve to some extent one or more symptoms associated with the infection. In some embodiments, the therapeutically effective amount is an amount that extends the survival of a patient.

[0073] As used herein, by "pharmaceutically acceptable" or "pharmacologically compatible" is meant a material that is not biologically or otherwise undesirable, *e.g.*, the material may be incorporated into a pharmaceutical composition administered to a patient without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained. Pharmaceutically acceptable carriers or excipients have preferably met the required standards of toxicological and manufacturing testing and/or are included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug administration.

[0074] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

[0075] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X".

[0076] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0077] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise.

Chimeric antibody/T cell receptor (caTCR) constructs

[0078] In one aspect, the present invention provides a target antigen-specific chimeric antibody/T cell receptor (caTCR) that specifically binds to a target antigen (such as a cell surface antigen or a peptide/MHC complex) and is capable of recruiting at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ), wherein the caTCR comprises at least one non-naturally occurring TCR domain (such as a non-naturally occurring TCR transmembrane domain). For example, in some embodiments, the TCRM comprises one or two non-naturally occurring TCR transmembrane domains. A non-naturally occurring TCR domain may be a corresponding domain of a naturally occurring TCR modified by substitution of one or

more amino acids, and/or by replacement of a portion of the corresponding domain with a portion of an analogous domain from another TCR.

[0079] In one aspect, the present invention provides a multispecific caTCR that specifically binds two or more target antigens or epitopes (such as two different cell surface antigens) and is capable of recruiting at least one TCR-associated signaling molecule (such as CD3δε, CD3γε, and/or ζζ). In some embodiments, the multispecific antigen-binding module comprises a dual variable domain (DVD), a cross-over dual variable (CODV) domain, or a scFv-Fab fusion domain. In some embodiments, the multispecific antigen-binding module comprises a first Fv and a second Fv, a first scFv and a second scFv, or a Fab and a scFv. In some embodiments, the TCRM comprises one or more non-naturally occurring TCR domains (*e.g.*, a non-naturally occurring TCR transmembrane domain). In some embodiments, the TCRM does not comprise a non-naturally occurring TCR transmembrane domain.

[0080] The caTCRs described herein comprise an antigen-binding module (*e.g.*, monospecific or multispecific antigen-binding module) that provides the antigen specificity and a T cell receptor module (TCRM) that allows for CD3 recruitment and signaling. The antigen-binding module is not a naturally occurring T cell receptor antigen-binding moiety. In some embodiments, the antigen-binding module is linked to the amino terminus of a polypeptide chain in the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). The TCRM comprises a transmembrane module derived from the transmembrane domains of one or more TCRs (TCR-TMs), such as an αβ and/or γδ TCR, and optionally further comprises one or both of the connecting peptides or fragments thereof of a TCR and/or one or more TCR intracellular domains or fragments thereof. In some embodiments, the TCRM comprises two polypeptide chains, each polypeptide chain comprising, from amino terminus to carboxy terminus, a connecting peptide, a transmembrane domain, and optionally a TCR intracellular domain. In some embodiments, the caTCR comprises a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the antigen-binding module and the TCRM. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the caTCR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the caTCR further comprises one or more T cell co-stimulatory

signaling sequences. The one or more co-stimulatory signaling sequences can be, individually, all or a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the one or more co-stimulatory signaling sequences are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more co-stimulatory signaling sequences are carboxy-terminal to the first TCRD and/or the second TCRD. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module is located between the antigen-binding module and the TCRM. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the spacer module comprises one or more peptide linkers connecting two caTCR modules or domains.

[0081] The caTCRs described herein, including caTCRs comprising non-naturally occurring transmembrane domains and multispecific caTCRs, may have one or more features described in this section. It is intended that any of the features for each component of the caTCR (*e.g.*, antigen-binding module, TCRD, TCR-TM, spacer module, stabilization module, T cell co-stimulation sequences, and various linkers etc.) described herein can be combined with each other as if each and every combination is individually described.

[0082] In some embodiments, the antigen-binding module (such as an antibody moiety) specifically binds to a target antigen with a) an affinity that is at least about 10 (including for example at least about any of 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for other molecules; or b) a K_d no more than about 1/10 (such as no more than about any of 1/10, 1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to other molecules. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, Biacore instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0083] Examples of stabilization domains include an Fc region; a hinge region; a C_H3 domain; a C_H4 domain; a C_H1 or C_L domain; a leucine zipper domain (*e.g.*, a jun/fos leucine zipper

domain, *see*, *e.g.*, Kostelney *et al*, *J. Immunol*, 148: 1547-1553, 1992; or a yeast GCN4 leucine zipper domain); an isoleucine zipper domain; a dimerizing region of a dimerizing cell-surface receptor (e.g., interleukin-8 receptor (IL-8R); or an integrin heterodimer such as LFA-1 or GPIIIb/IIIa); a dimerizing region of a secreted, dimerizing ligand (e.g., nerve growth factor (NGF), neurotrophin-3 (NT-3), interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), or brain-derived neurotrophic factor (BDNF); *see*, *e.g.*, Arakawa *et al*, *J. Biol. Chem.* 269:27833-27839, 1994, and Radziejewski *et al*, *Biochem.* 32: 1350, 1993); a coiled coil dimerization domain (*see*, for example, WO2014152878; Fletcher et al, *ACS Synth. Biol.* 1:240-250, 2012; and Thomas *et al.*, *J. Am. Chem. Soc.* 135(13):5161-5166, 2013); and a polypeptide comprising at least one cysteine residue (e.g., from about one, two, or three to about ten cysteine residues) such that disulfide bond(s) can form between the polypeptide and a second polypeptide comprising at least one cysteine residue.

[0084] In some embodiments, the TCRM described herein comprises a) a first T cell receptor domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and b) a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule, and wherein the first TCR-TM and the second TCR-TM are naturally occurring. In some embodiments, the TCRM described herein comprises a) a first T cell receptor domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and b) a second TCRD comprising a second TCR-TM, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule, and wherein the first TCR-TM and/or the second TCR-TM are non-naturally occurring. In some embodiments, both of the TCR-TMs are non-naturally occurring. In some embodiments, the first TCR-TM is derived from one of the transmembrane domains of a naturally occurring T cell receptor (such as an αβ TCR or a γδ TCR) and the second TCR-TM is derived from the other transmembrane domain of the naturally occurring T cell receptor. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the transmembrane domains of the naturally occurring T cell receptor. Recruitment of TCR-associated signaling molecules can be determined by methods known in the art, such as FACS analysis for TCR-CD3 complex surface expression or co-immunoprecipitation of CD3 subunits with the caTCR.

[0085] For example, in some embodiments, the first TCR-TM of a TCRM described herein comprises, consists essentially of, or consists of the transmembrane domain of the TCR α chain (*e.g.*, GenBank Accession No: CCI73895) or a variant thereof and the second TCR-TM of the

TCRM comprises, consists essentially of, or consists of the transmembrane domain of the TCR β chain (*e.g.*, GenBank Accession No: CCI73893) or a variant thereof. In some embodiments, the first TCR-TM comprises, consists essentially of, or consists of the transmembrane domain of the TCR δ chain (*e.g.*, GenBank Accession No: AAQ57272) or a variant thereof and the second TCR-TM comprises, consists essentially of, or consists of the transmembrane domain of the TCR γ chain (*e.g.*, GenBank Accession No: AGE91788) or a variant thereof. In some embodiments, the first and second TCR-TMs of a TCRM described herein comprise, consist essentially of, or consist of the transmembrane domain of a TCR α chain constant domain (*e.g.*, SEQ ID NO: 1) or a variant thereof and the transmembrane domain of a TCR β chain constant domain (*e.g.*, SEQ ID NO: 2) or a variant thereof, respectively. In some embodiments, the first and second TCR-TMs comprise, consist essentially of, or consist of the transmembrane domain of a TCR δ chain constant domain (*e.g.*, SEQ ID NO: 3) or a variant thereof and the transmembrane domain of a TCR γ chain constant domain (*e.g.*, SEQ ID NO: 4) or a variant thereof, respectively. In some embodiments, the first and second TCR-TMs comprise, consist essentially of, or consist of the amino acid sequences of SEQ ID NOs: 5 and 6, or variants thereof, respectively. In some embodiments, the first and second TCR-TMs comprise, consist essentially of, or consist of the amino acid sequences of SEQ ID NOs: 7 and 8, or variants thereof, respectively. Variants of the transmembrane domains include, without limitation, transmembrane domains with one or more amino acid substitutions compared to the reference sequence. In some embodiments, a variant transmembrane domain comprises no more than 5 amino acid substitutions compared to the reference sequence. In some embodiments, the first TCRD further comprises a first connecting peptide amino-terminal to the transmembrane domain and/or the second TCRD further comprises a second connecting peptide amino-terminal to the transmembrane domain. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and/or second connecting peptides comprise, consist essentially of, or consist of all or a portion of the connecting peptide of a TCR α chain constant domain (*e.g.*, SEQ ID NO: 1) or a variant thereof and all or a portion of the connecting peptide of a TCR β chain constant domain (*e.g.*, SEQ ID NO: 2) or a variant thereof, respectively. In some embodiments, the first and/or second connecting peptides comprise, consist essentially of, or consist of all or a portion of the connecting peptide of SEQ ID NO: 27

or 28, or a variant thereof, and all or a portion of the connecting peptide of SEQ ID NO: 29 or 30, or a variant thereof, respectively. In some embodiments, the first and/or second connecting peptides comprise, consist essentially of, or consist of all or a portion of the connecting peptide of a TCR δ chain constant domain (*e.g.*, SEQ ID NO: 3) or a variant thereof and all or a portion of the connecting peptide of a TCR γ chain constant domain (*e.g.*, SEQ ID NO: 4) or a variant thereof, respectively. In some embodiments, the first and/or second connecting peptides comprise, consist essentially of, or consist of all or a portion of the connecting peptide of SEQ ID NO: 31 or 32, or a variant thereof, and all or a portion of the connecting peptide of SEQ ID NO: 33 or 34, or a variant thereof, respectively. In some embodiments, the first TCRD further comprises a first TCR intracellular domain carboxy-terminal to the first TCR-TM and/or the second TCRD further comprises a second TCR intracellular domain carboxy-terminal to the second TCR-TM. In some embodiments, the first TCR intracellular domain comprises all or a portion of the intracellular domain of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second TCR intracellular domain comprises all or a portion of the intracellular domain of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the second TCR intracellular domains comprise any one of the amino acid sequences of SEQ ID NOs: 35-36, or variants thereof. In some embodiments, the first TCRD is a fragment of one chain of a naturally occurring TCR, or a variant thereof, and/or the second TCRD is a fragment of the other chain of the naturally occurring TCR, or a variant thereof. In some embodiments, at least one of the TCRDs is non-naturally occurring. In some embodiments, the first and second TCRDs are linked by a disulfide bond. In some embodiments, the first and second TCRDs are linked by a disulfide bond between a residue in the first connecting peptide and a residue in the second connecting peptide. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM is capable of recruiting each of CD3δε, CD3γε, and ζζ to form a caTCR-CD3 complex (*i.e.*, promotes caTCR-CD3 complex formation).

[0086] Contemplated caTCR constructs include, for example, caTCRs that specifically bind to cell surface antigens, caTCRs that specifically bind to cell surface-presented peptide/MHC complexes, and caTCRs that specifically bind to both cell surface antigens and cell surface-presented peptide/MHC complexes.

[0087] In some embodiments, the antigen-binding module is an antibody moiety selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some

embodiments, the antibody moiety is monospecific. In some embodiments, the antibody moiety is multi-specific. In some embodiments, the antibody moiety is bispecific. In some embodiments, the antibody moiety is a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, a dual variable domain (DVD) antibody, a chemically cross-linked antibody, a heteromultimeric antibody, or a heteroconjugate antibody. In some embodiments, the antibody moiety is a tandem scFv comprising two scFvs linked by a peptide linker. In some embodiments, the antibody moiety is two scFvs that are not directly linked. In some embodiments, the antibody moiety is fully human, semi-synthetic with human antibody framework regions, or humanized.

[0088] In some embodiments, the antigen-binding module comprises a first antigen-binding domain comprising a V_H antibody domain and a second antigen-binding domain comprising a V_L antibody domain. In some embodiments, the V_H antibody domain and V_L antibody domain CDRs are derived from the same antibody moiety. In some embodiments, some of the V_H antibody domain and V_L antibody domain CDRs are derived from different antibody moieties. In some embodiments, the V_H antibody domain and/or V_L antibody domain are human, humanized, chimeric, semi-synthetic, or fully synthetic.

[0089] In some embodiments, the antigen-binding module is an antibody moiety that is semi-synthetic, comprising fully human sequences and one or more synthetic regions. In some embodiments, the antigen-binding module is a semi-synthetic antibody moiety, comprising a fully human V_L and a semi-synthetic V_H comprising fully human FR1, HC-CDR1, FR2, HC-CDR2, FR3, and FR4 regions and a synthetic HC-CDR3. In some embodiments, the semi-synthetic V_H comprises a fully synthetic HC-CDR3 having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length. In some embodiments, the semi-synthetic V_H or the synthetic HC-CDR3 is obtained from a semi-synthetic library (such as a semi-synthetic human library) comprising fully synthetic HC-CDR3 regions having a sequence from about 5 to about 25 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25) amino acids in length, wherein each amino acid in the sequence is, independently from one another, randomly selected from the standard human amino acids, minus cysteine. In some embodiments, the synthetic HC-CDR3 is from about 10 to about 19 (such as about any of 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19) amino acids in length. In some embodiments, the antigen-binding module is a semi-synthetic antibody moiety, comprising a semi-synthetic V_L and a semi-synthetic V_H. In some embodiments, the antigen-binding module is a fully-synthetic antibody moiety, comprising

fixed human V_H/V_L framework pairings, but randomized and synthetic sequences for all 6 CDRs of both heavy and light chains.

[0090] The antigen-binding module in some embodiments is an antibody moiety comprising specific CDR sequences derived from one or more antibody moieties (such as a monoclonal antibody) or certain variants of such sequences comprising one or more amino acid substitutions. In some embodiments, the amino acid substitutions in the variant sequences do not substantially reduce the ability of the antigen-binding module to bind the target antigen. Alterations that substantially improve target antigen binding affinity or affect some other property, such as specificity and/or cross-reactivity with related variants of the target antigen, are also contemplated.

[0091] In some embodiments, the stabilization module is derived from an antibody moiety. For example, in some embodiments, the stabilization module comprises a fist stabilization domain comprising a C_H1 antibody domain or variant thereof and a second stabilization domain comprising a C_L antibody domain or variant thereof. In another embodiment, the stabilization module comprises a first stabilization domain comprising a C_H3 antibody domain or variant thereof and a second stabilization domain comprising a C_H3 antibody domain or a variant thereof. In some embodiments, antibody heavy chain constant domains (*e.g.*, C_H1 or C_H3) contained in the stabilization module are derived from an IgG (*e.g*, IgG1, IgG2, IgG3, or IgG4), IgA (*e.g.*, IgA1 or IgA2), IgD, IgM, or IgE heavy chain, optionally human. In some embodiments, an antibody heavy chain constant domain (*e.g.*, C_H1 or C_H3) contained in the stabilization module is a variant comprising one or more modifications (*e.g.*, amino acid substitutions, insertions, and/or deletions) compared to the sequence from which it is derived. In some embodiments, antibody light chain constant domains (C_L) contained in the stabilization module are derived from a kappa or lambda light chain, optionally human. In some embodiments, an antibody light chain constant domain (C_L) contained in the stabilization module is a variant comprising one or more modifications (*e.g.*, amino acid substitutions, insertions, and/or deletions) compared to the sequence from which it is derived. In some embodiments, the first and/or second stabilization domains comprise one or more modifications that do not substantially alter their binding affinity for each other. In some embodiments, the first and/or second stabilization domains comprise one or more modifications that increase their binding affinity for each other and/or introduce a non-naturally occurring disulfide bond. In some embodiments, the stabilization module comprises a knob-into-hole modification (*see*, for example, Carter P. *J Immunol Methods*. 248:7–15, 2001). For example, in some embodiments,

the stabilization module comprises antibody constant domain regions (*e.g.*, C_H3 domains) comprising a knob-into-hole modification. In some embodiments, the stabilization module comprises antibody constant domain regions (*e.g.*, C_H3 domains) modified by electrostatic steering to enhance their association (*see*, for example, WO2006106905 and Gunasekaran K, *et al. J Biol Chem.* 285:19637–46, 2010). In some embodiments, the first and second stabilization domains are linked by a disulfide bond.

[0092] In some embodiments, the caTCR comprises an antigen-binding module described herein linked to a TCRM described herein, optionally including a stabilization module. For example, in the some embodiments, the caTCR comprises the antigen-binding module linked to the N-terminus of one or both of the TCRDs. In some embodiments, the caTCR comprises a stabilization module between a TCRM and an antigen-binding module. In some embodiments, the caTCR further comprises a spacer module between any two caTCR modules or domains. In some embodiments, the spacer module comprises one or more peptide linkers between about 5 to about 70 (such as about any of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70, including any ranges between these values) amino acids in length. In some embodiments, the caTCR further comprises one or more accessory intracellular domains. In some embodiments, the one or more accessory intracellular domains are carboxy-terminal to the first and/or second TCRD. In some embodiments, the one or more accessory intracellular domains are between the first TCR-TM and the first TCR intracellular domain and/or between the second TCR-TM and the second TCR intracellular domain. In some embodiments, the one or more accessory intracellular domains comprise, individually, a TCR co-stimulatory domain. In some embodiments, the TCR co-stimulatory domain comprises all or a portion of the intracellular domain of an immune co-stimulatory molecule (such as CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like). In some embodiments, the TCR co-stimulatory domain comprises all or a portion of the amino acid sequence of any one of SEQ ID NOs: 49-51, or a variant thereof.

[0093] In some embodiments, the antigen-binding module specifically binds a cell surface antigen. In some embodiments, the cell surface antigen is selected from the group consisting of a protein, a carbohydrate, and a lipid. In some embodiments, the cell surface antigen is a disease-associated antigen expressed in a diseased cell. In some embodiments, the antigen-binding module specifically binds a complex comprising a peptide and an MHC protein. Peptide/MHC complexes include, for example, a surface-presented complex comprising a peptide derived from

a disease-associated antigen expressed in a diseased cell and an MHC protein. In some embodiments, the full-length disease-associated antigen is not normally expressed on the surface of the diseased cell (*e.g.*, the disease-associated antigen is an intracellular or secreted protein). In some embodiments, the disease is cancer and the disease-associated antigen is a tumor-associated antigen expressed in a cancer cell. In some embodiments, the tumor-associated antigen is an oncoprotein. In some embodiments, the oncoprotein is the result of a mutation in a proto-oncogene, and the oncoprotein comprises a neoepitope comprising the mutation. For example, in some embodiments, the antigen-binding module specifically binds a cell surface tumor-associated antigen (*e.g.*, an oncoprotein comprising a neoepitope). In some embodiments, the antigen-binding module specifically binds a complex comprising a peptide derived from a tumor-associated antigen (*e.g.*, an oncoprotein comprising a neoepitope) not normally expressed on the surface of a cancer cell (*e.g.*, an intracellular or secreted tumor-associated antigen) and an MHC protein. In some embodiments, the disease is viral infection and the disease-associated antigen is a virus-associated antigen expressed in an infected cell. For example, in some embodiments, the antigen-binding module specifically binds a cell surface virus-associated antigen. In some embodiments, the antigen-binding module specifically binds a complex comprising a peptide derived from a virus-associated antigen not normally expressed on the surface of a virus-infected cell (*e.g.*, an intracellular or secreted virus-associated antigen) and an MHC protein. In some embodiments, the caTCR construct binds the target antigen with a K_d between about 0.1 pM to about 500 nM (such as about any of 0.1 pM, 1.0 pM, 10 pM, 50 pM, 100 pM, 500 pM, 1 nM, 10 nM, 50 nM, 100 nM, or 500 nM, including any ranges between these values).

[0094] In some embodiments, the caTCR comprises an antigen-binding module that specifically binds to a cell surface antigen, wherein the cell surface antigen is CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, or FCRL5, including variants or mutants thereof. Specific binding to a full antigen, e.g., a cell surface antigen, is sometimes referred to as "non-MHC-restricted binding".

[0095] In some embodiments, the caTCR comprises an antigen-binding module that specifically binds to a complex comprising a peptide and an MHC protein, wherein the peptide is derived from a protein selected from the group consisting of WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. Specific binding to a complex comprising a peptide and an MHC protein is sometimes referred to as "MHC-restricted binding".

[0096] In some embodiments, the caTCR comprises an antigen-binding module that specifically binds to a complex comprising a peptide derived from a disease-associated antigen (such as a tumor-associated or virally-encoded antigen) and an MHC class I protein, wherein the MHC class I protein is HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G. In some embodiments, the MHC class I protein is HLA-A, HLA-B, or HLA-C. In some embodiments, the MHC class I protein is HLA-A. In some embodiments, the MHC class I protein is HLA-B. In some embodiments, the MHC class I protein is HLA-C. In some embodiments, the MHC class I protein is HLA-A01, HLA-A02, HLA-A03, HLA-A09, HLA-A10, HLA-A11, HLA-A19, HLA-A23, HLA-A24, HLA-A25, HLA-A26, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32, HLA-A33, HLA-A34, HLA-A36, HLA-A43, HLA-A66, HLA-A68, HLA-A69, HLA-A74, or HLA-A80. In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is any one of HLA-A*02:01-555, such as HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, HLA-A*02:08, HLA-A*02:09, HLA-A*02:10, HLA-A*02:11, HLA-A*02:12, HLA-A*02:13, HLA-A*02:14, HLA-A*02:15, HLA-A*02:16, HLA-A*02:17, HLA-A*02:18, HLA-A*02:19, HLA-A*02:20, HLA-A*02:21, HLA-A*02:22, or HLA-A*02:24. In some embodiments, the MHC class I protein is HLA-A*02:01.

[0097] In some embodiments, the caTCR comprises an antigen-binding module that specifically binds to a complex comprising a peptide derived from a disease-associated antigen (such as a tumor-associated or virally-encoded antigen) and an MHC class II protein, wherein the MHC class II protein is HLA-DP, HLA-DQ, or HLA-DR. In some embodiments, the MHC class II protein is HLA-DP. In some embodiments, the MHC class II protein is HLA-DQ. In some embodiments, the MHC class II protein is HLA-DR.

[0098] In some embodiments, there is provided a caTCR (such as an isolated caTCR) comprising a) an antigen-binding module that specifically binds to a target antigen, and b) a TCRM comprising first and second TCR-TMs derived from the transmembrane domains of a naturally occurring TCR (such as an αβTCR or a γδTCR), wherein the TCRM is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring. In some embodiments, the first and second TCR-TMs are non-naturally occurring. In some embodiments, the antigen-binding module is linked to the amino-terminus of one or more polypeptide chains in the TCRM. For example, in some embodiments, the TCRM comprises two polypeptide chains, and the antigen-binding module is linked to the amino-terminus of one or both of the TCRM polypeptide chains. In some

embodiments, the TCRM further comprises at least one connecting peptide or fragment thereof of the naturally occurring TCR amino-terminal to a TCR-TM. In some embodiments, the TCRM further comprises at least one TCR intracellular domain comprising a sequence from an intracellular domain of the naturally occurring TCR carboxy-terminal to a TCR-TM. In some embodiments, the TCRM comprises TCRDs derived from fragments of the naturally occurring TCR chains. At least one of the TCRDs is non-naturally occurring. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40) carboxy-terminal to a TCR-TM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety comprises a V_H antibody domain and a V_L antibody domain. In some embodiments, the antibody moiety is human, humanized, chimeric, semi-synthetic, or fully synthetic. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module comprises at least one disulfide bond linking the stabilization domains. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the caTCR is a heteromultimer, such as a heterodimer. For example, in some embodiments, the caTCR is a heterodimer comprising a first polypeptide chain comprising the first TCRD and a second polypeptide chain comprising the second TCRD, wherein the antigen-binding module is linked to the first and/or second polypeptide chains. In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0099] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR,

wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the naturally occurring TCR is an αβ TCR and the first and second TCR-TMs are derived from TCR α and β subunit transmembrane domains. In some embodiments, the naturally occurring TCR is a γδ TCR and the first and second TCR-TMs are derived from TCR γ and δ subunit transmembrane domains. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling

molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0100] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring αβ TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring αβ TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second

stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring αβ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0101] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring γδ TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring γδ TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit

from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring γδ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0102] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 and a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences

of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 1 and 2. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0103] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 and a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more

(such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a

stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 1 and 2. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0104] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 and a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 3 or 4, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 3 or 4. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 3 or 4. In some embodiments, the chimeric sequence in the first or second TCR-TM is from a transmembrane domain contained in SEQ ID NO: 3 and the chimeric sequence in the other TCR-TM is from a transmembrane domain contained in SEQ ID NO: 4. In some

embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 1 and 2. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is

a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0105] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 4 and a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L

antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 3 and 4. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0106] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 4 and a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one

another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 3 and 4. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0107] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from a transmembrane domain contained in one of the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 4 and

a second TCRD comprising a second TCR-TM derived from a transmembrane domain contained in the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 1 or 2, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 1 or 2. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from a transmembrane domain contained in SEQ ID NO: 1 or 2. In some embodiments, the chimeric sequence in the first or second TCR-TM is from a transmembrane domain contained in SEQ ID NO: 1 and the chimeric sequence in the other TCR-TM is from a transmembrane domain contained in SEQ ID NO: 2. In some embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of a connecting peptide contained in any one of the amino acid sequences of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of an intracellular domain contained in any one of SEQ ID NOs: 1-4, or a variant thereof. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular

domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of the transmembrane domains contained in SEQ ID NOs: 3 and 4. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0108] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise,

independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0109] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a

single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0110] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 7 or 8, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 7 or 8. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from SEQ ID NO: 7 or 8. In some embodiments, the chimeric sequence in the first or second TCR-TM is from SEQ ID NO: 7 and the chimeric sequence in the other TCR-TM is from SEQ ID NO: 8. In some embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization

domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0111] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that

stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0112] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the

second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0113] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 5 or 6, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically

binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 5 or 6. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from SEQ ID NO: 5 or 6. In some embodiments, the chimeric sequence in the first or second TCR-TM is from SEQ ID NO: 5 and the chimeric sequence in the other TCR-TM is from SEQ ID NO: 6. In some embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any

two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (such as bispecific).

[0114] In some embodiments, there is provided a complex comprising a caTCR according to any of the caTCRs described herein and at least one signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the complex comprises each of CD3δε, CD3γε, and ζζ. Thus, in some embodiments, there is provided a complex comprising the caTCR, CD3δε, CD3γε, and ζζ.

[0115] The different aspects are discussed in various sections below in further detail.

TCR-TM variants

[0116] The TCR-TMs of the caTCR described herein are derived from a naturally occurring T cell receptor. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. Non-naturally occurring TCR-TMs derived from a naturally occurring T cell receptor include a transmembrane domain from a naturally occurring T cell receptor that has been modified by substitution of one or more amino acids. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal (either in primary sequence or spatially) to an amino acid in the TCRM involved in binding CD3. For example, in some embodiments, at least one of the substituted amino acids is separated from an amino acid in the TCRM involved in binding CD3 by no more than 3 (such as 0, 1, 2, or 3) amino acids. In some embodiments, at least one of the substituted amino acids is separated from an amino acid in the TCRM involved in binding CD3 by no more than about 15 (such as no more than about any of 14, 12, 10, 8, 6, 4, 2, or 1) angstroms. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0117] For example, in some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor comprises, consists essentially of, or consists of the transmembrane domain of an α, β, γ, or δ TCR subunit modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the TCR subunit is modified by substitution of no more than 5 amino acid residues. In some embodiments, the transmembrane domain of the TCR subunit is modified by substitution of a single amino acid

residue. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0118] Thus, in some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of an α TCR subunit comprising the amino acid sequence of a transmembrane domain contained in SEQ ID NO: 1 (*e.g.*, SEQ ID NO: 5), modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the α TCR subunit is modified by substitution of no more than 5 amino acid residues in the transmembrane domain contained in SEQ ID NO: 1. In some embodiments, the transmembrane domain of the α TCR subunit is modified by substitution of a single amino acid residue in the transmembrane domain contained in SEQ ID NO: 1. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0119] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of an α TCR subunit comprising the amino acid sequence of SEQ ID NO: 5, modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the α TCR subunit is modified by substitution of no more than 5 amino acid residues in SEQ ID NO: 5. In some embodiments, the transmembrane domain of the α TCR subunit is modified by substitution of a single amino acid residue in SEQ ID NO: 5. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is

proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0120] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of a β TCR subunit comprising the amino acid sequence of a transmembrane domain contained in SEQ ID NO: 2 (*e.g.*, SEQ ID NO: 6), modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the β TCR subunit is modified by substitution of no more than 5 amino acid residues in the transmembrane domain contained in SEQ ID NO: 2. In some embodiments, the transmembrane domain of the β TCR subunit is modified by substitution of a single amino acid residue in the transmembrane domain contained in SEQ ID NO: 2. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0121] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of a β TCR subunit comprising the amino acid sequence of SEQ ID NO: 6, modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the β TCR subunit is modified by substitution of no more than 5 amino acid residues in SEQ ID NO: 6. In some embodiments, the transmembrane domain of the β TCR subunit is modified by substitution of a single amino acid residue in SEQ ID NO: 6. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0122] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of a δ TCR subunit comprising the amino acid sequence of a transmembrane domain contained in SEQ ID NO: 3 (*e.g.*, SEQ ID NO: 7), modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the δ TCR subunit is modified by substitution of no more than 5 amino acid residues in the transmembrane domain contained in SEQ ID NO: 3. In some embodiments, the transmembrane domain of the δ TCR subunit is modified by substitution of a single amino acid residue in the transmembrane domain contained in SEQ ID NO: 3. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0123] In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 3 (*e.g.*, SEQ ID NO: 7), modified by one or more substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to the following amino acids in SEQ ID NO: 7: L4, M6, V12, N15, F245, and L25. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 3 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to V12 and N15 in SEQ ID NO: 7. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 3 modified by one or more substitutions corresponding to the following substitutions in SEQ ID NO: 7: L4C, M6V, V12F, N15S, F245S, and L25S. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 3 modified by substitutions corresponding to V12F and N15S substitutions in SEQ ID NO: 7. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 9-13.

[0124] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of a δ TCR subunit comprising the amino acid sequence of SEQ ID NO:

7, modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the δ TCR subunit is modified by substitution of no more than 5 amino acid residues in SEQ ID NO: 7. In some embodiments, the transmembrane domain of the δ TCR subunit is modified by substitution of a single amino acid residue in SEQ ID NO: 7. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0125] In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 7 modified by one or more substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to the following amino acids in SEQ ID NO: 7: L4, M6, V12, N15, F245, and L25. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 7 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to V12 and N15 in SEQ ID NO: 7. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 7 modified by one or more substitutions corresponding to the following substitutions in SEQ ID NO: 7: L4C, M6V, V12F, N15S, F245S, and L25S. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 7 modified by substitutions corresponding to V12F and N15S substitutions in SEQ ID NO: 7. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 9-13.

[0126] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the transmembrane domain of a γ TCR subunit comprising the amino acid sequence of a transmembrane domain contained in SEQ ID NO: 4 (*e.g.*, SEQ ID NO: 8), modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the γ TCR subunit is modified by substitution of no more than 5 amino acid residues in the transmembrane domain contained in SEQ ID NO: 4. In some embodiments, the transmembrane domain of the γ TCR subunit is modified by substitution of a single amino acid residue in the transmembrane domain contained in SEQ ID NO: 4. In some embodiments, at

least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0127] In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 (*e.g.*, SEQ ID NO: 8), modified by one or more substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to the following amino acids in SEQ ID NO: 8: Y1, Y2, M3, L5, L8, V12, V13, F15, A16, I18, C19, C20, and C21. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to Y2, M3, A16, and I18 in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to L8, V12, and F15 in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by one or more substitutions corresponding to the following substitutions in SEQ ID NO: 8: Y1Q, Y2L, Y2I, M3V, M3I, L5C, L8F, V12F, V13Y, F15S, A16V, A16I, I18V, I18L, C19M, C20M, and C21G. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by substitutions corresponding to Y2L, M3V, A16V, and I18V substitutions in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by substitutions corresponding to Y2I, M3I, A16I, and I18L substitutions in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of the transmembrane domain contained in SEQ ID NO: 4 modified by substitutions corresponding to L8F, V12F, and F15S substitutions in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 14-26.

[0128] In some embodiments, a non-naturally occurring TCR-TM derived from a naturally occurring T cell receptor described herein comprises, consists essentially of, or consists of the

transmembrane domain of a γ TCR subunit comprising the amino acid sequence of SEQ ID NO: 8, modified by substitution of one or more amino acid residues. In some embodiments, the transmembrane domain of the γ TCR subunit is modified by substitution of no more than 5 amino acid residues in SEQ ID NO: 8. In some embodiments, the transmembrane domain of the γ TCR subunit is modified by substitution of a single amino acid residue in SEQ ID NO: 8. In some embodiments, at least one of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, each of the substituted amino acids is substituted with a residue more hydrophobic than the corresponding unsubstituted residue. In some embodiments, at least one of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3. In some embodiments, each of the substituted amino acids is proximal to an amino acid in the TCRM involved in binding CD3.

[0129] In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by one or more substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to the following amino acids in SEQ ID NO: 8: Y1, Y2, M3, L5, L8, V12, V13, F15, A16, I18, C19, C20, and C21. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to Y2, M3, A16, and I18 in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by substitutions (such as substitutions with more hydrophobic residues) in amino acids corresponding to L8, V12, and F15 in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by one or more substitutions corresponding to the following substitutions in SEQ ID NO: 8: Y1Q, Y2L, Y2I, M3V, M3I, L5C, L8F, V12F, V13Y, F15S, A16V, A16I, I18V, I18L, C19M, C20M, and C21G. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by substitutions corresponding to Y2L, M3V, A16V, and I18V substitutions in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by substitutions corresponding to Y2I, M3I, A16I, and I18L substitutions in SEQ ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of SEQ ID NO: 8 modified by substitutions corresponding to L8F, V12F, and F15S substitutions in SEQ

ID NO: 8. In some embodiments, the non-naturally occurring TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 14-26.

[0130] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM comprising, consisting essentially of, or consisting of any one of the amino acid sequences of SEQ ID NOs: 7 and 9-13, and a second TCRD comprising a second TCR-TM comprising, consisting essentially of, or consisting of the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCR-TM and second TCR-TM are selected according to any of the caTCRs listed in Table 2. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains

having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv).

Table 2

caTCR ID	First TCR-TM (δ subunit)	Second TCR-TM (γ subunit)
TM0	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVVYFAIITCCLL (SEQ ID NO: 8)
TM1	VLGLRMLFAKTVAVNFLLTAKLFSL (SEQ ID NO: 9)	YYMYLLLLLKSVVYFAIITCCLL (SEQ ID NO: 8)
TM2	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVYYFAIITCCLLRRTAF (SEQ ID NO: 14)
TM3	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVVYFAIITCGLLRRTAF (SEQ ID NO: 15)
TM4	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YLVYLLLLLKSVVYFVIVTCCLLRRTAF (SEQ ID NO: 16)
TM5	VLGLRVLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 10)	YLVYLLLLLKSVVYFVIVTCCLLRRTAF (SEQ ID NO: 16)
TM6	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YLMYLLLLLKSVVYFAIITCCLLRRTAF (SEQ ID NO: 17)
TM7	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYVYLLLLLKSVVYFAIITCCLLRRTAF (SEQ ID NO: 18)
TM8	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVVYFVIITCCLLRRTAF (SEQ ID NO: 19)
TM9	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVVYFAIVTCCLLRRTAF (SEQ ID NO: 20)
TM10	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYIYLLLLLKSVVYFAIITCCLLRRTAF (SEQ ID NO: 21)
TM11	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YIIYLLLLLKSVVYFIILTCCLLRRTAF (SEQ ID NO: 22)
TM12	VLGCRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 11)	YYMYCLLLLKSVVYFAIITCCLLRRTAF (SEQ ID NO: 23)
TM13	VLGLRMLFAKTFAVSFLLTAKLFFL (SEQ ID NO: 12)	YYMYLLLFLKSFVYSAIITCCLLRRTAF (SEQ ID NO: 24)
TM14	VLGLRMLFAKTVAVNFLLTAKLFFL (SEQ ID NO: 7)	YYMYLLLLLKSVVYFAIITMCLLRRTAF (SEQ ID NO: 25)
TM15	VLGLRMLFAKTVAVNFLLTAKLFFS (SEQ ID NO: 13)	QYMYLLLLLKSVVYFAIITCCLLRRTAF (SEQ ID NO: 26)

Antigen-binding modules

[0131] In some embodiments, according to any of the caTCRs described herein, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is selected

from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, where the antibody moiety is a multimer comprising a first antibody moiety chain and a second antibody moiety chain, the caTCR comprises the first TCRD linked to the first or second antibody moiety chain and the second TCRD linked to the other antibody moiety chain. In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof.

[0132] In some embodiments, according to any of the caTCRs described herein, the antigen-binding module is an antibody moiety comprising C_H1 and C_L domains. In some embodiments, the C_H1 domain is derived from an IgG (*e.g*, IgG1, IgG2, IgG3, or IgG4) heavy chain, optionally human. In some embodiments, the C_H1 domain is a variant comprising one or more modifications (*e.g.*, amino acid substitutions, insertions, and/or deletions) compared to the sequence from which it is derived. In some embodiments, the C_H1 domain comprises the amino acid sequence of any one of SEQ ID NOs: 37-47 and 86, or a variant thereof. In some embodiments, the C_H1 domain comprises the amino acid sequence of SEQ ID NO: 37, or a variant thereof. In some embodiments, the C_L domain is derived from a kappa or lambda light chain, optionally human. In some embodiments, the C_L domain is a variant comprising one or more modifications (*e.g.*, amino acid substitutions, insertions, and/or deletions) compared to the sequence from which it is derived. In some embodiments, the C_L domain comprises the amino acid sequence of SEQ ID NO: 48 or 87, or a variant thereof. In some embodiments, the C_H1 and/or C_L domains comprise one or more modifications that do not substantially alter their binding affinity for each other. In some embodiments, the C_H1 and/or C_L domains comprise one or more modifications that increase their binding affinity for each other and/or introduce a non-naturally occurring disulfide bond.

[0133] In some embodiments, according to any of the caTCRs described herein comprising an antibody moiety that specifically binds to a target antigen, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for the target antigen. In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*see*, *e.g.*, WO2017066136A2). In some embodiments, the antibody moiety comprises the CDRs or

variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 54 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 55, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD20 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 56 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 57, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*see*, *e.g.*, USSN 62/650,955 filed March 30, 2018, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 65 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 69, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*see*, *e.g.*, USSN 62/490,586 filed April 26, 2017, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 58 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 59, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR1 (*see*, *e.g.*, WO2016/187220 and WO2016/187216). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR2 (*see*, *e.g.*, WO2016/142768). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for BCMA (*see*, *e.g.*, WO2016/090327 and WO2016/090320). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPRC5D (*see*, *e.g.*, WO2016/090329 and WO2016/090312). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an

antibody moiety specific for FCRL5 (*see*, *e.g.*, WO2016/090337). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for WT-1 (*see*, *e.g.*, WO2012/135854, WO2015/070078, and WO2015/070061). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for AFP (*see*, *e.g.*, WO2016/161390). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for HPV16-E7 (*see*, *e.g.*, WO2016/182957). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for NY-ESO-1 (*see*, *e.g.*, WO2016/210365). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PRAME (*see*, *e.g.*, WO2016/191246). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for EBV-LMP2A (*see*, *e.g.*, WO2016/201124). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for KRAS (*see*, *e.g.*, WO2016/154047). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PSA (*see*, *e.g.*, WO2017/015634). In some embodiments, the antibody moiety is a Fab comprising one Fab chain comprising a V_H domain and a C_H1 domain, and another Fab chain comprising a V_L domain and a C_L domain. In some embodiments, the C_H1 domain comprises the amino acid sequence of any one of SEQ ID NOs: 37-47 and 86 and/or the C_L domain comprises the amino acid sequence of SEQ ID NO: 48 or 87. In some embodiments, the C_H1 domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 37 and the C_L domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 48.

caTCR Constructs

[0134] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antibody moiety that specifically binds to the target antigen,

wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the stabilization module is located between the TCRM and the antibody moiety.

[0135] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM comprising, consisting essentially of, or consisting of any one of the amino acid sequences of SEQ ID NOs: 7 and 9-13 and a second TCRD comprising a second TCR-TM comprising, consisting essentially of, or consisting of the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an antibody moiety that specifically binds to the target antigen, wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the first TCR-TM and second TCR-TM are selected according to any of the caTCRs listed in Table 2. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting

peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0136] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM and a second TCRD comprising a second TCR-TM, wherein the first and second TCR-TMs comprise, consist essentially of, or consist of the amino acid sequences of SEQ ID NOs: 9 and 8, 7 and 14, 7 and 15, 7 and 16, 10 and 16, 7 and 17, 7 and 18, 7 and 19, 7 and 20, 7 and 21, 7 and 22, 11 and 23, 12 and 24, 7 and 25, or 13 and 26, respectively, and wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antibody moiety that specifically binds to the target antigen, wherein the antibody moiety is linked to the first and/or second TCRDs. For example, in some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 9, and a second TCRD comprising a second TCR-TM comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 8, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and

b) an antibody moiety that specifically binds to the target antigen, wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0137] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM having the amino acid sequence

of SEQ ID NO: 10 and a second TCRD comprising a second TCR-TM having the amino acid sequence of SEQ ID NO: 16, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antibody moiety that specifically binds to the target antigen, wherein the antibody moiety is linked to the first and/or second TCRDs. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments,

the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0138] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first polypeptide chain comprising a first antigen-binding domain comprising a first Fab chain linked to a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a second Fab chain linked to a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, wherein the first and/or second TCR-TMs are non-naturally occurring, and wherein the first and second Fab chains form a Fab-like antigen-binding module that specifically binds the target antigen. In some embodiments, a) the first Fab chain comprises V_H and C_H1 antibody domains and the second Fab chain comprises V_L and C_L antibody domains; or b) the first Fab chain comprises V_L and C_L antibody domains and the second Fab chain comprises V_H and C_H1 antibody domains. For example, in some embodiments, the caTCR comprises a) a first polypeptide chain comprising a first Fab chain comprising V_H and C_H1 antibody domains linked to the first TCRD and b) a second Fab chain comprising V_L and C_L antibody domains linked to the second TCRD. In some embodiments, the caTCR comprises a) a first Fab chain comprising V_L and C_L antibody domains linked to the first TCRD and b) a second Fab chain comprising V_H and C_H1 antibody domains linked to the second TCRD. In some embodiments, there is a peptide linker between one or both of the TCRDs and their linked Fab chain. In some embodiments, there is a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, the C_H1 and/or C_L domains comprise one or more modifications that increase the binding affinity of the Fab chains for each other. In some embodiments, the C_H1 and C_L domains are swapped, such that one of the Fab chains comprises V_H and C_L antibody domains and the other Fab chain comprises V_L and C_H1 antibody domains. In some embodiments, the Fab-like antigen-binding module specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fab-like antigen-binding module specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization

module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first TCRD and its linked Fab chain and the other stabilization domain is located between the second TCRD and its linked Fab chain.

[0139] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first polypeptide chain comprising a first antigen-binding domain comprising a first Fab chain linked to a first TCRD comprising a first TCR-TM comprising, consisting essentially of, or consisting of any one of the amino acid sequences of SEQ ID NOs: 7 and 9-13; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a second Fab chain linked to a second TCRD comprising a second TCR-TM comprising, consisting essentially of, or consisting of the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, wherein the first and/or second TCR-TMs are non-naturally occurring, and wherein the first and second Fab chains form a Fab-like antigen-binding module that specifically binds the target antigen. In some embodiments, the first TCR-TM and second TCR-TM are selected according to any of the caTCRs listed in Table 2. In some embodiments, a) the first Fab chain comprises V_H and C_H1 antibody domains and the second Fab chain comprises V_L and C_L antibody domains; or b) the first Fab chain comprises V_L and C_L antibody domains and the second Fab chain comprises V_H and C_H1 antibody domains. In some embodiments, the C_H1 domain comprises the amino acid sequence of any one of SEQ ID NOs: 37-47 and 86 and/or the C_L domain comprises the amino acid sequence of SEQ ID NO: 48 or 87. In some embodiments, the C_H1 domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 37 and the C_L domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 48. In some embodiments, the Fab-like antigen-binding module specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fab-like antigen-binding module specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a

fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0140] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first polypeptide chain comprising a first antigen-binding domain comprising a first Fab chain linked to a first TCRD comprising a first TCR-TM and a second polypeptide chain comprising a second antigen-binding domain comprising a second Fab chain linked to a second TCRD comprising a second TCR-TM, wherein the first and second TCR-TMs comprise, consist essentially of, or consist of the amino acid sequences of SEQ ID NOs: 9 and 8, 7 and 14, 7 and 15, 7 and 16, 10 and 16, 7 and 17, 7 and 18, 7 and 19, 7 and 20, 7 and 21, 7 and 22, 11 and 23, 12 and 24, 7 and 25, or 13 and 26, respectively, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and second Fab chains form a Fab-like antigen-binding module that specifically binds the target antigen. In some embodiments, a) the first Fab chain comprises V_H and C_H1 antibody domains and the second Fab chain comprises V_L and C_L antibody domains; or b) the first Fab chain comprises V_L and C_L antibody domains and the second Fab

chain comprises V_H and C_H1 antibody domains. In some embodiments, the C_H1 domain comprises the amino acid sequence of any one of SEQ ID NOs: 37-47 and 86 and/or the C_L domain comprises the amino acid sequence of SEQ ID NO: 48 or 87. In some embodiments, the C_H1 domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 37 and the C_L domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 48. In some embodiments, the Fab-like antigen-binding module specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fab-like antigen-binding module specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments,

the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0141] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first polypeptide chain comprising a first antigen-binding domain comprising a first Fab chain linked to a first TCRD comprising a first TCR-TM having the amino acid sequence of SEQ ID NO: 10 and a second polypeptide chain comprising a second antigen-binding domain comprising a second Fab chain linked to a second TCRD comprising a second TCR-TM having the amino acid sequence of SEQ ID NO: 16, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and second Fab chains form a Fab-like antigen-binding module that specifically binds the target antigen. In some embodiments, a) the first Fab chain comprises V_H and C_H1 antibody domains and the second Fab chain comprises V_L and C_L antibody domains; or b) the first Fab chain comprises V_L and C_L antibody domains and the second Fab chain comprises V_H and C_H1 antibody domains. In some embodiments, the C_H1 domain comprises the amino acid sequence of any one of SEQ ID NOs: 37-47 and 86 and/or the C_L domain comprises the amino acid sequence of SEQ ID NO: 48 or 87. In some embodiments, the C_H1 domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 37 and the C_L domain comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 48. In some embodiments, the Fab-like antigen-binding module specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fab-like antigen-binding module specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain

comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains.

[0142] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) a Fab' that specifically binds to the target antigen, wherein the Fab' comprises a first Fab' chain comprising V_H, C_H1, and partial hinge antibody domains and a second Fab' chain comprising V_L and C_L antibody domains, and wherein the first Fab' chain is linked to the first or second TCRD and the second Fab' chain is linked to the other TCRD. In some embodiments, there is a peptide linker between one or both of the TCRDs and their linked Fab' chain. In some embodiments, there is a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain. In some embodiments, the C_H1 and/or C_L domains comprise one or more modifications that increase the binding affinity of the Fab' chains for each other. In some embodiments, the C_H1 and C_L domains are swapped, such that the first Fab' chain comprises V_H, C_L, and partial hinge antibody domains and the second Fab' chain comprises V_L and C_H1 domains. In some embodiments, the Fab' specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fab'

specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first TCRD and its linked Fab' chain and the other stabilization domain is located between the second TCRD and its linked Fab' chain.

[0143] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) a (Fab')2 that specifically binds to the target antigen, wherein the (Fab')2 comprises first and second (Fab')2 chains comprising V_H, C_H1, and partial hinge antibody domains and third and fourth (Fab')2 chains comprising V_L and C_L antibody domains, and wherein the first (Fab')2 chain is linked to the first or second TCRD and the second (Fab')2 chain is linked to the other TCRD. In some embodiments, there is a peptide linker between one or both of the TCRDs and their linked (Fab')2 chain. In some embodiments, there is a disulfide bond between a residue in a C_H1 domain and a residue in a C_L domain. In some embodiments, the C_H1 and/or C_L domains comprise one or more modifications that increase the binding affinity of the (Fab')2 chains for each other. In some embodiments, the C_H1 and C_L domains are swapped, such that the first and second (Fab')2 chains comprise V_H, C_L, and partial hinge antibody domains and the third and fourth (Fab')2 chains comprise V_L and C_H1 domains. In some embodiments, the (Fab')2 specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the (Fab')2 specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second

stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first TCRD and its linked (Fab')2 chain and the other stabilization domain is located between the second TCRD and its linked (Fab')2 chain.

[0144] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) an Fv that specifically binds to the target antigen, wherein the Fv comprises a first Fv chain comprising a V_H antibody domain and a second Fv chain comprising a V_L antibody domain, and wherein the first Fv chain is linked to the first or second TCRD and the second Fv chain is linked to the other TCRD. In some embodiments, there is a peptide linker between one or both of the TCRDs and their linked Fv chain. In some embodiments, the Fv specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the Fv specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first TCRD and its linked Fv chain and the other stabilization domain is located between the second TCRD and its linked Fv chain.

[0145] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) a first scFv that specifically binds to the target antigen, wherein the

first scFv comprises V_H and V_L antibody domains, and wherein the first scFv is linked to the first or second TCRD. In some embodiments, the caTCR further comprises a second antigen-binding module linked to the first scFv or to the TCRD that is not linked to the first scFv. In some embodiments, the second antigen-binding module specifically binds to the target antigen. In some embodiments, the second antigen-binding module specifically binds to an antigen other than the target antigen. In some embodiments, the second antigen-binding module is a second scFv. In some embodiments, there is a peptide linker between the first scFv and its linked TCRD and/or between the second antigen-binding module and its linked scFv or TCRD. In some embodiments, the scFv specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the scFv specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first scFv and its linked TCRD and the other stabilization domain is linked to the second TCRD.

[0146] In some embodiments, there is provided a caTCR that specifically binds a target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule, and wherein the first and/or second TCR-TMs are non-naturally occurring; and b) a first scFv that specifically binds to the target antigen and a second scFv, wherein the first and second scFvs comprise V_H and V_L antibody domains, and wherein the first scFv is linked to the first or second TCRD and the second scFv is linked to the other TCRD. In some embodiments, the second TCRD specifically binds to the target antigen. In some embodiments, the second scFv comprises, consists essentially of, or consists of the amino acid sequence of the first scFv. In some embodiments, the second scFv specifically binds to an antigen other than the target antigen. In some embodiments, the first and/or second scFvs specifically bind, individually, a cell surface antigen including, without limitation, CD19, CD20,

CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the first and/or second scFvs specifically bind, individually, a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first or second stabilization domain is located between first scFv and its linked TCRD and the other stabilization domain is located between the second scFv and its linked TCRD.

Multispecific caTCRs

[0147] One aspect of the present application provides multispecific caTCRs that specifically binds to two or more (*e.g.*, 2, 3, 4, or more) different target antigens or epitopes. In some embodiments, the multispecific caTCR specifically binds to two or more (*e.g.*, 2, 3, 4, or more) different target antigens. In some embodiments, the multispecific caTCR specifically binds to two or more (*e.g.*, 2, 3, 4, or more) different epitopes on the same target antigen. In some embodiments, the multispecific caTCR comprises an antigen-binding module for each antigen or epitope. In some embodiments, the multispecific caTCR comprises more than two antigen-binding module for at least one antigen or epitope. In some embodiments, the multispecific caTCR comprises a multispecific antigen-binding module comprising two or more (*e.g.*, 2, 3, 4, or more) antigen-binding domains each specifically binding to an antigen or epitope. In some embodiments, the multispecific caTCR is bispecific. In some embodiments, the multispecific caTCR is trispecific.

[0148] Multi-specific molecules are molecules that have binding specificities for at least two different antigens or epitopes *(e.g.,* bispecific antibodies have binding specificities for two antigens or epitopes). Multi-specific caTCRs with more than two valencies and/or specificities are also contemplated. Bispecific antibodies have been described, *e.g.*, see, Brinkmann U. and Kontermann R.E. (2017) *MABS*, 9(2), 182-212. Trispecific antibodies can be prepared. *See*, Tutt *et al. J. Immunol.* 147: 60 (1991). It is to be appreciated that one of skill in the art could select appropriate features of individual multi-specific molecules known in the art to form a multi-specific caTCR.

[0149] In some embodiments, there is provided a caTCR (also referred herein as "multispecific caTCR") comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module

comprising a first antigen-binding domain that specifically binds to a first target antigen and a second antigen-binding domain that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring αβ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR

modules or domains. In some embodiments, both the first TCR-TM and the second TCR-TM are naturally occurring. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first TCR-TM comprises up to 5 amino acid substitutions (*e.g.*, a single amino acid substitution) compared to the transmembrane domain from which it is derived and/or the second TCR-TM comprises up to 5 amino acid substitutions (*e.g.*, a single amino acid substitution) compared to the transmembrane domain from which it is derived. In some embodiments, a substituted amino acid in the first TCR-TM is proximal to a substituted amino acid in the second TCR-TM. In some embodiments, one or more substituted amino acids are proximal to an amino acid in the first or second TCR-TM involved in binding to CD3. In some embodiments, one or more (*e.g.*, each) substituted amino acids are more hydrophobic than their corresponding unsubstituted amino acid. In some embodiments, the first TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 7 and 9-13, and wherein the second TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26.

[0150] Exemplary structures of bispecific caTCRs are shown in FIGs. 13A-13E, in which the target antigens are CD19 and CD22, but a skilled person in the art would readily appreciate that bispecific caTCRs targeting other target antigens or epitopes may be prepared using the same structural formats.

[0151] For example, dual-variable domains (DVD) derived from DVD IgGs (*see*, DiGiammarino *et al.*, mAbs 3(5): 487-494) can be used as a bispecific antigen-binding module in the caTCR (FIG. 13A). Various linkers for fusion of the outer variable domain and inner variable domain have been developed and optimized for DVD-Igs, which may be useful in constructing bispecific caTCRs having a DVD module. However, the variable domain stacking approach in DVD modules may affect the folding and target binding affinity of the inner variable domain. The linkers between the two variable domains and the order of the two variable domains may affect the efficacy of the caTCR.

[0152] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a Fv that specifically binds to a first target antigen and a Fab that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[0153] In some embodiments, there is provided a caTCR comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H 1-L1- V_H2-C_H1-TCRD1; and a

second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_L2-C_L-TCRD2; (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_H2-C_H1-TCRD2; (iii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1- V_H2-C_H1-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_L2-C_L-TCRD2; or (iv) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_H2-C_H1-TCRD2, wherein V_H1 and V_L1 form a first antigen-binding domain that specifically binds to a first target antigen, and V_H2 and V_L2 form a second antigen-binding domain that specifically binds to a second target antigen, wherein TCRD1 and TCRD2 form a TCRM that facilitates recruitment of at least one TCR-associated signaling molecule, and wherein L1 and L2 are peptide linkers. In some embodiments, L1 and/or L2 are about 5 to about 50 (*e.g.*, about 5-10, about 10-15, or about 15-30) amino acids long. In some embodiments, L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 83-85. In some embodiments, L1 and L2 have the same length. In some embodiment, L1 and L2 have the same amino acid sequence. In some embodiments, L1 and L2 have different lengths. In some embodiments, L1 and L2 have different amino acid sequences. An exemplary bispecific caTCR is shown in FIG. 13A.

[0154] Cross-over dual variable domains (CODV) derived from CODV-IgGs (*see*, Steinmetz, *et al.*; mAbs (2016), 8(5): 867-878) can be used as a bispecific antigen-binding module in the caTCR (FIG. 13B). CODV allows relatively unobstructed antigen-binding sites for each Fv. Various linkers for fusion of the heavy chain and light chain variable regions have been developed and optimized for CODV-Igs, which may be useful in constructing bispecific caTCRs having a CODV module. However, proper folding of the CODV module can be challenging, and long linkers used in the CODV module can be a potential source of immunogenicity and susceptible to proteolytic cleavage.

[0155] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first Fv that specifically binds to a first target antigen and a second Fv that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-

associated signaling molecule. In some embodiments, the caTCR further comprises a C_H1 and a C_L.

[0156] In some embodiments, there is provided a caTCR comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1- V_H2- C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L2-L2-V_L1-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_L2-C_L-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H2-L2- V_H1-C_H1-TCRD2, wherein V_H1 and V_L1 form a first antigen-binding domain that specifically binds to a first target antigen, and V_H2 and V_L2 form a second antigen-binding domain that specifically binds to a second target antigen, wherein TCRD1 and TCRD2 form a TCRM that facilitates recruitment of at least one TCR-associated signaling molecule, and wherein L1 and L2 are peptide linkers. In some embodiments, L1 and/or L2 are about 5 to about 50 (*e.g.*, about 5-20, about 15-30, or about 30-50) amino acids long. In some embodiments, L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 83-85. In some embodiments, L1 and L2 have the same length. In some embodiment, L1 and L2 have the same amino acid sequence. In some embodiments, L1 and L2 have different lengths. In some embodiments, L1 and L2 have different amino acid sequences. An exemplary bispecific caTCR is shown in FIG. 13B.

[0157] Bispecific antigen-binding modules derived from scFv-fusion proteins, such as those described in Chen *et al.*, mAbs 8(4): 761-774, may be used in a bispecific caTCR (FIG. 13C). Expression of bispecific antibodies having a similar fusion format has demonstrated proper folding and stability of this format. Various linkers for fusion of the scFvs to the constant domains have been developed and optimized for these bispecific antibodies, which may be useful in constructing bispecific caTCRs having a similar scFv fusion domain. However, steric hindrance between the scFvs may compromise binding of the scFvs to their target antigens.

[0158] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first scFv that specifically binds to a first target antigen and a second scFv that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the caTCR further comprises a C_H1 and a C_L.

[0159] In some embodiments, there is provided a caTCR comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L2-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L2-C_L-TCRD2; wherein scFv1 specifically binds to a first target antigen and scFv2 specifically binds to a second target antigen, wherein TCRD1 and TCRD2 form a TCRM that facilitates recruitment of at least one TCR-associated signaling molecule, and wherein L1 and L2 are peptide linkers. In some embodiments, L1 and/or L2 are about 5 to about 50 (*e.g.*, about 5-10, about 10-15, or about 15-30) amino acids long. In some embodiments, L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 83-85. In some embodiments, L1 and L2 have the same length. In some embodiment, L1 and L2 have the same amino acid sequence. In some embodiments, L1 and L2 have different lengths. In some embodiments, L1 and L2 have different amino acid sequences. An exemplary bispecific caTCR is shown in FIG. 13C.

[0160] Bispecific antigen-binding modules derived from an IgG-scFv bispecific antibody or a Fab-scFv-Fc bispecific antibody may be used in a bispecific caTCR. In one format (FIG. 13D), an scFv is attached to either the V_H or V_L of a Fab, which allows greater flexibility of the scFv and thus greater access of the Fab to its target antigen. However, the scFv-Fab module may have stability issues. In a second format (FIG. 13E), a Fab is fused to a first TCRD and an scFv is fused to a second TCRD.

[0161] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a scFv that specifically binds to a first target antigen and a Fab that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[0162] In some embodiments, there is provided a caTCR comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-V_L-C_L-TCRD2; wherein the scFv specifically binds to a first target antigen, and the V_H

and V_L form a second antigen-binding domain that specifically binds to a second target antigen, wherein TCRD1 and TCRD2 form a TCRM that facilitates recruitment of at least one TCR-associated signaling molecule, and wherein L1 and L2 are peptide linkers. In some embodiments, L1 and/or L2 are about 5 to about 50 (*e.g.*, about 5-10, about 10-15, or about 15-30) amino acids long. In some embodiments, L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 83-85. An exemplary bispecific caTCR is shown in FIG. 13D.

[0163] In some embodiments, there is provided a caTCR comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1, and a third polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-TCRD2; (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L, and a third polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-TCRD2; (iii) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1, and a third polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-L2-TCRD2; or (iv) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L, and a third polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-L2-TCRD2; wherein the scFv specifically binds to a first target antigen, and the V_H and V_L form a second antigen-binding domain that specifically binds to a second target antigen, wherein TCRD1 and TCRD2 form a TCRM that facilitates recruitment of at least one TCR-associated signaling molecule, and wherein L1 and L2 are peptide linkers. In some embodiments, L1 and/or L2 are about 5 to about 50 (*e.g.*, about 5-10, about 10-15, or about 15-30) amino acids long. In some embodiments, L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 83-85. An exemplary bispecific caTCR is shown in FIG. 13E. The length of the peptide linker between the scFv and the TCRD and the length of the peptide linker between the Fab and the TCRD can be optimized as they may affect the accessibility of the scFv and the Fab to their target antigens.

[0164] The multispecific antigen-binding module of the multispecific caTCR may specifically bind to any suitable combination of target antigens or epitopes. In some embodiments, the multispecific antigen-binding module specifically binds to at least one cell surface antigen. In some embodiments, the at least one cell surface antigen is selected from the group consisting of

CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the multispecific antigen-binding module specifically binds to at least one peptide/MHC complex. In some embodiments, the at least one peptide/MHC complex comprises a peptide derived from a protein selected from the group consisting of WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the multispecific antigen-binding module specifically binds to a first cell surface antigen and a second cell surface antigen. In some embodiments, the multispecific antigen-binding module specifically binds to CD19 and CD22. In some embodiments, the multispecific antigen-binding module specifically binds to CD19 and CD20. In some embodiments, the multispecific antigen-binding module specifically binds to a first peptide/MHC complex and a second peptide/MHC complex. In some embodiments, the multispecific antigen-binding module specifically binds a cell surface antigen and a peptide/MHC complex.

[0165] The first antigen-binding domain and the second antigen-binding domain may be antibody moieties derived from suitable antibodies. Exemplary anti-CD19 and anti-CD22 antibodies are known in the art. *See*, for example, anti-CD19 antibodies described in WO2017066136A2, and anti-CD22 antibodies described in USSN 62/650,955 filed March 30, 2018, the contents of which are incorporated herein by reference in their entireties.

[0166] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first antigen-binding domain that specifically binds to CD19 and a second antigen-binding domain that specifically binds to CD22; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[0167] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising: (i) a first antigen-binding domain that specifically binds to CD19 comprising a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 74, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 78; and (ii) a second antigen-binding domain that specifically binds to CD22 comprising a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 65, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 69; and b) a TCRM comprising a first

TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 71, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 72, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 73; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 75, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 76, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 77. In some embodiments, the second antigen-binding domain specifically binding to CD22 comprises a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 62, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 63, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 64; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 66, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 67, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 68. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 74 and a V_L comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, the second antigen-binding domain specifically binding to CD22 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 65 and a V_L comprising the amino acid sequence of SEQ ID NO: 69. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a scFv comprising the amino acid sequence of SEQ ID NO: 79. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a scFv comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, the TCRD1 comprises the amino acid sequence of SEQ ID NO: 80 and the TCRD2 comprises the amino acid sequence of SEQ ID NO: 81, or the TCRD1 comprises the amino acid sequence of SEQ ID NO: 81 and the TCRD2 comprises the amino acid sequence of SEQ ID NO: 80. In some embodiments, the caTCR comprises a C_H1 fused to the TCRD1 and a C_L fused to the TCRD2, or a C_L fused to the TCRD1 and a C_H1 fused to the TCRD2. In some embodiments, the C_H1 comprises the amino acid sequence of SEQ ID NO: 37, and the C_L comprises the amino acid sequence of SEQ ID NO: 48. In some embodiments, the C_H1 and the C_L comprise one or more mutations that stabilize the dimerization. In some embodiments, the C_H1 comprises the amino acid sequence of SEQ ID NO: 86, and the C_L comprises the amino acid sequence of SEQ ID NO: 87. In some embodiments, the first antigen-binding domain or a fragment thereof (*e.g.,* V_H or V_L) is fused to

the second antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) via a peptide linker. In some embodiments, the first antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) is fused to the C_H1 or C_L via a peptide linker. In some embodiments, the second antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) is fused to the C_H1 or C_L via a peptide linker. In some embodiments, the peptide linker is about 5 to about 50 amino acids long. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 83-85.

[0168] In some embodiments, there is provided a caTCR that specifically binds to CD19 and CD20 comprising: (i) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 88, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 89; (ii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 90, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 91; (iii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 92, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 93; (iv) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 94, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 95; (v) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 96, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 97; (vi) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 98, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 99; (vii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 100, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 101; (viii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 102, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 103; (ix) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 104, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 105; (x) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 106, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 107; (xi) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 108, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 109; (xii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 110, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 111; or (xiii) a first polypeptide comprising the amino acid sequence of SEQ ID NO: 112, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 113.

[0169] In some embodiments, there is provided a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first antigen-binding domain that specifically

binds to CD19 and a second antigen-binding domain that specifically binds to CD20; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 74, and a V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, the second antigen-binding domain specifically binding to CD20 comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 56, and a V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 57. In some embodiments, the first antigen-binding domain specifically binding to CD19 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 74, and a V_L comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the second antigen-binding domain specifically binding to CD20 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 56, and a V_L comprises the amino acid sequence of SEQ ID NO: 57. In some embodiments, the TCRD1 comprises the amino acid sequence of SEQ ID NO: 80 and the TCRD2 comprises the amino acid sequence of SEQ ID NO: 81, or the TCRD1 comprises the amino acid sequence of SEQ ID NO: 81 and the TCRD2 comprises the amino acid sequence of SEQ ID NO: 80. In some embodiments, the caTCR comprises a C_H1 fused to the TCRD1 and a C_L fused to the TCRD2, or a C_L fused to the TCRD1 and a C_H1 fused to the TCRD2. In some embodiments, the C_H1 comprises the amino acid sequence of SEQ ID NO: 37, and the C_L comprises the amino acid sequence of SEQ ID NO: 48. In some embodiments, the C_H1 and the C_L comprise one or more mutations that stabilize the dimerization. In some embodiments, the C_H1 comprises the amino acid sequence of SEQ ID NO: 86, and the C_L comprises the amino acid sequence of SEQ ID NO: 87. In some embodiments, the first antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) is fused to the second antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) via a peptide linker. In some embodiments, the first antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) is fused to the C_H1 or C_L via a peptide linker. In some embodiments, the second antigen-binding domain or a fragment thereof (*e.g.*, V_H or V_L) is fused to the C_H1 or C_L via a peptide linker. In some embodiments, the peptide linker is about 5 to about 50 amino acids long. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 83-85.

Chimeric co-stimulatory receptor (CSR) constructs

[0170] In some embodiments, there is provided an immune cell (such as a T cell) comprising any one of the caTCRs described herein and a chimeric co-stimulatory receptor (CSR) comprising: i) a ligand-binding module that is capable of binding or interacting with a target ligand; ii) a transmembrane module; and iii) a co-stimulatory immune cell signaling module that is capable of providing a co-stimulatory signal to the immune cell, wherein the ligand-binding module and the co-stimulatory immune cell signaling module are not derived from the same molecule, and wherein the CSR lacks a functional primary immune cell signaling domain. Such an immune cell is also referred herein as a "caTCR plus CSR immune cell."

[0171] The ligand-specific chimeric co-stimulatory receptor (CSR) described herein specifically binds to a target ligand (such as a cell surface antigen or a peptide/MHC complex) and is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. The CSR comprises a ligand-binding module that provides the ligand-binding specificity, a transmembrane module, and a co-stimulatory immune cell signaling module that allows for stimulating the immune cell. The CSR lacks a functional primary immune cell signaling sequence. In some embodiments, the CSR lacks any primary immune cell signaling sequence. In some embodiments, the CSR comprises a single polypeptide chain comprising the ligand-binding module, transmembrane module, and co-stimulatory signaling module. In some embodiments, the CSR comprises a first polypeptide chain and a second polypeptide chain, wherein the first and second polypeptide chains together form the ligand-binding module, transmembrane module, and co-stimulatory signaling module. In some embodiments, the first and second polypeptide chains are separate polypeptide chains, and the CSR is a multimer, such as a dimer. In some embodiments, the first and second polypeptide chains are covalently linked, such as by a peptide linkage, or by another chemical linkage, such as a disulfide linkage. In some embodiments, the first polypeptide chain and the second polypeptide chain are linked by at least one disulfide bond. In some embodiments, the expression of the CSR in the caTCR plus CSR immune cell is inducible. In some embodiments, the expression of the caTCR plus CSR immune cell is inducible upon signaling through the caTCR.

[0172] Examples of co-stimulatory immune cell signaling domains for use in the CSRs of the invention include the cytoplasmic sequences of co-receptors of the T cell receptor (TCR), which can act in concert with a caTCR to initiate signal transduction following caTCR engagement, as

well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[0173] Under some circumstances, signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, in some embodimetns, T cell activation is mediated by two distinct classes of intracellular signaling sequence: those that initiate antigen-dependent primary activation through the TCR (referred to herein as "primary T cell signaling sequences") and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (referred to herein as "co-stimulatory T cell signaling sequences").

[0174] Primary immune cell signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. Examples of ITAM-containing primary immune cell signaling sequences include those derived from TCRζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, and CD66d. A "functional" primary immune cell signaling sequence is a sequence that is capable of transducing an immune cell activation signal when operably coupled to an appropriate receptor. "Non-functional" primary immune cell signaling sequences, which may comprises fragments or variants of primary immune cell signaling sequences, are unable to transduce an immune cell activation signal. The CSRs described herein lack a functional primary immune cell signaling sequence, such as a functional signaling sequence comprising an ITAM. In some embodiments, the CSRs lack any primary immune cell signaling sequence.

[0175] The co-stimulatory immune cell signaling sequence can be a portion of the intracellular domain of a co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD27, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like.

[0176] In some embodiments, the target ligand is a cell surface antigen. In some embodiments, the target ligand is a peptide/MHC complex. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different than the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a molecule presented on the surface of a cell presenting the target antigen. For example, in some embodiments, the target antigen of the caTCR is a cancer-associated antigen presented on a cancer cell, and the target ligand is a ubiquitous molecule expressed on the surface of the cancer cell, such as an integrin. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target

ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is, for example, CD19, CD20, CD22, CD47, IL4, GPC-3, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the cancer-associated ligand is a peptide/MHC complex comprising a peptide derived from a protein including WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, and PSA. In some embodiments, the target ligand is a virus-associated ligand. In some embodiments, the target ligand is an immune checkpoint molecule. In some embodiments, the immune checkpoint molecule includes PD-L1, PD-L2, CD80, CD86, ICOSL, B7-H3, B7-H4, HVEM, 4-1BBL, OX40L, CD70, CD40, and GAL9. In some embodiments, the target ligand is an apoptotic molecule. In some embodiments, the apoptotic molecule includes FasL, FasR, TNFR1, and TNFR2.

[0177] In some embodiments, the ligand-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, and PSA. In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*see*, *e.g.*, WO2017066136A2). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 54 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 55, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD20 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 56 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 57, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*see*, *e.g.*, USSN 62/650,955 filed March 30, 2018, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ

ID NO: 65 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 69, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*see*, *e.g.*, USSN 62/490,586 filed April 26, 2017, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 58 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 59, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR1 (*see*, *e.g.*, WO2016/187220 and WO2016/187216). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR2 (*see*, *e.g.*, WO2016/142768). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for BCMA (*see*, *e.g.*, WO2016/090327 and WO2016/090320). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPRC5D (*see*, *e.g.*, WO2016/090329 and WO2016/090312). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for FCRL5 (*see*, *e.g.*, WO2016/090337). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for WT-1 (*see*, *e.g.*, WO2012/135854, WO2015/070078, and WO2015/070061). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for AFP (*see*, *e.g.*, WO2016/161390). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for HPV16-E7 (*see*, *e.g.*, WO2016/182957). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for NY-ESO-1 (*see*, *e.g.*, WO2016/210365). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PRAME (*see*, *e.g.*, WO2016/191246). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for EBV-LMP2A (*see*, *e.g.*, WO2016/201124). In some embodiments, the antibody moiety comprises the CDRs or variables

domains (V_H and/or V_L domains) of an antibody moiety specific for KRAS (*see*, *e.g.*, WO2016/154047). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PSA (*see*, *e.g.*, WO2017/015634).

[0178] In some embodiments, the ligand-binding module is (or is derived from) all or a portion of the extracellular domain of a receptor for the target ligand. In some embodiments, the receptor includes, for example, FasR, TNFR1, TNFR2, PD-1, CD28, CTLA-4, ICOS, BTLA, KIR, LAG-3, 4-1BB, OX40, CD27, and TIM-3.

[0179] In some embodiments, the transmembrane module comprises one or more transmembrane domains derived from, for example, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154.

[0180] In some embodiments, the co-stimulatory signaling module comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of CD28 comprising the amino acid sequence of SEQ ID NO: 49 or 118. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of 4-1BB comprising the amino acid sequence of SEQ ID NO: 50 or 119. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of OX40 comprising the amino acid sequence of SEQ ID NO: 51 or 120. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of CD27 comprising the amino acid sequence of SEQ ID NO: 122 or 123. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of CD30 comprising the amino acid sequence of SEQ ID NO: 124 or 125. In some embodiments, the co-stimulatory signaling molecule comprises a fragment of CD8 comprising the amino acid sequence of SEQ ID NO: 121.

[0181] In some embodiments, the CSR further comprises a spacer module between any of the ligand-binding module, the transmembrane module, and the co-stimulatory signaling module. In some embodiments, the spacer module comprises one or more peptide linkers connecting two CSR modules. In some embodiments, the spacer module comprises one or more peptide linkers between about 5 to about 70 (such as about any of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70, including any ranges between these values) amino acids in length.

[0182] In some embodiments, the ligand-binding module (such as an antibody moiety) specifically binds to a target antigen with a) an affinity that is at least about 10 (including for example at least about any of 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for other molecules; or b) a K_d no more than about 1/10 (such as no more than about any of 1/10, 1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to other molecules. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, Biacore instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0183] In some embodiments, the CSR described herein specifically binds to a target ligand (such as a cell surface antigen or a peptide/MHC complex), comprising a) a target ligand-binding domain (LBD); b) a transmembrane domain; and c) and a co-stimulatory signaling domain, wherein the CSR is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. In some embodiments, the target ligand is a cell surface antigen. In some embodiments, the target ligand is a peptide/MHC complex. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different from the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is, for example, CD19, CD20, CD22, CD47, IL4, GPC-3, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the cancer-associated ligand is a peptide/MHC complex comprising a peptide derived from a protein including WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, and PSA. In some embodiments, the target ligand is a virus-associated ligand. In some embodiments, the target ligand is an immune checkpoint molecule. In some embodiments, the immune checkpoint molecule includes PD-L1, PD-L2, CD80, CD86, ICOSL, B7-H3, B7-H4, HVEM, 4-1BBL, OX40L, CD70, CD40, and GAL9. In some embodiments, the target ligand is an apoptotic molecule. In some embodiments, the apoptotic molecule includes FasL, FasR, TNFR1, and TNFR2. In some embodiments, the ligand-binding domain is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the ligand-binding domain is (or is derived from) all or a portion of the extracellular domain of a receptor for the target ligand. In some embodiments, the receptor

includes, for example, FasR, TNFR1, TNFR2, PD-1, CD28, CTLA-4, ICOS, BTLA, KIR, LAG-3, 4-1BB, OX40, CD27, and TIM-3. In some embodiments, the transmembrane domain comprises a transmembrane domain derived from a transmembrane protein including, for example, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the CSR comprises a fragment of a transmembrane protein (fTMP), wherein the fTMP comprises the CSR transmembrane domain. In some embodiments, the co-stimulatory signaling domain comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the CSR comprises a fragment of an immune cell co-stimulatory molecule (fCSM), wherein the fCSM comprises the CSR transmembrane domain and CSR co-stimulatory signaling domain. In some embodiments, the CSR further comprises a spacer domain between any of the ligand-binding domain, the transmembrane domain, and the co-stimulatory signaling domain. In some embodiments, the spacer domain comprises a peptide linker connecting two CSR domains.

[0184] In some embodiments, the CSR described herein specifically binds to a target ligand, comprising a) a target ligand-binding domain; b) a transmembrane domain; and c) and a co-stimulatory signaling domain, wherein the target ligand is a cell surface antigen, and wherein the CSR is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different from the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is, for example, CD19, CD20, CD22, CD47, IL4, GPC-3, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the target ligand is a virus-associated ligand. In some embodiments, the target ligand is an immune checkpoint molecule. In some embodiments, the immune checkpoint molecule includes PD-L1, PD-L2, CD80, CD86, ICOSL, B7-H3, B7-H4, HVEM, 4-1BBL, OX40L, CD70, CD40, and GAL9. In some embodiments, the target ligand is an apoptotic molecule. In some embodiments, the apoptotic molecule includes FasL, FasR, TNFR1, and TNFR2. In some embodiments, the ligand-binding domain is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain

Fv (scFv). In some embodiments, the ligand-binding domain is (or is derived from) all or a portion of the extracellular domain of a receptor for the target ligand. In some embodiments, the receptor includes, for example, FasR, TNFR1, TNFR2, PD-1, CD28, CTLA-4, ICOS, BTLA, KIR, LAG-3, 4-1BB, OX40, CD27, and TIM-3. In some embodiments, the transmembrane domain comprises a transmembrane domain derived from a transmembrane protein including, for example, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the CSR comprises a fragment of a transmembrane protein (fTMP), wherein the fTMP comprises the CSR transmembrane domain. In some embodiments, the co-stimulatory signaling domain comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the CSR comprises a fragment of an immune cell co-stimulatory molecule (fCSM), wherein the fCSM comprises the CSR transmembrane domain and CSR co-stimulatory signaling domain. In some embodiments, the CSR further comprises a spacer domain between any of the ligand-binding domain, the transmembrane domain, and the co-stimulatory signaling domain. In some embodiments, the spacer domain comprises a peptide linker connecting two CSR domains.

[0185] In some embodiments, the CSR described herein specifically binds to a target ligand, comprising a) a target ligand-binding domain; b) a transmembrane domain; and c) and a co-stimulatory signaling domain, wherein the target ligand is a peptide/MHC complex, and wherein the CSR is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different from the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is a peptide/MHC complex comprising a peptide derived from a protein including WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, and PSA. In some embodiments, the target ligand is a virus-associated ligand. In some embodiments, the ligand-binding domain is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the transmembrane domain comprises a transmembrane domain derived from a transmembrane protein including, for example, CD28,

CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the CSR comprises a fragment of a transmembrane protein (fTMP), wherein the fTMP comprises the CSR transmembrane domain. In some embodiments, the co-stimulatory signaling domain comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the CSR comprises a fragment of an immune cell co-stimulatory molecule (fCSM), wherein the fCSM comprises the CSR transmembrane domain and CSR co-stimulatory signaling domain. In some embodiments, the CSR further comprises a spacer domain between any of the ligand-binding domain, the transmembrane domain, and the co-stimulatory signaling domain. In some embodiments, the spacer domain comprises a peptide linker connecting two CSR domains.

[0186] In some embodiments, the CSR described herein specifically binds to a target ligand (such as a cell surface antigen or a peptide/MHC complex), comprising a) a target ligand-binding domain; b) a transmembrane domain; and c) and a co-stimulatory signaling domain, wherein the ligand-binding domain is an antibody moiety, and wherein the CSR is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. In some embodiments, the target ligand is a cell surface antigen. In some embodiments, the target ligand is a peptide/MHC complex. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different from the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is, for example, CD19, CD20, CD22, CD47, IL4, GPC-3, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the cancer-associated ligand is a peptide/MHC complex comprising a peptide derived from a protein including WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, and PSA. In some embodiments, the target ligand is a virus-associated ligand. In some embodiments, the target ligand is an immune checkpoint molecule. In some embodiments, the immune checkpoint molecule includes PD-L1, PD-L2, CD80, CD86, ICOSL, B7-H3, B7-H4, HVEM, 4-1BBL, OX40L, CD70, CD40, and GAL9. In some embodiments, the target ligand is an apoptotic molecule. In some embodiments, the apoptotic molecule includes FasL, FasR, TNFR1, and TNFR2. In some embodiments, the

antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the transmembrane domain comprises a transmembrane domain derived from a transmembrane protein including, for example, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the CSR comprises a fragment of a transmembrane protein (fTMP), wherein the fTMP comprises the CSR transmembrane domain. In some embodiments, the co-stimulatory signaling domain comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the CSR comprises a fragment of an immune cell co-stimulatory molecule (fCSM), wherein the fCSM comprises the CSR transmembrane domain and CSR co-stimulatory signaling domain. In some embodiments, the CSR further comprises a spacer domain between any of the ligand-binding domain, the transmembrane domain, and the co-stimulatory signaling domain. In some embodiments, the spacer domain comprises a peptide linker connecting two CSR domains.

[0187] In some embodiments, the CSR comprises an fCSM of CD28. In some embodiments, the CSR comprises: a) a target ligand-binding domain; and b) a fragment of CD28 comprising the amino acid sequence of SEQ ID NO: 118. In some embodiments, the CSR comprises: a target ligand-binding domain and a CSR domain comprising the amino acid sequence of SEQ ID NO: 126.

[0188] In some embodiments, the CSR described herein specifically binds to a target ligand (such as a cell surface antigen or a peptide/MHC complex), comprising a) a target ligand-binding domain; b) a transmembrane domain; and c) and a co-stimulatory signaling domain, wherein the ligand-binding domain is (or is derived from) all or a portion of the extracellular domain of a receptor for the target ligand, and wherein the CSR is capable of stimulating an immune cell on the surface of which it is functionally expressed upon target ligand binding. In some embodiments, the target ligand is a cell surface antigen. In some embodiments, the target ligand is the same as the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is different from the target antigen of a caTCR expressed in the same immune cell. In some embodiments, the target ligand is a disease-associated ligand. In some embodiments, the target ligand is a cancer-associated ligand. In some embodiments, the cancer-associated ligand is, for example, CD19, CD20, CD22, CD47, IL4, GPC-3, ROR1,

ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the target ligand is an immune checkpoint molecule. In some embodiments, the immune checkpoint molecule includes PD-L1, PD-L2, CD80, CD86, ICOSL, B7-H3, B7-H4, HVEM, 4-1BBL, OX40L, CD70, CD40, and GAL9. In some embodiments, the target ligand is an apoptotic molecule. In some embodiments, the apoptotic molecule includes FasL, FasR, TNFR1, and TNFR2. In some embodiments, the target ligand receptor includes, for example, FasR, TNFR1, TNFR2, PD-1, CD28, CTLA-4, ICOS, BTLA, KIR, LAG-3, 4-1BB, OX40, CD27, and TIM-3. In some embodiments, the transmembrane domain comprises a transmembrane domain derived from, for example, CD28, CD3ε, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, or CD154. In some embodiments, the co-stimulatory signaling domain comprises, consists essentially of, or consists of all or a portion of the intracellular domain of an immune cell co-stimulatory molecule including, for example, CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and the like. In some embodiments, the CSR further comprises a spacer domain between any of the ligand-binding domain, the transmembrane domain, and the co-stimulatory signaling domain. In some embodiments, the spacer domain comprises a peptide linker connecting two CSR domains.

[0189] In some embodiments, the CSR described herein specifically binds to CD19, comprising a) an scFv comprising a V_H domain having the amino acid sequence of SEQ ID NO: 74 and a V_L domain having the amino acid sequence of SEQ ID NO: 78; and b) a fragment of CD28 comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 49 or 118. In some embodiments, the fragment of CD28 comprises the amino acid sequence of SEQ ID NO: 118. In some embodiments, the CSR comprises, from amino terminus to carboxy terminus, the scFv, a peptide linker, and the fragment of CD28. In some embodiments, the CSR comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 127.

[0190] In some embodiments, the CSR described herein specifically binds to GPC3, comprising a) an scFv comprising a V_H domain having the amino acid sequence of SEQ ID NO: 58 and a V_L domain having the amino acid sequence of SEQ ID NO: 59; and b) a fragment of CD28 comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 49 or 80. In some embodiments, the scFv comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 117. In some embodiments, the fragment of CD28 comprises the amino acid sequence of SEQ ID NO: 118. In some embodiments, the CSR

comprises, from amino terminus to carboxy terminus, the scFv, a peptide linker, and the fragment of CD28. In some embodiments, the CSR comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 128.

[0191] In some embodiments, the expression of the CSR in the caTCR plus CSR immune cell is inducible. In some embodiments, the caTCR plus CSR immune cell comprises a nucleic acid sequence encoding the CSR operably linked to an inducible promoter, including any of the inducible promoters described herein. In some embodiments, the expression of the CSR in the caTCR plus CSR immune cell is inducible upon signaling through the caTCR. In some such embodiments, the caTCR plus CSR immune cell comprises a nucleic acid sequence encoding the CSR operably linked to a promoter or regulatory element responsive to signaling through the caTCR. In some embodiments, the nucleic acid sequence encoding the CSR is operably linked to a nuclear-factor of the activated T-cell (NFAT)-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter (*see* for example Durand, D. *et. al.*, *Molec. Cell. Biol.* 8, 1715-1724 (1988); Clipstone, NA, Crabtree, GR. *Nature. 1992* 357(6380): 695-7; Chmielewski, M., et al. *Cancer research* 71.17 (2011): 5697-5706; and Zhang, L., et al. *Molecular therapy* 19.4 (2011): 751-759). In some embodiments, the NFAT-derived promoter comprises the nucleotide sequence of SEQ ID NO: 116. In some embodiments, the nucleic acid sequence encoding the CSR is operably linked to an IL-2 promoter.

Secretory secondary effector (SSE) constructs

[0192] In some embodiments, there is provided an immune cell (such as a T cell) comprising any one of the caTCRs described herein, wherein the immune cell is capable of secreting a secretory secondary effector (SSE). Such an immune cell is also referred herein as a "caTCR plus SSE immune cell." The SSE enhances the immune response mediated by a caTCR plus SSE immune cell in which it is functionally expressed and secreted from. In some embodiments, the SSE is capable of redirecting other immune cells (such as bystander T cells or NK cells) to target disease cells (such as target cancer cells). In some embodiments, the SSE is a multispecific antibody (such as a bispecific antibody) targeting an immune cell (such as a T cell or NK cell) and a disease cell (such as a cancer cell). In some embodiments, the SSE protects the caTCR plus SSE immune cell from an immunosuppressive environment, such as an immunosuppressive tumor environment. In some embodiments, the SSE provides autocrine activation of stimulatory receptors on the caTCR plus SSE immune cell. In some embodiments, the SSE is an exogenous growth factor or stimulatory cytokine. In some embodiments, the expression of the SSE in the caTCR plus SSE immune cell is inducible. In some embodiments, the expression of the SSE in

the caTCR plus SSE immune cell is inducible upon signaling through the caTCR. In some embodiemnts, the caTCR plus SSE immune cell further comprises any one of the CSRs described herein.

[0193] In some embodiments, the SSE is a multispecific antibody (such as a bispecific antibody) targeting a T cell and a disease cell. In some embodiments, the SSE comprises an antibody moiety that specifically binds to a surface antigen of a T cell. In some embodiments, the T cell surface antigen is CD3. In some embodiments, the SSE comprises an antibody moiety that specifically binds to a disease-associated antigen (such as a cancer-associated antigen). In some embodiments, the disease-associated antigen is a surface antigen of a disease cell (such as a cancer cell). In some embodiments, the disease-associated antigen is glypican-3 (GPC3), CD47, mucin-16 (MUC16), CD19, CD20, CD22, EpCAM, EGFR, HER2, CEA, PSMA, AFP, PSA, BCMA, FCRL5, NY-ESO, HPV16, or FoxP3, including variants or mutants thereof. In some embodiments, the SSE is a multispecific antibody selected from the group consisting of a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, and a dual variable domain (DVD) antibody. In some embodiments, the SSE is a bispecific antibody. In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting the T cell surface antigen and a second scFv targeting the disease-associated antigen.

[0194] In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD3 and a second scFv targeting a disease-associated antigen. In some embodiments, the disease-associated antigen is GPC3, CD47, MUC16, CD19, CD20, CD22, EpCAM, EGFR, HER2, CEA, PSMA, AFP, PSA, BCMA, FCRL5, NY-ESO, HPV16, or FoxP3, including variants or mutants thereof.

[0195] In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD3 and a second scFv targeting GPC3. In some embodiments, the second scFv comprises a V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 58 and a V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 59. In some embodiments, the V_H domain is amino-terminal to the V_L domain. In some embodiments, the V_L domain is amino-terminal to the V_H domain. In some embodiments, the second scFv comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 117. In some embodiments, the first scFv is amino-terminal to the second scFv. In some embodiments, the second scFv is amino-terminal to the first scFv. In some embodiments, the SSE comprises, consists essentially of, or consists of the amino acid sequence of SEQ ID NO: 129.

[0196] In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD3 and a second scFv targeting CD47. In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD3 and a second scFv targeting MUC16.

[0197] In some embodiments, the SSE is a multispecific antibody (such as a bispecific antibody) targeting an NK cell and a disease-associated antigen (such as a cancer-associated antigen). In some embodiments, the SSE comprises an antibody moiety that specifically binds to a surface antigen of an NK cell. In some embodiments, the NK cell surface antigen is CD16a. In some embodiments, the SSE comprises an antibody moiety that specifically binds to a disease-associated antigen (such as a cancer-associated antigen). In some embodiments, the disease-associated antigen is a surface antigen of a disease cell (such as a cancer cell). In some embodiments, the disease-associated antigen is GPC3, CD47, MUC16, CD19, CD20, CD22, EpCAM, EGFR, HER2, CEA, PSMA, AFP, PSA, BCMA, FCRL5, NY-ESO, HPV16, or FoxP3, including variants or mutants thereof. In some embodiments, the SSE is a multispecific antibody selected from the group consisting of a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, and a dual variable domain (DVD) antibody. In some embodiments, the SSE is a bispecific antibody. In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting the NK cell surface antigen and a second scFv targeting the disease-associated antigen.

[0198] In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD16a and a second scFv targeting a disease-associated antigen. In some embodiments, the disease-associated antigen is GPC3, CD47, MUC16, CD19, CD20, CD22, EpCAM, EGFR, HER2, CEA, PSMA, AFP, PSA, BCMA, FCRL5, NY-ESO, HPV16, or FoxP3, including variants or mutants thereof.

[0199] In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD16a and a second scFv targeting GPC3. In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD16a and a second scFv targeting CD47. In some embodiments, the SSE is a tandem scFv comprising a first scFv targeting CD16a and a second scFv targeting MUC16.

[0200] In some embodiments, the SSEs described herein comprises an antibody moiety that specifically binds to a disease-associated antigen, wherein the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for the disease-associated antigen. In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*see*, *e.g.*,

WO2017066136A2). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD19 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 54 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 55, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD20 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 56 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 57, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*see*, *e.g.*, USSN 62/650,955 filed March 30, 2018, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for CD22 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 65 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 69, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*see*, *e.g.*, USSN 62/490,586 filed April 26, 2017, the contents of which are incorporated herein by reference in their entirety). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPC3 (*e.g.*, V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 58 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 59, or CDRs contained therein). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR1 (*see*, *e.g.*, WO2016/187220 and WO2016/187216). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for ROR2 (*see*, *e.g.*, WO2016/142768). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for BCMA (*see*, *e.g.*, WO2016/090327 and WO2016/090320). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for GPRC5D (*see*, *e.g.*, WO2016/090329 and WO2016/090312). In some embodiments, the

antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for FCRL5 (*see*, *e.g.*, WO2016/090337). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for WT-1 (*see*, *e.g.*, WO2012/135854, WO2015/070078, and WO2015/070061). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for AFP (*see*, *e.g.*, WO2016/161390). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for HPV16-E7 (*see*, *e.g.*, WO2016/182957). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for NY-ESO-1 (*see*, *e.g.*, WO2016/210365). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PRAME (*see*, *e.g.*, WO2016/191246). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for EBV-LMP2A (*see*, *e.g.*, WO2016/201124). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for KRAS (*see*, *e.g.*, WO2016/154047). In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antibody moiety specific for PSA (*see*, *e.g.*, WO2017/015634). In some embodiments, the antibody moiety is an scFv. In some embodiments, the SSE is a tandem scFv comprising a) a first scFv that specifically binds to a surface antigen of a T cell (such as CD3) or an NK cell (such as CD16a) and b) the antibody moiety, wherein the antibody moiety is a second scFv. In some embodiments, the SSE comprises the first and second scFvs connected by a peptide linker. In some embodiments, the first scFv is amino-terminal to the second scFv. In some embodiments, the second scFv is amino-terminal to the first scFv.

[0201] In some embodiments, the SSE is a multispecific antibody (such as a bispecific antibody) targeting one or more soluble immunosuppressive agents. Such an SSE can act as a trap to sequester the soluble immunosuppressive agents from their targets, thereby reducing their immunosuppressive effects. In some embodiments, the SSE comprises one or more antibody moieties that specifically bind to one or more soluble immunosuppressive agents. In some embodiments, the immunosuppressive agents are immunosuppressive cytokines. In some embodiments, the immunosuppressive cytokines include TGF-β family members (such as TGF-β 1 to 4), IL-4, and IL-10, including variants or mutants thereof. In some embodiments, the SSE

is a multispecific antibody selected from the group consisting of a tandem scFv, a diabody (Db), a single chain diabody (scDb), a dual-affinity retargeting (DART) antibody, and a dual variable domain (DVD) antibody. In some embodiments, the SSE is a bispecific antibody. For example, in some embodiments, the SSE is a tandem scFv comprising a first scFv targeting a first immunosuppressive cytokine (such as TGFβ) and a second scFv targeting a second immunosuppressive cytokine (such as IL-4).

[0202] In some embodiments, the SSE is an antibody moiety targeting an immune checkpoint molecule. In some embodiments, the SSE is an antagonist of an inhibitory immune checkpoint molecule. In some embodiments, the inhibitory immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, CTLA-4, HVEM, BTLA, KIR, LAG-3, TIM-3, and A2aR. In some embodiments, the SSE is an agonist of a stimulatory immune checkpoint molecule. In some embodiments, the stimulatory immune checkpoint molecule is selected from the group consisting of CD28, ICOS, 4-1BB, OX40, CD27, and CD40. In some embodiments, the antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antibody moiety is an scFv.

[0203] In some embodiments, the SSE is an antagonistic antibody moiety targeting PD-1. In some embodiments, the antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antagonistic antibody moiety specific for PD-1 (*see*, *e.g.*, WO2016/210129). In some embodiments, the antagonistic antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antagonistic antibody moiety is an scFv.

[0204] In some embodiments, the SSE is an antagonistic antibody moiety targeting CD47. In some embodiments, the antagonistic antibody moiety comprises the CDRs or variables domains (V_H and/or V_L domains) of an antagonistic antibody moiety specific for CD47 (e.g., V_H domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 60 and/or V_L domain comprising, consisting essentially of, or consisting of the amino acid sequence of SEQ ID NO: 61, or CDRs contained therein). In some embodiments, the antagonistic antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antagonistic antibody moiety is an scFv.

[0205] In some embodiments, the SSE comprises an antibody moiety that binds to a target antigen with a) an affinity that is at least about 10 (including for example at least about any of 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or more) times its binding affinity for other molecules; or b) a K_d no more than about 1/10 (such as no more than about any of 1/10,

1/20, 1/30, 1/40, 1/50, 1/75, 1/100, 1/200, 1/300, 1/400, 1/500, 1/750, 1/1000 or less) times its K_d for binding to other molecules. Binding affinity can be determined by methods known in the art, such as ELISA, fluorescence activated cell sorting (FACS) analysis, or radioimmunoprecipitation assay (RIA). K_d can be determined by methods known in the art, such as surface plasmon resonance (SPR) assay utilizing, for example, Biacore instruments, or kinetic exclusion assay (KinExA) utilizing, for example, Sapidyne instruments.

[0206] In some embodiments, the SSE is a soluble molecule that specifically binds a ligand of an immunosuppressive receptor. In some embodiments, the SSE comprises a ligand-binding domain derived from the extracellular domain of the immunosuppressive receptor. In some embodiments, the ligand-binding domain is a portion of the extracellular domain of the receptor. In some embodiments, the immunosuppressive receptor is selected from the group consisting of FasR, TNFR1, TNFR2, SIRPα, PD-1, CD28, CTLA-4, ICOS, BTLA, KIR, LAG-3, 4-1BB, OX40, CD27, CD40, and TIM-3.

[0207] In some embodiments, the SSE is a soluble molecule that specifically binds to and antagonizes an immunosuppressive receptor. In some embodiments, the SSE comprises a receptor-binding domain derived from the extracellular domain of a ligand for the immunosuppressive receptor. In some embodiments, the receptor-binding domain is a portion of the extracellular domain of the ligand. In some embodiments, the ligand is selected from the group consisting of FasL, PD-L1, PD-L2, CD47, CD80, CD86, ICOSL, HVEM, 4-1BBL, OX40L, CD70, CD40L, and GAL9.

[0208] In some embodiments, the SSE is an exogenous stimulatory cytokine. An exogenous cytokine described herein is a cytokine expressed from an exogenous gene. In some embodiments, the exogenous stimulatory cytokine is an IL-12 family member. In some embodiments, the IL-12 family member is IL-12, IL-23, IL-27, or IL-35. In some embodiments, the exogenous stimulatory cytokine is IL-2, IL-15, IL-18, or IL-21. In some embodiments, the exogenous stimulatory cytokine is capable of providing autocrine activation of receptors for the cytokine on the caTCR plus SSE immune cell.

[0209] In some embodiments, the expression of the SSE in the caTCR plus SSE immune cell is inducible. In some embodiments, the caTCR plus SSE immune cell comprises a nucleic acid sequence encoding the SSE operably linked to an inducible promoter, including any of the inducible promoters described herein. In some embodiments, the expression of the SSE in the caTCR plus SSE immune cell is inducible upon signaling through the caTCR. In some such embodiments, the caTCR plus SSE immune cell comprises a nucleic acid sequence encoding the

SSE operably linked to a promoter or regulatory element responsive to signaling through the caTCR. In some embodiments, the nucleic acid sequence encoding the SSE is operably linked to a nuclear-factor of the activated T-cell (NFAT)-derived promoter. In some embodiments, the NFAT-derived promoter is an NFAT-derived minimal promoter (*see* for example Durand, D. *et. al.*, *Molec. Cell. Biol.* 8, 1715-1724 (1988); Clipstone, NA, Crabtree, GR. *Nature. 1992* 357(6380): 695-7; Chmielewski, M., et al. *Cancer research* 71.17 (2011): 5697-5706; and Zhang, L., et al. *Molecular therapy* 19.4 (2011): 751-759). In some embodiments, the NFAT-derived promoter comprises the nucleotide sequence of SEQ ID NO: 116. In some embodiments, the nucleic acid sequence encoding the SSE is operably linked to an IL-2 promoter.

Nucleic Acids

[0210] Nucleic acid molecules encoding the caTCRs, CSRs and/or SSEs described herein are also contemplated. In some embodiments, according to any of the caTCRs, CSRs and/or SSEs described herein described herein, there is provided a nucleic acid (or a set of nucleic acids) encoding the caTCR, CSR and/or SSE.

[0211] The present invention also provides vectors in which a nucleic acid of the present invention is inserted.

[0212] In brief summary, the expression of a caTCR by a nucleic acid encoding the caTCR can be achieved by inserting the nucleic acid into an appropriate expression vector, such that the nucleic acid is operably linked to 5' and 3' regulatory elements, including for example a promoter (*e.g.*, a lymphocyte-specific promoter) and a 3' untranslated region (UTR). The vectors can be suitable for replication and integration in eukaryotic host cells. Typical cloning and expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0213] The nucleic acids of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In some embodiments, the invention provides a gene therapy vector.

[0214] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to, a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0215] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (*see*, *e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0216] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In some embodiments, lentivirus vectors are used. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0217] Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline.

[0218] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1α (EF-1α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian

virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter.

[0219] Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Exemplary inducible promoter systems for use in eukaryotic cells include, but are not limited to, hormone-regulated elements (*e.g.*, see Mader, S. and White, J. H. (1993) Proc. Natl. Acad. Sci. USA 90:5603-5607), synthetic ligand-regulated elements (see, *e.g.*, Spencer, D. M. et al 1993) Science 262: 1019-1024) and ionizing radiation-regulated elements (*e.g.*, see Manome, Y. et al. (1993) Biochemistry 32: 10607-10613; Datta, R. et al. (1992) Proc. Natl. Acad. Sci. USA 89: 1014- 10153). Further exemplary inducible promoter systems for use in *in vitro* or *in vivo* mammalian systems are reviewed in Gingrich et al. (1998) Annual Rev. Neurosci 21:377-405.

[0220] An exemplary inducible promoter system for use in the present invention is the Tet system. Such systems are based on the Tet system described by Gossen et al. (1993). In an exemplary embodiment, a polynucleotide of interest is under the control of a promoter that comprises one or more Tet operator (TetO) sites. In the inactive state, Tet repressor (TetR) will bind to the TetO sites and repress transcription from the promoter. In the active state, *e.g.*, in the presence of an inducing agent such as tetracycline (Tc), anhydrotetracycline, doxycycline (Dox), or an active analog thereof, the inducing agent causes release of TetR from TetO, thereby allowing transcription to take place. Doxycycline is a member of the tetracycline family of antibiotics having the chemical name of 1-dimethylamino-2,4a,5,7,12-pentahydroxy-11-methyl-4,6-dioxo-1,4a,11,11a,12,12a-hexahydrotetracene-3-carboxamide.

[0221] In one embodiment, a TetR is codon-optimized for expression in mammalian cells, *e.g.*, murine or human cells. Most amino acids are encoded by more than one codon due to the degeneracy of the genetic code, allowing for substantial variations in the nucleotide sequence of a given nucleic acid without any alteration in the amino acid sequence encoded by the nucleic acid. However, many organisms display differences in codon usage, also known as "codon bias" (*i.e.*, bias for use of a particular codon(s) for a given amino acid). Codon bias often correlates

with the presence of a predominant species of tRNA for a particular codon, which in turn increases efficiency of mRNA translation. Accordingly, a coding sequence derived from a particular organism (*e.g.*, a prokaryote) may be tailored for improved expression in a different organism (*e.g.*, a eukaryote) through codon optimization.

[0222] Other specific variations of the Tet system include the following "Tet-Off" and "Tet-On" systems. In the Tet-Off system, transcription is inactive in the presence of Tc or Dox. In that system, a tetracycline-controlled transactivator protein (tTA), which is composed of TetR fused to the strong transactivating domain of VP16 from Herpes simplex virus, regulates expression of a target nucleic acid that is under transcriptional control of a tetracycline-responsive promoter element (TRE). The TRE is made up of TetO sequence concatamers fused to a promoter (commonly the minimal promoter sequence derived from the human cytomegalovirus (hCMV) immediate-early promoter). In the absence of Tc or Dox, tTA binds to the TRE and activates transcription of the target gene. In the presence of Tc or Dox, tTA cannot bind to the TRE, and expression from the target gene remains inactive.

[0223] Conversely, in the Tet-On system, transcription is active in the presence of Tc or Dox. The Tet-On system is based on a reverse tetracycline-controlled transactivator, rtTA. Like tTA, rtTA is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain. However, a four amino acid change in the TetR DNA binding moiety alters rtTA's binding characteristics such that it can only recognize the tetO sequences in the TRE of the target transgene in the presence of Dox. Thus, in the Tet-On system, transcription of the TRE-regulated target gene is stimulated by rtTA only in the presence of Dox.

[0224] Another inducible promoter system is the lac repressor system from *E. coli*. (See, Brown et al., Cell 49:603-612 (1987). The lac repressor system functions by regulating transcription of a polynucleotide of interest operably linked to a promoter comprising the lac operator (lacO). The lac repressor (lacR) binds to LacO, thus preventing transcription of the polynucleotide of interest. Expression of the polynucleotide of interest is induced by a suitable inducing agent, *e.g.*, isopropyl-β-D-thiogalactopyranoside (IPTG).

[0225] In order to assess the expression of a polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to

enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[0226] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, β-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tel *et al.*, 2000 *FEBS Letters* 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5′ flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0227] In some embodiments, there is provided nucleic acid encoding a caTCR according to any of the caTCRs described herein. In some embodiments, the nucleic acid encoding the caTCR comprises one or more nucleic acid sequences encoding all of the polypeptide chains of the caTCR. In some embodiments, each of the one or more nucleic acid sequences is located on separate vectors. In some embodiments, at least some of the nucleic acid sequences are located on the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses). For example, in some embodiments, the caTCR is a dimer comprising a first caTCR polypeptide chain and a second caTCR polypeptide chain, and the nucleic acid encoding the caTCR comprises a first nucleic acid sequence encoding the first caTCR polypeptide chain and a second nucleic acid encoding the second caTCR chain. In some embodiments, the first nucleic acid sequence is located on a first vector and the second nucleic acid sequence is located on a second vector. In some embodiments, the first and second nucleic acid sequences are located on the same vector. In some embodiments, the first nucleic acid sequence is under the control of a first promoter and the second nucleic acid sequence is under the control of a second promoter. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and second nucleic acid sequences are expressed as a single transcript under the control of a single

promoter in a multicistronic (such as a bicistronic) vector. *See* for example Kim, JH, *et al.*, *PLoS One* 6(4):e18556, 2011. In some embodiments, the first, second, and/or single promoters are inducible. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is about the same as the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression level of the second nucleic acid sequence in the host cell. Expression can be determined at the mRNA or protein level. The level of mRNA expression can be determined by measuring the amount of mRNA transcribed from the nucleic acid using various well-known methods, including Northern blotting, quantitative RT-PCR, microarray analysis and the like. The level of protein expression can be measured by known methods including immunocytochemical staining, enzyme-linked immunosorbent assay (ELISA), western blot analysis, luminescent assays, mass spectrometry, high performance liquid chromatography, high-pressure liquid chromatography-tandem mass spectrometry, and the like. It is to be understood that the features of the embodiments described herein can be adapted and combined to encompass embodiments where the nucleic acid encoding the caTCR comprises three or more nucleic acid sequences (*e.g.*, where the caTCR comprises 3 or more distinct polypeptide chains), or a single nucleic acid sequence (*e.g.*, where the caTCR comprises a single polypeptide chain).

[0228] Thus, in some embodiments, there is provided nucleic acid encoding a dimeric caTCR comprising a first polypeptide chain and a second polypeptide chain according to any of the caTCRs described herein comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR, and b) a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first nucleic acid sequence is located on a first vector (such as a lentiviral vector) and operably linked to a first promoter and the second nucleic acid sequence is located on a second vector (such as a lentiviral vector) and operably linked to a second promoter. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is about the same as the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the

first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first and/or second vectors are viral vectors (such as lentiviral vectors).

[0229] In some embodiments, there is provided a vector (such as a lentiviral vector) comprising nucleic acid encoding a dimeric caTCR comprising a first polypeptide chain and a second polypeptide chain according to any of the caTCRs described herein comprising a) a first promoter operably linked to a first nucleic acid sequence encoding a first polypeptide chain of the caTCR; and b) a second promoter operably linked to a second nucleic acid sequence encoding a second polypeptide chain of the caTCR. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is about the same as the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the first nucleic acid sequence has an expression level in a host cell (such as a T cell) that is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression level of the second nucleic acid sequence in the host cell. In some embodiments, the vector is a viral vector (such as a lentiviral vector).

[0230] In some embodiments, there is provided a vector (such as a lentiviral vector) comprising nucleic acid encoding a dimeric caTCR comprising a first polypeptide chain and a second polypeptide chain according to any of the caTCRs described herein comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR; and b) a second nucleic acid sequence encoding a second polypeptide chain of the caTCR; wherein the first and second nucleic acid sequences are under the control of a single promoter. In some embodiments, the promoter is operably linked to the 5' end of the first nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the

3' end of first nucleic acid sequence to the 5' end of the second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, the promoter is operably linked to the 5' end of the second nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the 3' end of second nucleic acid sequence to the 5' end of the first nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, the promoter is inducible. In some embodiments, the vector is a viral vector (such as a lentiviral vector).

[0231] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[0232] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook *et al.* (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). In some embodiments, the introduction of a polynucleotide into a host cell is carried out by calcium phosphate transfection.

[0233] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human, cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus 1, adenoviruses and adeno-associated viruses, and the like. *See*, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0234] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

[0235] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic

acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0236] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

caTCR effector cells

[0237] In some embodiments, there is provided an effector cell (such as a T cell) presenting on its surface a caTCR according to any of the caTCRs described herein. In some embodiments, the effector cell comprises nucleic acid encoding the caTCR, wherein the caTCR is expressed from the nucleic acid and localized to the effector cell surface. In some embodiments, the caTCR is exogenously expressed and combined with the effector cell. In some embodiments, the effector cell is a T cell. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and

the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the T cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. Modifications of cells to disrupt gene expression include any such techniques known in the art, including for example RNA interference (*e.g.*, siRNA, shRNA, miRNA), gene editing (*e.g.*, CRISPR- or TALEN-based gene knockout), and the like. In some embodiments, the effector cell further comprises a chimeric co-stimulatory receptor (CSR). In some embodiments, the effector cell is capable of secreting a secretory secondary effector (SSE) capable of enhancing an immune response mediated by the caTCR.

[0238] For example, in some embodiments, there is provided an effector cell (such as a T cell) comprising nucleic acid encoding a caTCR according to any of the caTCRs described herein, wherein the caTCR is expressed from the nucleic acid and localized to the effector cell surface. In some embodiments, the nucleic acid encoding the caTCR comprises a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and a second nucleic acid sequence encoding a second polypeptide chain of the caTCR. In some embodiments, the first nucleic acid sequence is located on a first vector and the second nucleic acid sequence is located on a second vector. In some embodiments, the first and second nucleic acid sequences are located on the same vector. Vectors may be selected, for example, from the group consisting of mammalian expression vectors and viral vectors (such as those derived from retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses). In some embodiments, one or more of the vectors is integrated into the host genome of the effector cell. In some embodiments, the first nucleic acid sequence is under the control of a first promoter and the second nucleic acid sequence is under the control of a second promoter. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and second nucleic acids are under the control of a single promoter. In some embodiments, the first, second, and/or single promoters are inducible. In some embodiments, the expression of the first polypeptide chain is about the same

as the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression of the second polypeptide chain. Expression can be determined at the mRNA or protein level. The level of mRNA expression can be determined by measuring the amount of mRNA transcribed from the nucleic acid using various well-known methods, including Northern blotting, quantitative RT-PCR, microarray analysis and the like. The level of protein expression can be measured by known methods including immunocytochemical staining, enzyme-linked immunosorbent assay (ELISA), western blot analysis, luminescent assays, mass spectrometry, high performance liquid chromatography, high-pressure liquid chromatography-tandem mass spectrometry, and the like. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

[0239] Thus, in some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a) a first nucleic acid comprising a first promoter operably linked to a nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second nucleic acid comprising a second promoter operably linked to a nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first polypeptide chain is expressed from the first nucleic acid and the second polypeptide chain is expressed from the second nucleic acid to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the expression of the first polypeptide chain is about the same as the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression of the second polypeptide chain. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ

and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the vector is a viral vector (such as a lentiviral vector) integrated into the host genome of the effector cell.

[0240] In some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a) a first vector comprising a first promoter operably linked to a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second vector comprising a second promoter operably linked to a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first polypeptide chain is expressed from the first nucleic acid sequence and the second polypeptide chain is expressed from the second nucleic acid sequence to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the expression of the first polypeptide chain is about the same as the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression of the second polypeptide chain. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In

some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the first and second vectors are viral vectors (such as lentiviral vectors) integrated into the host genome of the effector cell.

[0241] In some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a vector comprising a) a first promoter operably linked to a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second promoter operably linked to a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first polypeptide chain is expressed from the first nucleic acid sequence and the second polypeptide chain is expressed from the second nucleic acid sequence to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the expression of the first polypeptide chain is about the same as the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression of the second polypeptide chain. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the

effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the first and second vectors are viral vectors (such as lentiviral vectors) integrated into the host genome of the effector cell.

[0242] In some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a host genome-integrated lentiviral vector comprising a) a first promoter operably linked to a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second promoter operably linked to a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first polypeptide chain is expressed from the first nucleic acid sequence and the second polypeptide chain is expressed from the second nucleic acid sequence to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, the first and second promoters have the same sequence. In some embodiments, the first and second promoters have different sequences. In some embodiments, the first and/or second promoters are inducible. In some embodiments, the expression of the first polypeptide chain is about the same as the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is at least about two (such as at least about any of 2, 3, 4, 5, or more) times the expression of the second polypeptide chain. In some embodiments, the expression of the first polypeptide chain is no more than about 1/2 (such as no more than about any of 1/2, 1/3, 1/4, 1/5 or less) times the expression of the second polypeptide chain. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise

sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

[0243] In some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a vector comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first and second nucleic acid sequences are under the control of a single promoter, wherein the first polypeptide chain is expressed from the first nucleic acid sequence and the second polypeptide chain is expressed from the second nucleic acid sequence to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, the promoter is operably linked to the 5' end of the first nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the 3' end of first nucleic acid sequence to the 5' end of the second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, the promoter is operably linked to the 5' end of the second nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the 3' end of second nucleic acid sequence to the 5' end of the first nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, the promoter is inducible. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the

TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the vector is a viral vector (such as a lentiviral vector) integrated into the host genome of the effector cell.

[0244] In some embodiments, there is provided a caTCR effector cell (such as a T cell) expressing on its surface a caTCR according to any of the caTCRs described herein, wherein the caTCR effector cell comprises a host genome-integrated lentiviral vector comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and b) a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first and second nucleic acid sequences are under the control of a single promoter, wherein the first polypeptide chain is expressed from the first nucleic acid sequence and the second polypeptide chain is expressed from the second nucleic acid sequence to form the caTCR, and wherein the caTCR localizes to the surface of the effector cell. In some embodiments, there is a promoter operably linked to the 5' end of the first nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the 3' end of first nucleic acid sequence to the 5' end of the second nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, there is a promoter operably linked to the 5' end of the second nucleic acid sequence, and there is nucleic acid linker selected from the group consisting of an internal ribosomal entry site (IRES) and a nucleic acid encoding a self-cleaving 2A peptide (such as P2A, T2A, E2A, or F2A) linking the 3' end of second nucleic acid sequence to the 5' end of the first nucleic acid sequence, wherein the first nucleic acid sequence and the second nucleic acid sequence are transcribed as a single RNA under the control of the promoter. In some embodiments, the promoter is inducible. In some embodiments, the effector cell does not express the TCR subunits from which the TCR-TMs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR δ and γ chains, or the effector cell is a γδ T cell and the TCR-TMs of the introduced caTCR comprise sequences derived from TCR α and β chains. In some embodiments, the effector cell is modified to block or decrease the expression of one or

both of the endogenous TCR subunits from which the TCRDs of the caTCR are derived. For example, in some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

Preparation of caTCRs, CSRs and SSEs

[0245] In some embodiments, according to any of the caTCRs, CSRs and SSEs described herein, the antigen-binding module comprises a Fab-like antigen-binding module comprising sequences from a monoclonal antibody. In some embodiments, the Fab-like antigen-binding module comprises V_H, C_H1, V_L, and C_L domains from the monoclonal antibody. In some embodiments, the antigen-binding module comprises a Fv or a scFv comprising sequences from a monoclonal antibody. In some embodiments, the antigen-binding module comprises bispecific fusion domains comprising one or more Fabs, Fvs, and scFvs derived from monoclonal antibodies. Monoclonal antibodies can be prepared, *e.g.,* using hybridoma methods, such as those described by Kohler and Milstein, *Nature,* 256:495 (1975) and Sergeeva *et al.*, *Blood*, 117(16):4262-4272.

[0246] In a hybridoma method, a hamster, mouse, or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro.* The immunizing agent can include a polypeptide or a fusion protein of the protein of interest, or a complex comprising at least two molecules, such as a complex comprising a peptide and an MHC protein. Generally, peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. *See, e.g.,* Goding, Monoclonal Antibodies: Principles and Practice (New York: Academic Press, 1986), pp. 59-103. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the

unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which prevents the growth of HGPRT-deficient cells.

[0247] In some embodiments, the immortalized cell lines fuse efficiently, support stable high-level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. In some embodiments, the immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. Kozbor, *J. Immunol.,* 133:3001 (1984); Brodeur *et al.* Monoclonal Antibody Production Techniques and Applications (Marcel Dekker, Inc.: New York, 1987) pp. 51-63.

[0248] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptide. The binding specificity of monoclonal antibodies produced by the hybridoma cells can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.,* 107:220 (1980).

[0249] After the desired hybridoma cells are identified, the clones can be sub-cloned by limiting dilution procedures and grown by standard methods. Goding, *supra.* Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[0250] The monoclonal antibodies secreted by the sub-clones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0251] In some embodiments, according to any of the caTCRs, CSRs and SSEs described herein, the antigen-binding module is an antibody moiety comprising sequences from a clone selected from an antibody moiety library (such as a phage library presenting scFv or Fab fragments). The clone may be identified by screening combinatorial libraries for antibody fragments with the desired activity or activities. For example, a variety of methods are known in

the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.*, *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, N.J., 2001) and further described, *e.g.*, in McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, N.J., 2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0252] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0253] The antibody moiety can be prepared using phage display to screen libraries for antibodies specific to the target antigen (such as a peptide/MHC class I/II complex or a cell surface antigen). The library can be a human scFv phage display library having a diversity of at least one x 10^9 (such as at least about any of 1 x 10^9, 2.5 x 10^9, 5 x 10^9, 7.5 x 10^9, 1 x 10^{10}, 2.5 x 10^{10}, 5 x 10^{10}, 7.5 x 10^{10}, or 1 x 10^{11}) unique human antibody fragments. In some embodiments, the library is a naïve human library constructed from DNA extracted from human PMBCs and spleens from healthy donors, encompassing all human heavy and light chain subfamilies. In some embodiments, the library is a naïve human library constructed from DNA extracted from

PBMCs isolated from patients with various diseases, such as patients with autoimmune diseases, cancer patients, and patients with infectious diseases. In some embodiments, the library is a semi-synthetic human library, wherein heavy chain CDR3 is completely randomized, with all amino acids (with the exception of cysteine) equally likely to be present at any given position (*see, e.g.,* Hoet, R.M. *et al.*, *Nat. Biotechnol.* 23(3):344-348, 2005). In some embodiments, the heavy chain CDR3 of the semi-synthetic human library has a length from about 5 to about 24 (such as about any of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24) amino acids. In some embodiments, the library is a fully-synthetic phage display library. In some embodiments, the library is a non-human phage display library.

[0254] Phage clones that bind to the target antigen with high affinity can be selected by iterative binding of phage to the target antigen, which is bound to a solid support (such as, for example, beads for solution panning or mammalian cells for cell panning), followed by removal of non-bound phage and by elution of specifically bound phage. In an example of solution panning, the target antigen can be biotinylated for immobilization to a solid support. The biotinylated target antigen is mixed with the phage library and a solid support, such as streptavidin-conjugated Dynabeads M-280, and then target antigen-phage-bead complexes are isolated. The bound phage clones are then eluted and used to infect an appropriate host cell, such as *E. coli* XL1-Blue, for expression and purification. In an example of cell panning, T2 cells (a TAP-deficient, HLA-A*02:01^{+} lymphoblast cell line) loaded with an AFP peptide are mixed with the phage library, after which the cells are collected and the bound clones are eluted and used to infect an appropriate host cell for expression and purification. The panning can be performed for multiple (such as about any of 2, 3, 4, 5, 6 or more) rounds with either solution panning, cell panning, or a combination of both, to enrich for phage clones binding specifically to the target antigen. Enriched phage clones can be tested for specific binding to the target antigen by any methods known in the art, including for example ELISA and FACS.

Human and Humanized Antibody Moieties

[0255] The caTCR, CSR and SSE antibody moieties can be human or humanized. Humanized forms of non-human *(e.g.,* murine) antibody moieties are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$, scFv, or other antigen-binding subsequences of antibodies) that typically contain minimal sequence derived from non-human immunoglobulin. Humanized antibody moieties include human immunoglobulins, immunoglobulin chains, or fragments thereof (recipient antibody) in which residues from a CDR of the recipient 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 antibody moieties can also comprise residues that are found neither in the recipient antibody moiety nor in the imported CDR or framework sequences. In general, the humanized antibody moiety can comprise 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. *See*, *e.g.*, Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al., Nature,* 332: 323-329 (1988); Presta, *Curr. Op. Struct. Biol.,* 2:593-596 (1992).

[0256] Generally, a humanized antibody moiety 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. According to some embodiments, humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature,* 321: 522-525 (1986); Riechmann *et al.*, *Nature,* 332: 323-327 (1988); Verhoeyen *et al.*, *Science,* 239: 1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody moiety. Accordingly, such "humanized" antibody moieties are antibody moieties (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibody moieties are typically human antibody moieties in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0257] As an alternative to humanization, human antibody moieties can be generated. For example, it is now possible to produce 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. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) 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 into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.,* Jakobovits *et al.*, *PNAS USA,* 90:2551 (1993); Jakobovits *et al., Nature,* 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.,* 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852. Alternatively, human antibodies can be made by introducing human immunoglobulin loci into

transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al., Bio/Technology,* 10: 779-783 (1992); Lonberg *et al., Nature,* 368: 856-859 (1994); Morrison, *Nature,* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology,* 14: 845-851 (1996); Neuberger, *Nature Biotechnology,* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.,* 13: 65-93 (1995).

[0258] Human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275) or by using various techniques known in the art, including phage display libraries. Hoogenboom and Winter, *J. Mol. Biol.,* 227:381 (1991); Marks *et al., J. Mol. Biol.,* 222:581 (1991). The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies. Cole *et al., Monoclonal Antibodies and Cancer Therapy,* Alan R. Liss, p. 77 (1985) and Boerner *et al., J. Immunol.,* 147(1): 86-95 (1991).

Variants

[0259] In some embodiments, amino acid sequence variants of the antigen-binding modules provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antigen-binding module. Amino acid sequence variants of an antigen-binding module may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antigen-binding module, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antigen-binding module. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

[0260] In some embodiments, antigen-binding module variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs of antibody moieties. Amino acid substitutions may be introduced into an antigen-binding module of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding or decreased immunogenicity.

[0261] Conservative substitutions are shown in Table 3 below.

TABLE 3: CONSERVATIVE SUBSTITITIONS

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0262] Amino acids may be grouped into different classes according to common side-chain properties:

a. hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

b. neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

c. acidic: Asp, Glu;

d. basic: His, Lys, Arg;

e. residues that influence chain orientation: Gly, Pro;

f. aromatic: Trp, Tyr, Phe.

[0263] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0264] An exemplary substitutional variant is an affinity matured antibody moiety, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques. Briefly, one or more CDR residues are mutated and the variant antibody moieties displayed on phage and screened for a particular biological activity *(e.g.*, binding affinity). Alterations *(e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody moiety affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see*, *e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or specificity determining residues (SDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, (2001).)

[0265] In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods *(e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody moiety variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues *(e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0266] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody moiety to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In some embodiments of the variant V_H and V_L sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0267] A useful method for identification of residues or regions of an antigen-binding module that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antigen-binding module with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional

sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antigen-binding module complex can be determined to identify contact points between the antigen-binding module and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0268] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antigen-binding module with an N-terminal methionyl residue. Other insertional variants of the antigen-binding module include the fusion to the N- or C-terminus of the antigen-binding module to an enzyme (*e.g.,* for ADEPT) or a polypeptide which increases the serum half-life of the antigen-binding module.

Derivatives

[0269] In some embodiments, a caTCR according to any of the caTCRs described herein, a CSR according to any of the CSRs described herein, and/or a SSE according to any of the SSEs described herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the caTCR and/or CSR and/or SSE include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the caTCR and/or CSR and/or SSE may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the caTCR and/or CSR and/or SSE to be improved, whether the caTCR and/or CSR and/or SSE derivative will be used in a therapy under defined conditions, etc.

[0270] In some embodiments, conjugates of the caTCR and/or CSR and/or SSE and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the caTCR- and/or CSR- and/or SSE-nonproteinaceous moiety are killed.

Preparation of caTCR effector cells

[0271] The present invention in one aspect provides effector cells (such as lymphocytes, for example T cells) expressing a caTCR. Exemplary methods of preparing effector cells (such as T cells) expressing the caTCRs (caTCR effector cells, such as caTCR T cells) are provided herein.

[0272] In some embodiments, a caTCR effector cell (such as a caTCR T cell) can be generated by introducing one or more nucleic acids (including for example a lentiviral vector) encoding a caTCR (such as any of the caTCRs described herein) that specifically binds to a target antigen (such as a disease-associated antigen) into the effector cell. The introduction of the one or more nucleic acids into the effector cell can be accomplished using techniques known in the art, such as those described herein for Nucleic Acids. In some embodiments, the caTCR effector cells (such as caTCR T cells) of the invention are able to replicate *in vivo*, resulting in long-term persistence that can lead to sustained control of a disease associated with expression of the target antigen (such as cancer or viral infection).

[0273] In some embodiments, the invention relates to administering a genetically modified T cell expressing a caTCR that specifically binds to a target antigen according to any of the caTCRs described herein for the treatment of a patient having or at risk of developing a disease and/or disorder associated with expression of the target antigen (also referred to herein as a "target antigen-positive" or "TA-positive" disease or disorder), including, for example, cancer or viral infection, using lymphocyte infusion. In some embodiments, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a patient in need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the patient.

[0274] In some embodiments, there is provided a T cell expressing a caTCR that specifically binds to a target antigen according to any of the caTCRs described herein (also referred to herein as an "caTCR T cell"). The caTCR T cells of the invention can undergo robust *in vivo* T cell expansion and can establish target antigen-specific memory cells that persist at high levels for an

extended amount of time in blood and bone marrow. In some embodiments, the caTCR T cells of the invention infused into a patient can eliminate target antigen-presenting cells, such as target antigen-presenting cancer or virally-infected cells, *in vivo* in patients having a target antigen-associated disease. In some embodiments, the caTCR T cells of the invention infused into a patient can eliminate target antigen-presenting cells, such as target antigen-presenting cancer or virally-infected cells, *in vivo* in patients having a target antigen-associated disease that is refractory to at least one conventional treatment.

[0275] Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In some embodiments of the present invention, any number of T cell lines available in the art may be used. In some embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as FICOLL™ separation. In some embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In some embodiments, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate buffered saline (PBS). In some embodiments, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solutions with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0276] In some embodiments, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$ T cells, can be further

isolated by positive or negative selection techniques. For example, in some embodiments, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3×28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In some embodiments, the time period is about 30 minutes. In some embodiments, the time period ranges from 30 minutes to 36 hours or longer (including all ranges between these values). In some embodiments, the time period is at least one, 2, 3, 4, 5, or 6 hours. In some embodiments, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such as in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of $CD8^+$ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In some embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[0277] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD 14, CD20, CD11b, CD 16, HLA-DR, and CD8. In some embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, CD62Lhi, $GITR^+$, and $FoxP3^+$. Alternatively, in some embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar methods of selection.

[0278] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In some embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in some embodiments, a concentration of about 2 billion cells/ml is used. In some embodiments, a concentration of about 1 billion cells/ml is used. In some embodiments, greater than about 100 million cells/ml is used. In some embodiments, a concentration of cells of about any of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In some embodiments, a concentration of cells of about any of 75, 80, 85, 90, 95, or 100 million cells/ml is used. In some embodiments, a concentration of about 125 or about 150 million cells/ml is used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[0279] In some embodiments of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in some embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

[0280] Whether prior to or after genetic modification of the T cells to express a desirable caTCR, the T cells can be activated and expanded generally using methods as described, for

example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[0281] Generally, the T cells of the invention are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg *et al.*, *Transplant Proc.* 30(8):3975-3977, 1998; Haanen *et al.*, *J. Exp. Med.* 190(9):13191328, 1999; Garland *et al.*, *J. Immunol. Meth.* 227(1-2):53-63, 1999).

[0282] In some of any such embodiments described herein, preparation of caTCR effector cells results in minimal or substantially no exhaustion of the caTCR effector cells. For example, in some embodiments, preparation results in fewer than about 50% (such as fewer than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of the caTCR effector cells becoming exhausted. Effector cell exhaustion can be determined by any means known in the art, including any means described herein.

[0283] In some of any such embodiments described herein, preparation of caTCR effector cells results in minimal or substantially no terminal differentiation of the caTCR effector cells. For example, in some embodiments, preparation results in fewer than about 50% (such as fewer than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of the caTCR effector cells becoming terminally differentiated. Effector cell differentiation can be determined by any means known in the art, including any means described herein.

[0284] In some of any such embodiments described herein, preparation of caTCR effector cells results in minimal or substantially no internalization of caTCRs on the caTCR effector cells. For example, in some embodiments, preparation results in less than about 50% (such as less than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of caTCRs on the caTCR effector

cells becoming internalized. Internalization of caTCRs on caTCR effector cells can be determined by any means known in the art, including any means described herein.

Genetic modification

[0285] In some embodiments, the caTCR effector cells (such as caTCR T cells) of the invention are generated by transducing effector cells (such as T cells prepared by the methods described herein) with a viral vector encoding a caTCR as described herein. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the effector cell. For a review of gene therapy procedures, see Anderson, Science 256:808-813 (1992); Nabel & Feigner, TIBTECH 11 :211 - 217 (1993); Mitani & Caskey, TIBTECH 11 :162-166 (1993); Dillon, TIBTECH 11 : 167-175 (1993); Miller, Nature 357:455-460 (1992); Van Brunt, Biotechnology 6(10): 1149-1 154 (1988); Vigne, Restorative Neurology and Neuroscience 8:35-36 (1995); Kremer & Perricaudet, British Medical Bulletin 51(l):31-44 (1995); and Yu et al., Gene Therapy 1 :13-26 (1994). In some embodiments, the viral vector is a lentiviral vector, and the caTCR effector cell comprises the lentiviral vector integrated into the caTCR effector cell genome. In some embodiments, the caTCR effector cell is a caTCR T cell comprising the lentiviral vector integrated into its genome.

[0286] In some embodiments, the caTCR effector cell is a T cell modified to block or decrease the expression of one or both of its endogenous TCR chains. For example, in some embodiments, the caTCR effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR α and β chains, or the caTCR effector cell is a γδ T cell modified to block or decrease the expression of the TCR γ and/or δ chains and the TCRDs of the introduced caTCR comprise sequences derived from TCR γ and δ chains. Modifications of cells to disrupt gene expression include any such techniques known in the art, including for example RNA interference (*e.g.*, siRNA, shRNA, miRNA), gene editing (*e.g.*, CRISPR- or TALEN-based gene knockout), and the like.

[0287] In some embodiments, caTCR T cells with reduced expression of one or both of the endogenous TCR chains of the T cell are generated using the CRISPR/Cas system. For a review of the CRISPR/Cas system of gene editing, *see* for example Jian W & Marraffini LA, *Annu. Rev. Microbiol.* 69, 2015; Hsu PD *et al.*, *Cell*, 157(6):1262-1278, 2014; and O'Connell MR *et al.*, *Nature***Volume:** 516:Pages:263–266, 2014. In some embodiments, caTCR T cells with reduced

expression of one or both of the endogenous TCR chains of the T cell are generated using TALEN-based genome editing.

Enrichment

[0288] In some embodiments, there is provided a method of enriching a heterogeneous cell population for a caTCR effector cell according to any of the caTCR effector cells described herein.

[0289] A specific subpopulation of caTCR effector cells (such as caTCR T cells) that specifically bind to a target antigen can be enriched for by positive selection techniques. For example, in some embodiments, caTCR effector cells (such as caTCR T cells) are enriched for by incubation with target antigen-conjugated beads for a time period sufficient for positive selection of the desired caTCR effector cells. In some embodiments, the time period is about 30 minutes. In some embodiments, the time period ranges from 30 minutes to 36 hours or longer (including all ranges between these values). In some embodiments, the time period is at least one, 2, 3, 4, 5, or 6 hours. In some embodiments, the time period is 10 to 24 hours. In some embodiments, the incubation time period is 24 hours. For isolation of caTCR effector cells present at low levels in the heterogeneous cell population, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate caTCR effector cells in any situation where there are few caTCR effector cells as compared to other cell types. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention.

[0290] For isolation of a desired population of caTCR effector cells by positive selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In some embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in some embodiments, a concentration of about 2 billion cells/ml is used. In some embodiments, a concentration of about 1 billion cells/ml is used. In some embodiments, greater than about 100 million cells/ml is used. In some embodiments, a concentration of cells of about any of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In some embodiments, a concentration of cells of about any of 75, 80, 85, 90, 95, or 100 million cells/ml is used. In some embodiments, a concentration of about 125 or about 150 million cells/ml is used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of caTCR effector cells that may weakly express the caTCR.

[0291] In some of any such embodiments described herein, enrichment results in minimal or substantially no exhaustion of the caTCR effector cells. For example, in some embodiments, enrichment results in fewer than about 50% (such as fewer than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of the caTCR effector cells becoming exhausted. Effector cell exhaustion can be determined by any means known in the art, including any means described herein.

[0292] In some of any such embodiments described herein, enrichment results in minimal or substantially no terminal differentiation of the caTCR effector cells. For example, in some embodiments, enrichment results in fewer than about 50% (such as fewer than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of the caTCR effector cells becoming terminally differentiated. Effector cell differentiation can be determined by any means known in the art, including any means described herein.

[0293] In some of any such embodiments described herein, enrichment results in minimal or substantially no internalization of caTCRs on the caTCR effector cells. For example, in some embodiments, enrichment results in less than about 50% (such as less than about any of 45, 40, 35, 30, 25, 20, 15, 10, or 5%) of caTCRs on the caTCR effector cells becoming internalized. Internalization of caTCRs on caTCR effector cells can be determined by any means known in the art, including any means described herein.

[0294] In some of any such embodiments described herein, enrichment results in increased proliferation of the caTCR effector cells. For example, in some embodiments, enrichment results in an increase of at least about 10% (such as at least about any of 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 1000% or more) in the number of caTCR effector cells following enrichment.

[0295] Thus, in some embodiments, there is provided a method of enriching a heterogeneous cell population for caTCR effector cells expressing a caTCR that specifically binds to a target antigen comprising: a) contacting the heterogeneous cell population with a capture molecule comprising the target antigen or one or more epitopes contained therein to form complexes comprising the caTCR effector cell bound to the capture molecule; and b) separating the complexes from the heterogeneous cell population, thereby generating a cell population enriched for the caTCR effector cells. In some embodiments, the capture molecule is immobilized to a solid support. In some embodiments, the solid support is particulate (such as beads). In some embodiments, the solid support is a surface (such as the bottom of a well). In some embodiments, the capture molecule is labelled with a tag. In some embodiments, the tag is a fluorescent molecule, an affinity tag, or a magnetic tag. In some embodiments, the method

further comprises eluting the caTCR effector cells from the capture molecule and recovering the eluate.

Library screening

[0296] In some embodiments, to isolate candidate caTCR constructs specific for a target antigen, a caTCR library, for example cells expressing a library of nucleic acids encoding a plurality of caTCRs, may be exposed to a capture molecule comprising the target antigen or one or more epitopes contained therein, followed by isolation of affinity members of the library that specifically bind the capture molecule. In some embodiments, the capture molecule is immobilized on a solid support. In some embodiments, the support may be the surfaces of beads, microtiter plates, immunotubes, or any material known in the art useful for such purposes. In some embodiments, the interaction takes place in solution with a tagged capture molecule (e.g. biotinylated capture molecule). In some embodiments, the procedure involves one or more washing steps to remove unspecific and non-reactive library members (panning). In some embodiments, to purify complexes in solution, they are collected by either immobilization or by centrifugation. In some embodiments, affinity members are captured on a soluble biotinylated capture molecule, followed by immobilization of the affinity complex (affinity member and capture molecule) on streptavidin beads. In some embodiments, the solid support is a bead. In some embodiments, the beads include, for example, magnetic beads (e.g. from Bangs Laboratories, Polysciences inc., Dynal Biotech, Miltenyi Biotech or Quantum Magnetic), nonmagnetic beads (e.g. Pierce and Upstate technology), monodisperse beads (e.g. Dynal Biotech and Microparticle Gmbh), and polydisperse beads (e.g. Chemagen). The use of magnetic beads has been described exhaustingly in literature (Uhlen, M, et al (1994) in Advances in Biomagnetic Separation, BioTechniques press, Westborough, MA). In some embodiments, the affinity members are purified by positive selection. In some embodiments, the affinity members are purified by negative selection to remove unwanted library members. In some embodiments, the affinity members are purified by both positive and negative selection steps.

[0297] Generally, the techniques used to prepare the library constructs will be based on known genetic engineering techniques. In this regard, nucleic acid sequences encoding the caTCRs to be expressed in the library are incorporated into expression vectors appropriate for the type of expression system to be used. Appropriate expression vectors for use in display in cells, such as CD3+ cells, are well known and described in the art. For example, in some embodiments, the expression vector is a viral vector, such as a lentiviral vector.

[0298] In some embodiments, there is provided a nucleic acid library comprising sequences encoding a plurality of caTCRs according to any one of the embodiments described herein. In some embodiments, the nucleic acid library comprises viral vectors encoding the plurality of caTCRs. In some embodiments, the viral vectors are lentiviral vectors.

[0299] In some embodiments, there is provided a method of screening a nucleic acid library according to any of the embodiments described herein for sequences encoding caTCRs specific for a target antigen, comprising: a) introducing the nucleic acid library into a plurality of cells, such that the caTCRs are expressed on the surface of the plurality of cells; b) incubating the plurality of cells with a capture molecule comprising the target antigen or one or more epitopes contained therein; c) collecting cells bound to the capture molecule; and d) isolating sequences encoding the caTCRs from cells collected in step c), thereby identifying caTCRs specific for the target antigen. In some embodiments, the method further comprises one or more wash steps. In some embodiments, the one or more wash steps are carried out between steps b) and c). In some embodiments, the plurality of cells is a plurality of CD3+ cells. In some embodiments, the capture molecule is immobilized on a solid support. In some embodiments, the solid support is a bead. In some embodiments, collecting cells bound to the capture molecule comprises eluting cells from the capture ligand bound to the solid support and collecting the eluate. In some embodiments, the capture molecule is labelled with a tag. In some embodiments, the tag is a fluorescent molecule, an affinity tag, or a magnetic tag. In some embodiments, collecting cells bound to the capture molecule comprises isolating complexes comprising the cells and the labelled ligand. In some embodiments, the cells are dissociated from the complexes.

MHC proteins

[0300] MHC class I proteins are one of two primary classes of major histocompatibility complex (MHC) molecules (the other being MHC class II) and are found on nearly every nucleated cell of the body. Their function is to display fragments of proteins from within the cell to T cells; healthy cells will be ignored, while cells containing foreign or mutated proteins will be attacked by the immune system. Because MHC class I proteins present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called the cytosolic or endogenous pathway. Class I MHC molecules bind peptides generated mainly from degradation of cytosolic proteins by the proteasome. The MHC I:peptide complex is then inserted into the plasma membrane of the cell. The peptide is bound to the extracellular part of the class I MHC molecule. Thus, the function of the class I MHC is to display intracellular proteins to cytotoxic T

cells (CTLs). However, class I MHC can also present peptides generated from exogenous proteins, in a process known as cross-presentation.

[0301] MHC class I proteins consist of two polypeptide chains, α and β2-microglobulin (β2M). The two chains are linked noncovalently via interaction of β2M and the α3 domain. Only the α chain is polymorphic and encoded by a HLA gene, while the β2M subunit is not polymorphic and encoded by the β-2 microglobulin gene. The α3 domain is plasma membrane-spanning and interacts with the CD8 co-receptor of T-cells. The α3-CD8 interaction holds the MHC I molecule in place while the T cell receptor (TCR) on the surface of the cytotoxic T cell binds its α1-α2 heterodimer ligand, and checks the coupled peptide for antigenicity. The α1 and α2 domains fold to make up a groove for peptides to bind. MHC class I proteins bind peptides that are 8-10 amino acid in length.

[0302] MHC class II molecules are a family of molecules normally found only on antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. The antigens presented by class II peptides are derived from extracellular proteins (not cytosolic as in class I); hence, the MHC class II-dependent pathway of antigen presentation is called the endocytic or exogenous pathway. Loading of an MHC class II molecule occurs by phagocytosis; extracellular proteins are endocytosed, digested in lysosomes, and the resulting epitopic peptide fragments are loaded onto MHC class II molecules prior to their migration to the cell surface.

[0303] Like MHC class I molecules, class II molecules are also heterodimers, but in this case consist of two homogenous peptides, an α and β chain. The subdesignation α1, α2, etc. refers to separate domains within the HLA gene; each domain is usually encoded by a different exon within the gene, and some genes have further domains that encode leader sequences, transmembrane sequences, etc. Because the antigen-binding groove of MHC class II molecules is open at both ends while the corresponding groove on class I molecules is closed at each end, the antigens presented by MHC class II molecules are longer, generally between 15 and 24 amino acid residues long.

[0304] The human leukocyte antigen (HLA) genes are the human versions of the MHC genes. The three major MHC class I proteins in humans are HLA-A, HLA-B, and HLA-C, while the 3 minor ones are HLA-E, HLA-F, and HLA-G. The three major MHC class II proteins involved in antigen presentation in humans are HLA-DP, HLDA-DQ, and HLA-DR, while the other MHC class II proteins, HLA-DM and HLA-DO, are involved in the internal processing and loading of antigens. HLA-A is ranked among the genes in humans with the fastest-evolving coding

sequence. As of December 2013, there were 2432 known HLA-A alleles coding for 1740 active proteins and 117 null proteins. The HLA-A gene is located on the short arm of chromosome 6 and encodes the larger, α-chain, constituent of HLA-A. Variation of HLA-A α-chain is key to HLA function. This variation promotes genetic diversity in the population. Since each HLA has a different affinity for peptides of certain structures, greater variety of HLAs means greater variety of antigens to be 'presented' on the cell surface, enhancing the likelihood that a subset of the population will be resistant to any given foreign invader. This decreases the likelihood that a single pathogen has the capability to wipe out the entire human population. Each individual can express up to two types of HLA-A, one from each of their parents. Some individuals will inherit the same HLA-A from both parents, decreasing their individual HLA diversity; however, the majority of individuals will receive two different copies of HLA-A. This same pattern follows for all HLA groups. In other words, a person can only express either one or two of the 2432 known HLA-A alleles.

[0305] All alleles receive at least a four digit classification, *e.g.*, HLA-A*02:12. The A signifies which HLA gene the allele belongs to. There are many HLA-A alleles, so that classification by serotype simplifies categorization. The next pair of digits indicates this assignment. For example, HLA-A*02:02, HLA-A*02:04, and HLA-A*02:324 are all members of the A2 serotype (designated by the *02 prefix). This group is the primary factor responsible for HLA compatibility. All numbers after this cannot be determined by serotyping and are designated through gene sequencing. The second set of digits indicates what HLA protein is produced. These are assigned in order of discovery and as of December 2013 there are 456 different HLA-A02 proteins known (assigned names HLA-A*02:01 to HLA-A*02:456). The shortest possible HLA name includes both of these details. Each extension beyond that signifies a nucleotide change that may or may not change the protein.

[0306] In some embodiments, the Fab-like antigen-binding module specifically binds to a complex comprising a peptide derived from a disease-associated antigen (such as a tumor-associated or virally-encoded antigen) and an MHC class I protein, wherein the MHC class I protein is HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, or HLA-G. In some embodiments, the MHC class I protein is HLA-A, HLA-B, or HLA-C. In some embodiments, the MHC class I protein is HLA-A. In some embodiments, the MHC class I protein is HLA-B. In some embodiments, the MHC class I protein is HLA-C. In some embodiments, the MHC class I protein is HLA-A01, HLA-A02, HLA-A03, HLA-A09, HLA-A10, HLA-A11, HLA-A19, HLA-A23, HLA-A24, HLA-A25, HLA-A26, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32,

HLA-A33, HLA-A34, HLA-A36, HLA-A43, HLA-A66, HLA-A68, HLA-A69, HLA-A74, or HLA-A80. In some embodiments, the MHC class I protein is HLA-A02. In some embodiments, the MHC class I protein is any one of HLA-A*02:01-555, such as HLA-A*02:01, HLA-A*02:02, HLA-A*02:03, HLA-A*02:04, HLA-A*02:05, HLA-A*02:06, HLA-A*02:07, HLA-A*02:08, HLA-A*02:09, HLA-A*02:10, HLA-A*02:11, HLA-A*02:12, HLA-A*02:13, HLA-A*02:14, HLA-A*02:15, HLA-A*02:16, HLA-A*02:17, HLA-A*02:18, HLA-A*02:19, HLA-A*02:20, HLA-A*02:21, HLA-A*02:22, or HLA-A*02:24. In some embodiments, the MHC class I protein is HLA-A*02:01. HLA-A*02:01 is expressed in 39-46% of all Caucasians, and therefore represents a suitable choice of MHC class I protein for use in the present invention.

[0307] In some embodiments, the Fab-like antigen-binding module specifically binds to a complex comprising a peptide derived from a disease-associated antigen (such as a tumor-associated or virally-encoded antigen) and an MHC class II protein, wherein the MHC class II protein is HLA-DP, HLA-DQ, or HLA-DR. In some embodiments, the MHC class II protein is HLA-DP. In some embodiments, the MHC class II protein is HLA-DQ. In some embodiments, the MHC class II protein is HLA-DR.

[0308] Peptides suitable for use in generating Fab-like antigen-binding modules can be determined, for example, based on the presence of HLA (such as HLA-A*02:01) binding motifs and cleavage sites for proteasomes and immune-proteasomes using computer prediction models known to those of skill in the art. For predicting MHC binding sites, such models include, but are not limited to, ProPred1 (described in more detail in Singh and Raghava, *ProPred: prediction of HLA-DR binding sites. BIOINFORMATICS* 17(12):1236-1237, 2001), and SYFPEITHI (see Schuler *et al. SYFPEITHI, Database for Searching and T-Cell Epitope Prediction. in Immunoinformatics Methods in Molecular Biology*, vol 409(1): 75-93, 2007).

[0309] Once appropriate peptides have been identified, peptide synthesis may be done in accordance with protocols well known to those of skill in the art. Because of their relatively small size, the peptides of the invention may be directly synthesized in solution or on a solid support in accordance with conventional peptide synthesis techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. The synthesis of peptides in solution phase has become a well-established procedure for large-scale production of synthetic peptides and as such is a suitable alternative method for preparing the peptides of the invention (See for example, Solid Phase Peptide Synthesis by John Morrow Stewart and Martin *et al. Application of Almez-mediated Amidation Reactions to Solution Phase Peptide Synthesis*, Tetrahedron Letters Vol. 39, pages 1517-1520, 1998).

Pharmaceutical compositions

[0310] Also provided herein are compositions (such as pharmaceutical compositions, also referred to herein as formulations) comprising an caTCR according to any of the embodiments described herein, a nucleic acid encoding an caTCR according to any of the embodiments described herein, or an caTCR effector cell according to any of the embodiments described herein. In some embodiments, the composition is an caTCR effector cell composition (such as a pharmaceutical composition) comprising an effector cell (such as a T cell) presenting on its surface an caTCR according to any of the caTCRs described herein. In some embodiments, the caTCR effector cell composition is a pharmaceutical composition.

[0311] The composition may comprise a homogenous cell population comprising caTCR effector cells of the same cell type and expressing the same caTCR, or a heterogeneous cell population comprising a plurality of caTCR effector cell populations comprising caTCR effector cells of different cell types and/or expressing different caTCRs. The composition may further comprise cells that are not caTCR effector cells.

[0312] Thus, in some embodiments, there is provided a caTCR effector cell composition comprising a homogeneous cell population of caTCR effector cells (such as caTCR T cells) of the same cell type and expressing the same caTCR. In some embodiments, the caTCR effector cell is a T cell. In some embodiments, the caTCR effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell. In some embodiments, the caTCR effector cell composition is a pharmaceutical composition.

[0313] In some embodiments, there is provided a caTCR effector cell composition comprising a heterogeneous cell population comprising a plurality of caTCR effector cell populations comprising caTCR effector cells of different cell types and/or expressing different caTCRs. In some embodiments, the caTCR effector cells are T cells. In some embodiments, each population of caTCR effector cells is, independently from one another, of a cell type selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, all of the caTCR effector cells in the composition are of the same cell type (*e.g.*, all of the caTCR effector cells are cytotoxic T cells). In some embodiments, at least one population of caTCR effector cells is of a different cell type than the others (*e.g.*, one population of caTCR effector cells consists of cytotoxic T cells and the other populations of caTCR effector cells consist of natural killer T cells). In some embodiments, each population of caTCR effector cells expresses the same caTCR. In some embodiments, at least one population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each

population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each population of caTCR effector cells expresses a caTCR that specifically binds to the same target antigen. In some embodiments, at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen than the others (*e.g.*, one population of caTCR effector cells specifically binds to a pMHC complex and the other populations of caTCR effector cells specifically bind to a cell surface receptor). In some embodiments, where at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen, each population of caTCR effector cells expresses a caTCR that specifically binds to a target antigen associated with the same disease or disorder (*e.g.*, each of the target antigens are associated with a cancer, such as breast cancer). In some embodiments, the caTCR effector cell composition is a pharmaceutical composition.

[0314] Thus, in some embodiments, there is provided a caTCR effector cell composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein all of the caTCR effector cells in the composition are of the same cell type (*e.g.*, all of the caTCR effector cells are cytotoxic T cells), and wherein each population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, the caTCR effector cells are T cells. In some embodiments, the caTCR effector cells are selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, each population of caTCR effector cells expresses a caTCR that specifically binds to the same target antigen. In some embodiments, at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen than the others (*e.g.*, one population of caTCR effector cells specifically binds to a pMHC complex and the other populations of caTCR effector cells specifically bind to a cell surface receptor). In some embodiments, where at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen, each population of caTCR effector cells expresses a caTCR that specifically binds to a target antigen associated with the same disease or disorder (*e.g.*, each of the target antigens are associated with a cancer, such as breast cancer). In some embodiments, the caTCR effector cell composition is a pharmaceutical composition.

[0315] In some embodiments, there is provided a composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein at least one population of caTCR effector cells is of a different cell type than the others. In some embodiments, all of the populations of caTCR effector cells are of different cell types. In some

embodiments, the caTCR effector cells are T cells. In some embodiments, each population of caTCR effector cells is, independently from one another, of a cell type selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, each population of caTCR effector cells expresses the same caTCR. In some embodiments, at least one population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each population of caTCR effector cells expresses a caTCR that specifically binds to the same target antigen. In some embodiments, at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen than the others (*e.g.*, one population of caTCR effector cells specifically binds to a pMHC complex and the other populations of caTCR effector cells specifically bind to a cell surface receptor). In some embodiments, where at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen, each population of caTCR effector cells expresses a caTCR that specifically binds to a target antigen associated with the same disease or disorder (*e.g.*, each of the target antigens are associated with a cancer, such as breast cancer). In some embodiments, the caTCR effector cell composition is a pharmaceutical composition.

[0316] At various points during preparation of a composition, it can be necessary or beneficial to cryopreserve a cell. The terms "frozen/freezing" and "cryopreserved/cryopreserving" can be used interchangeably. Freezing includes freeze drying.

[0317] As is understood by one of ordinary skill in the art, the freezing of cells can be destructive (see Mazur, P., 1977, Cryobiology 14:251 -272) but there are numerous procedures available to prevent such damage. For example, damage can be avoided by (a) use of a cryoprotective agent, (b) control of the freezing rate, and/or (c) storage at a temperature sufficiently low to minimize degradative reactions. Exemplary cryoprotective agents include dimethyl sulfoxide (DMSO) (Lovelock and Bishop, 1959, Nature 183:1394- 1395; Ashwood-Smith, 1961 , Nature 190:1204-1205), glycerol, polyvinylpyrrolidine (Rinfret, 1960, Ann. N.Y. Acad. Sci. 85:576), polyethylene glycol (Sloviter and Ravdin, 1962, Nature 196:548), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe et al., 1962, Fed. Proc. 21 :157), D-sorbitol, i-inositol, D-lactose, choline chloride (Bender et al., 1960, J. Appl. Physiol. 15:520), amino acids (Phan The Tran and Bender, 1960, Exp. Cell Res. 20:651), methanol, acetamide, glycerol monoacetate (Lovelock, 1954, Biochem. J. 56:265), and inorganic salts (Phan The Tran and Bender, 1960, Proc. Soc. Exp. Biol. Med. 104:388; Phan The Tran and

Bender, 1961 , in Radiobiology, Proceedings of the Third Australian Conference on Radiobiology, llbery ed., Butterworth, London, p. 59). In particular embodiments, DMSO can be used. Addition of plasma (*e.g.*, to a concentration of 20-25%) can augment the protective effects of DMSO. After addition of DMSO, cells can be kept at 0° C until freezing, because DMSO concentrations of 1% can be toxic at temperatures above 4° C.

[0318] In the cryopreservation of cells, slow controlled cooling rates can be critical and different cryoprotective agents (Rapatz et al., 1968, Cryobiology 5(1): 18-25) and different cell types have different optimal cooling rates (see *e.g.*, Rowe and Rinfret, 1962, Blood 20:636; Rowe, 1966, Cryobiology 3(1):12-18; Lewis, et al., 1967, Transfusion 7(1):17-32; and Mazur, 1970, Science 168:939- 949 for effects of cooling velocity on survival of stem cells and on their transplantation potential). The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, *e.g.*, a programmable freezing device or a methanol bath procedure. Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling.

[0319] In particular embodiments, DMSO-treated cells can be pre-cooled on ice and transferred to a tray containing chilled methanol which is placed, in turn, in a mechanical refrigerator (*e.g.*, Harris or Revco) at -80° C. Thermocouple measurements of the methanol bath and the samples indicate a cooling rate of 1° to 3°C/minute can be preferred. After at least two hours, the specimens can have reached a temperature of - 80° C and can be placed directly into liquid nitrogen (-196° C).

[0320] After thorough freezing, the cells can be rapidly transferred to a long-term cryogenic storage vessel. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196° C) or vapor (-1° C). Such storage is facilitated by the availability of highly efficient liquid nitrogen refrigerators.

[0321] Further considerations and procedures for the manipulation, cryopreservation, and long-term storage of cells, can be found in the following exemplary references: U.S. Patent Nos. 4,199,022; 3,753,357; and 4,559,298; Gorin, 1986, Clinics In Haematology 15(1):19-48; Bone-Marrow Conservation, Culture and Transplantation, Proceedings of a Panel, Moscow, July 22-26, 1968, International Atomic Energy Agency, Vienna, pp. 107- 186; Livesey and Linner, 1987, Nature 327:255; Linner et al., 1986, J. Histochem. Cytochem. 34(9):1 123-1 135; Simione, 1992, J. Parenter. Sci. Technol. 46(6):226-32).

[0322] Following cryopreservation, frozen cells can be thawed for use in accordance with methods known to those of ordinary skill in the art. Frozen cells are preferably thawed quickly

and chilled immediately upon thawing. In particular embodiments, the vial containing the frozen cells can be immersed up to its neck in a warm water bath; gentle rotation will ensure mixing of the cell suspension as it thaws and increase heat transfer from the warm water to the internal ice mass. As soon as the ice has completely melted, the vial can be immediately placed on ice.

[0323] In particular embodiments, methods can be used to prevent cellular clumping during thawing. Exemplary methods include: the addition before and/or after freezing of DNase (Spitzer et al., 1980, Cancer 45:3075-3085), low molecular weight dextran and citrate, hydroxyethyl starch (Stiff et al., 1983, Cryobiology 20:17-24), etc. [0162] As is understood by one of ordinary skill in the art, if a cryoprotective agent that is toxic to humans is used, it should be removed prior to therapeutic use. DMSO has no serious toxicity.

[0324] Exemplary carriers and modes of administration of cells are described at pages 14-15 of U.S. Patent Publication No. 2010/0183564. Additional pharmaceutical carriers are described in Remington: The Science and Practice of Pharmacy, 21 st Edition, David B. Troy, ed., Lippicott Williams & Wilkins (2005).

[0325] In particular embodiments, cells can be harvested from a culture medium, and washed and concentrated into a carrier in a therapeutically-effective amount. Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Nonnosol-R (Abbott Labs), Plasma-Lyte A(R) (Baxter Laboratories, Inc., Morton Grove, IL), glycerol, ethanol, and combinations thereof.

[0326] In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% HAS or dextrose. Additional isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0327] Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0328] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2- phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol, and cyclitols, such as inositol; PEG;

amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (*i.e.*, <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

[0329] Where necessary or beneficial, compositions can include a local anesthetic such as lidocaine to ease pain at a site of injection.

[0330] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0331] Therapeutically effective amounts of cells within compositions can be greater than 10^2 cells, greater than 10^3 cells, greater than 10^4 cells, greater than 10^5 cells, greater than 10^6 cells, greater than 10^7 cells, greater than 10^8 cells, greater than 10^9 cells, greater than 10^{10} cells, or greater than 10^{11} cells.

[0332] In compositions and formulations disclosed herein, cells are generally in a volume of a liter or less, 500 ml or less, 250 ml or less or 100 ml or less. Hence the density of administered cells is typically greater than 10^4 cells/ml, 10^7 cells/ml or 10^8 cells/ml.

[0333] Also provided herein are caTCR nucleic acid compositions (such as pharmaceutical compositions, also referred to herein as formulations) comprising any of the nucleic acids encoding a caTCR described herein. In some embodiments, the caTCR nucleic acid composition is a pharmaceutical composition. In some embodiments, the caTCR nucleic acid composition further comprises any of an isotonizing agent, an excipient, a diluent, a thickener, a stabilizer, a buffer, and/or a preservative; and/or an aqueous vehicle, such as purified water, an aqueous sugar solution, a buffer solution, physiological saline, an aqueous polymer solution, or RNase free water. The amounts of such additives and aqueous vehicles to be added can be suitably selected according to the form of use of the caTCR nucleic acid composition.

[0334] The compositions and formulations disclosed herein can be prepared for administration by, for example, injection, infusion, perfusion, or lavage. The compositions and formulations can further be formulated for bone marrow, intravenous, intradermal, intraarterial, intranodal, intralymphatic, intraperitoneal, intralesional, intraprostatic, intravaginal, intrarectal, topical, intrathecal, intratumoral, intramuscular, intravesicular, and/or subcutaneous injection.

[0335] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by, *e.g.*, filtration through sterile filtration membranes.

Methods of treatment using caTCRs

[0336] The caTCRs and/or compositions of the invention can be administered to individuals (*e.g.*, mammals such as humans) to treat a disease and/or disorder associated with target antigen (TA) expression (also referred to herein as a "target-antigen positive" or "TA-positive" disease or disorder), including, for example, cancer and infectious disease (such as viral infection). The present application thus in some embodiments provides a method for treating a target antigen-positive disease (such as cancer or viral infection) in an individual comprising administering to the individual an effective amount of a composition (such as a pharmaceutical composition) comprising a caTCR comprising an antibody moiety, such as any one of the caTCRs described herein. In some embodiments, the composition further comprises a cell (such as an effector cell) associated with the caTCR. In some embodiments, the cancer is selected, for example, from the group consisting of adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer and thyroid cancer. In some embodiments, the viral infection is caused by a virus selected, for example, from the group consisting of Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Kaposi's Sarcoma associated herpesvirus (KSHV), Human papillomavirus (HPV), Molluscum contagiosum virus (MCV), Human T cell leukemia virus 1 (HTLV-1), HIV (Human immunodeficiency virus), and Hepatitis C Virus (HCV).

[0337] For example, in some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR (such as an isolated caTCR) comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring TCR, wherein at least one of the TCR-TMs is non-naturally occurring and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-

binding module is linked to the first and/or second TCRDs. In some embodiments, the naturally occurring TCR is an αβ TCR and the first and second TCR-TMs are derived from TCR α and β subunit transmembrane domains. In some embodiments, the naturally occurring TCR is a γδ TCR and the first and second TCR-TMs are derived from TCR γ and δ subunit transmembrane domains. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding

module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific). In some embodiments, the target antigen is a cell surface antigen. In some embodiments, the cell surface antigen is selected from the group consisting of a protein, a carbohydrate, and a lipid. In some embodiments, the cell surface antigen is a disease-associated antigen, such as a tumor-associated or virally-encoded antigen. In some embodiments, the cell surface antigen is CD19, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some embodiments, the target antigen is a surface-presented peptide/MHC complex. In some embodiments, the peptide/MHC complex comprises a peptide derived from a disease-associated antigen (such as a tumor-associated or virally-encoded antigen) and an MHC protein. In some embodiments, the peptide/MHC complex comprises a peptide and an MHC protein, wherein the peptide is derived from a protein selected from the group consisting of WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the MHC protein is an MHC class I protein. In some embodiments, the MHC class I protein is HLA-A. In some embodiments, the HLA-A is HLA-A02. In some embodiments, the HLA-A02 is HLA-A*02:01. In some embodiments, the effector cell is a γδ T cell. In some embodiments, the effector cell is an αβ T cell modified to block or decrease the expression of the TCR α and/or β chains. In some embodiments, the effector cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

[0338] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring αβ TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring αβ TCR, wherein at least one of the TCR-TMs is non-naturally occurring and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment

thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring αβ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0339] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising

administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the transmembrane domains of a naturally occurring γδ TCR and a second TCRD comprising a second TCR-TM derived from the other transmembrane domain of the naturally occurring γδ TCR, wherein at least one of the TCR-TMs is non-naturally occurring and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting

of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring γδ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0340] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs is non-naturally occurring and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that

stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0341] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the

substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0342] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically

recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 7 or 8, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 7 or 8. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from SEQ ID NO: 7 or 8. In some embodiments, the chimeric sequence in the first or second TCR-TM is from SEQ ID NO: 7 and the chimeric sequence in the other TCR-TM is from SEQ ID NO: 8. In some embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L

antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 5 and 6. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0343] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs is non-naturally occurring and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a

stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the first TCR-TM and second TCR-TM are selected according to any of the caTCRs listed in Table 2. In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0344] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, one or more (such as 2, 3, 4, 5, or more) amino acid substitutions compared to the amino acid sequence from which it is derived. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, no more than 5 amino acid substitutions compared to the amino acid sequences from which they are derived. In some embodiments, at least one of the TCR-TMs comprises a

single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, each of the TCR-TMs comprises a single amino acid substitution compared to the amino acid sequence from which it is derived. In some embodiments, at least one of the substituted amino acids in the first TCR-TM is positioned such that in the caTCR it can interact with at least one of the substituted amino acids in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the first TCR-TM and second TCR-TM are selected according to any of the

caTCRs listed in Table 2. In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0345] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM and a second TCRD comprising a second TCR-TM, wherein the first and second TCR-TMs comprise, consist essentially of, or consist of the amino acid sequences of SEQ ID NOs: 9 and 8, 7 and 14, 7 and 15, 7 and 16, 10 and 16, 7 and 17, 7 and 18, 7 and 19, 7 and 20, 7 and 21, 7 and 22, 11 and 23, 12 and 24, 7 and 25, or 13 and 26, respectively, and wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further

comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0346] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM having the amino acid sequence of SEQ ID NO: 10 and a second TCRD comprising a second TCR-TM having the amino acid sequence of SEQ ID NO: 16, wherein the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, the antibody moiety is selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv). In some embodiments, the antibody moiety specifically binds a cell surface antigen including, without limitation, CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5, including variants or mutants thereof. In some embodiments, the antibody moiety specifically binds a peptide/MHC complex, wherein the peptide is derived from a protein including, without limitation, WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA, including variants or mutants thereof. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises the amino acid sequence SEQ ID NO: 31 or 32, or a variant thereof, and/or the second connecting peptide comprises the amino acid sequence SEQ ID NO: 33 or 34, or a variant thereof. In some

embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the second TCR intracellular domain comprises the amino acid sequence of SEQ ID NO: 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0347] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR that specifically recognizes the target antigen comprising a) a first TCRD comprising a first TCR-TM derived from one of the amino acid sequences of SEQ ID NO: 7 and SEQ ID NO: 8 and a second TCRD comprising a second TCR-TM derived from the other amino acid sequence, wherein at least one of the TCR-TMs comprises a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 5 or 6, and the first and second TCRDs form a TCRM that is capable of recruiting at least one TCR-associated signaling molecule; and b) an antigen-binding module that specifically binds to the target antigen, wherein the antigen-binding module is linked to the first and/or second TCRDs. In some embodiments, each of the TCR-TMs comprises, independently from one another, a chimeric sequence comprising a portion of consecutive amino acids from SEQ ID NO: 5 or 6. In some embodiments, the first TCR-TM and/or the second TCR-TM each comprise, independently from one another, a chimeric sequence comprising a

portion of no more than about 10 (such as no more than about 9, 8, 7, 6, 5, or fewer) consecutive amino acids from SEQ ID NO: 7 or 8. In some embodiments, the chimeric sequence in the first or second TCR-TM is from SEQ ID NO: 5 and the chimeric sequence in the other TCR-TM is from SEQ ID NO: 6. In some embodiments, the chimeric sequence in the first TCR-TM is positioned such that it can interact with the chimeric sequence in the second TCR-TM. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide and the second connecting peptide each comprise, independently from one another, the amino acid sequence of any one of SEQ ID NOs: 27-34, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain and the second TCR intracellular domain each comprise, independently from one another, the amino acid sequence of SEQ ID NO: 35 or 36, or a variant thereof. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising T cell receptor transmembrane domains having the sequences of SEQ ID NOs: 7 and 8. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any of the antigen-binding module, the stabilization module, and the TCRM. In some embodiments, the antigen-binding module is an antibody moiety. In some embodiments, the antibody moiety is a Fab, a Fab', a (Fab')2, an Fv, or a single chain Fv (scFv). In some embodiments, the antigen-binding module is multispecific (*e.g.*, bispecific).

[0348] In some embodiments, there is provided a method of treating a disease associate with a first target antigen and a second target antigen (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a composition comprising effector cells (such as T cells or natural killer cells) presenting on their surface a caTCR comprising: a) a multispecific (*e.g.*, bispecific) antigen-binding module comprising a first antigen-binding domain that specifically binds to the first target antigen and a second antigen-binding domain that specifically binds to the second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule. In some embodiments, the first TCRD further comprises a first TCR connecting peptide or a fragment thereof and/or the second TCRD further comprises a second TCR connecting peptide or a fragment thereof. In some embodiments, the first connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the first TCR-TM is derived, or a variant thereof, and/or the second connecting peptide comprises all or a portion of the connecting peptide of the TCR subunit from which the second TCR-TM is derived, or a variant thereof. In some embodiments, the first and second connecting peptides are linked by a disulfide bond. In some embodiments, the first TCRD further comprises a first TCR intracellular domain and/or the second TCRD further comprises a second TCR intracellular domain. In some embodiments, the first TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the first TCR-TM is derived and/or the second TCR intracellular domain comprises a sequence from the intracellular domain of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the first TCRD is a fragment of the TCR subunit from which the first TCR-TM is derived and/or the second TCRD is a fragment of the TCR subunit from which the second TCR-TM is derived. In some embodiments, the caTCR further comprises at least one accessory intracellular domain comprising a T cell co-stimulatory signaling sequence (such as from CD27, CD28, 4-1BB (CD137), OX40, CD30, or CD40). In some embodiments, the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR. In some embodiments, the first and second stabilization domains are linked by a disulfide bond. In some embodiments, the first and second stabilization domains comprise an antibody moiety, such as C_H1 and C_L antibody domains, or variants thereof. In some embodiments, the TCRM is capable of recruiting at least one TCR-associated signaling

molecule selected from the group consisting of CD3δε, CD3γε, and ζζ. In some embodiments, the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the naturally occurring αβ T cell receptor transmembrane domains. In some embodiments, the TCRM promotes caTCR-CD3 complex formation. In some embodiments, there is a spacer module between any two caTCR modules or domains. In some embodiments, both the first TCR-TM and the second TCR-TM are naturally occurring. In some embodiments, at least one of the TCR-TMs is non-naturally occurring. In some embodiments, the first target antigen is CD19 and the second target antigen is CD22, or the first target antigen is CD22 and the second target antigen is CD19. In some embodiments, the first target antigen is CD19 and the second target antigen is CD20, or the first target antigen is CD20 and the second target antigen is CD19. In some embodiments, the disease is leukemia.

[0349] Also contemplated are methods of treating a target antigen-associated disease in an individual in need thereof comprising administering to the individual a composition comprising a plurality of effector cells expressing different caTCRs. Thus, in some embodiments, according to any of the methods for treating a target antigen-associated disease in an individual described herein, the composition is a heterogeneous caTCR effector cell composition as described herein.

[0350] For example, in some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a heterogeneous caTCR effector cell composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein all of the caTCR effector cells in the composition are of the same cell type (*e.g.*, all of the caTCR effector cells are cytotoxic T cells), wherein each population of caTCR effector cells expresses a different caTCR than the others, and wherein at least one population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, the caTCR effector cells are T cells. In some embodiments, the caTCR effector cells are selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, each population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen. In some embodiments, where at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen, each of the different target antigens is associated with the target antigen-associated disease.

[0351] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising administering to the individual an effective amount of a heterogeneous caTCR effector cell composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein at least one population of caTCR effector cells is of a different cell type than the others, and wherein at least one population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, all of the populations of caTCR effector cells are of different cell types. In some embodiments, the caTCR effector cells are T cells. In some embodiments, each population of caTCR effector cells is, independently from one another, of a cell type selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, each population of caTCR effector cells expresses the same caTCR. In some embodiments, at least one population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each population of caTCR effector cells expresses a different caTCR than the others. In some embodiments, each population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen. In some embodiments, where at least one population of caTCR effector cells expresses a caTCR that specifically binds to a different target antigen, each of the different target antigens is associated with the target antigen-associated disease.

[0352] In some embodiments, there is provided a method of treating a disease associated with a plurality of target antigens in an individual in need thereof comprising administering to the individual an effective amount of a heterogeneous caTCR effector cell composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein all of the caTCR effector cells in the composition are of the same cell type (*e.g.*, all of the caTCR effector cells are cytotoxic T cells), wherein each population of caTCR effector cells expresses a different caTCR than the others, and wherein for each target antigen of the plurality of target antigens, at least one population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, the caTCR effector cells are T cells. In some embodiments, the caTCR effector cells are selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells.

[0353] In some embodiments, there is provided a method of treating a disease associate with a plurality of target antigens in an individual in need thereof comprising administering to the

individual an effective amount of a heterogeneous caTCR effector cell composition comprising a plurality of caTCR effector cell populations according to any of the embodiments described herein, wherein at least one population of caTCR effector cells is of a different cell type than the others, and wherein for each target antigen of the plurality of target antigens, at least one population of caTCR effector cells expresses a caTCR that specifically binds to the target antigen. In some embodiments, all of the populations of caTCR effector cells are of different cell types. In some embodiments, the caTCR effector cells are T cells. In some embodiments, each population of caTCR effector cells is, independently from one another, of a cell type selected from the group consisting of cytotoxic T cells, helper T cells, natural killer T cells, and suppressor T cells. In some embodiments, each population of caTCR effector cells expresses a different caTCR than the others.

[0354] In some embodiments, the individual is a mammal (*e.g.*, human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc.). In some embodiments, the individual is a human. In some embodiments, the individual is a clinical patient, a clinical trial volunteer, an experimental animal, etc. In some embodiments, the individual is younger than about 60 years old (including for example younger than about any of 50, 40, 30, 25, 20, 15, or 10 years old). In some embodiments, the individual is older than about 60 years old (including for example older than about any of 70, 80, 90, or 100 years old). In some embodiments, the individual is diagnosed with or environmentally or genetically prone to one or more of the diseases or disorders described herein (such as cancer or viral infection). In some embodiments, the individual has one or more risk factors associated with one or more diseases or disorders described herein.

[0355] In some embodiments, the caTCR effector cell compositions of the invention are administered in combination with a second, third, or fourth agent (including, *e.g.,* an antineoplastic agent, a growth inhibitory agent, a cytotoxic agent, or a chemotherapeutic agent) to treat diseases or disorders involving target antigen expression. In some embodiments, the caTCR effector cell composition is administered in combination with a cytokine (such as IL-2). In some embodiments, the caTCR is administered in combination with an agent that increases the expression of MHC proteins and/or enhances the surface presentation of peptides by MHC proteins. In some embodiments, the agent includes, for example, IFN receptor agonists, Hsp90 inhibitors, enhancers of p53 expression, and chemotherapeutic agents. In some embodiments, the agent is an IFN receptor agonist including, for example, IFNγ, IFNβ, and IFNα. In some embodiments, the agent is an Hsp90 inhibitor including, for example, tanespimycin (17-AAG),

alvespimycin (17-DMAG), retaspimycin (IPI-504), IPI-493, CNF2024/BIIB021, MPC-3100, Debio 0932 (CUDC-305), PU-H71, Ganetespib (STA-9090), NVP-AUY922 (VER-52269), HSP990, KW-2478, AT13387, SNX-5422, DS-2248, and XL888. In some embodiments, the agent is an enhancer of p53 expression including, for example, 5-fluorouracil and nutlin-3. In some embodiments, the agent is a chemotherapeutic agent including, for example, topotecan, etoposide, cisplatin, paclitaxel, and vinblastine.

[0356] In some embodiments, there is provided a method of treating a target antigen-positive disease in an individual in need thereof comprising administering to the individual a caTCR effector cell composition according to any of the embodiments described herein in combination with a cytokine (such as IL-2). In some embodiments, the caTCR effector cell composition and the cytokine are administered simultaneously. In some embodiments, the caTCR effector cell composition and the cytokine are administered sequentially.

[0357] In some embodiments, there is provided a method of treating a target antigen-positive disease in an individual in need thereof, wherein the cells expressing the target antigen do not normally present, or present at relatively low levels, a complex comprising the target antigen and an MHC class I protein on their surface, the method comprising administering to the individual a caTCR effector cell compositions according to any of the embodiments described herein in combination with an agent that increases the expression of MHC class I proteins and/or enhances the surface presentation of target antigens by MHC class I proteins. In some embodiments, the agent includes, for example, IFN receptor agonists, Hsp90 inhibitors, enhancers of p53 expression, and chemotherapeutic agents. In some embodiments, the agent is an IFN receptor agonist including, for example, IFNγ, IFNβ, and IFNα. In some embodiments, the agent is an Hsp90 inhibitor including, for example, tanespimycin (17-AAG), alvespimycin (17-DMAG), retaspimycin (IPI-504), IPI-493, CNF2024/BIIB021, MPC-3100, Debio 0932 (CUDC-305), PU-H71, Ganetespib (STA-9090), NVP-AUY922 (VER-52269), HSP990, KW-2478, AT13387, SNX-5422, DS-2248, and XL888. In some embodiments, the agent is an enhancer of p53 expression including, for example, 5-fluorouracil and nutlin-3. In some embodiments, the agent is a chemotherapeutic agent including, for example, topotecan, etoposide, cisplatin, paclitaxel, and vinblastine. In some embodiments, the caTCR effector cell composition and the agent are administered simultaneously. In some embodiments, the caTCR effector cell composition and the agent are administered sequentially.

[0358] In some embodiments, there is provided a method of treating a target antigen-associated disease (such as cancer or viral infection) in an individual in need thereof comprising

administering to the individual an effective amount of a composition comprising nucleic acid encoding a caTCR according to any of the embodiments described herein. Methods for gene delivery are known in the art. See, *e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties.

[0359] Cancer treatments can be evaluated, for example, by tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to determining efficacy of the therapy can be employed, including for example, measurement of response through radiological imaging.

[0360] In some embodiments, the efficacy of treatment is measured as the percentage tumor growth inhibition (% TGI), calculated using the equation 100-(T/C x 100), where T is the mean relative tumor volume of the treated tumor, and C is the mean relative tumor volume of a non-treated tumor. In some embodiments, the %TGI is about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94% , about 95%, or more than 95%.

[0361] Viral infection treatments can be evaluated, for example, by viral load, duration of survival, quality of life, protein expression and/or activity.

Diseases

[0362] The caTCR effector cells in some embodiments can be useful for treating cancers associated with a target antigen. Cancers that may be treated using any of the methods described herein include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the caTCR effector cells of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0363] Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-

Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, plasmacytoma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

[0364] Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include adrenocortical carcinoma, cholangiocarcinoma, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, stomach cancer, lymphoid malignancy, pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, thyroid cancer (*e.g.*, medullary thyroid carcinoma and papillary thyroid carcinoma), pheochromocytomas sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer (*e.g.*, cervical carcinoma and pre-invasive cervical dysplasia), colorectal cancer, cancer of the anus, anal canal, or anorectum, vaginal cancer, cancer of the vulva (*e.g.*, squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, and fibrosarcoma), penile cancer, oropharyngeal cancer, esophageal cancer, head cancers (*e.g.*, squamous cell carcinoma), neck cancers (*e.g.*, squamous cell carcinoma), testicular cancer (*e.g.*, seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, Leydig cell tumor, fibroma, fibroadenoma, adenomatoid tumors, and lipoma), bladder carcinoma, kidney cancer, melanoma, cancer of the uterus (*e.g.*, endometrial carcinoma), urothelial cancers (*e.g.*, squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma, ureter cancer, and urinary bladder cancer), and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

[0365] Cancer treatments can be evaluated, for example, by tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity.

Approaches to determining efficacy of the therapy can be employed, including for example, measurement of response through radiological imaging.

[0366] The caTCR effector cells in other embodiments can be useful for treating infectious diseases by targeting pathogen-associated (such as virally-encoded) antigens. The infection to be prevented or treated, for example, may be caused by a virus, bacteria, protozoa, or parasite. The target antigen may be a pathogenic protein, polypeptide or peptide that is responsible for a disease caused by the pathogen, or is capable of inducing an immunological response in a host infected by the pathogen. Pathogenic antigens which can be targeted by caTCR effector cells include, but are not limited to, antigens derived from Acinetobacter baumannii, Anaplasma genus, Anaplasma phagocytophilum, Ancylostoma braziliense, Ancylostoma duodenale, Arcanobacterium haemolyticum, Ascaris lumbricoides, Aspergillus genus, Astroviridae, Babesia genus, Bacillus anthracis, Bacillus cereus, Bartonella henselae, BK virus, Blastocystis hominis, Blastomyces dermatitidis, Bordetella pertussis, Borrelia burgdorferi, Borrelia genus, Borrelia spp, Brucella genus, Brugia malayi, Bunyaviridae family, Burkholderia cepacia and other Burkholderia species, Burkholderia mallei, Burkholderia pseudomallei, Caliciviridae family, Campylobacter genus, Candida albicans, Candida spp, Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, CJD prion, Clonorchis sinensis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium perfringens, Clostridium spp, Clostridium tetani, Coccidioides spp, coronaviruses, Corynebacterium diphtheriae, Coxiella burnetii, Crimean-Congo hemorrhagic fever virus, Cryptococcus neoformans, Cryptosporidium genus, Cytomegalovirus (CMV), Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), Dientamoeba fragilis, Ebolavirus (EBOV), Echinococcus genus, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia genus, Entamoeba histolytica, Enterococcus genus, Enterovirus genus, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71), Epidermophyton spp, Epstein-Barr Virus (EBV), Escherichia coli O157:H7, O111 and O104:H4, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily, Flaviviruses, Francisella tularensis, Fusobacterium genus, Geotrichum candidum, Giardia intestinalis, Gnathostoma spp, GSS prion, Guanarito virus, Haemophilus ducreyi, Haemophilus influenzae, Helicobacter pylori, Henipavirus (Hendra virus Nipah virus), Hepatitis A Virus, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Histoplasma capsulatum, HIV (Human immunodeficiency virus), Hortaea werneckii, Human bocavirus (HBoV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Human parainfluenza viruses

(HPIV), Human T cell leukemia virus 1 (HTLV-1), Japanese encephalitis virus, JC virus, Junin virus, Kaposi's Sarcoma associated herpesvirus (KSHV), Kingella kingae, Klebsiella granulomatis, Kuru prion, Lassa virus, Legionella pneumophila, Leishmania genus, Leptospira genus, Listeria monocytogenes, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, Malassezia spp, Marburg virus, Measles virus, Metagonimus yokagawai, Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Mycobacterium leprae and Mycobacterium lepromatosis, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Naegleria fowleri, Necator americanus, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Nocardia spp, Onchocerca volvulus, Orientia tsutsugamushi, Orthomyxoviridae family (Influenza), Paracoccidioides brasiliensis, Paragonimus spp, Paragonimus westermani, Parvovirus B19, Pasteurella genus, Plasmodium genus, Pneumocystis jirovecii, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhinovirus, rhinoviruses, Rickettsia akari, Rickettsia genus, Rickettsia prowazekii, Rickettsia rickettsii, Rickettsia typhi, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, Salmonella genus, Sarcoptes scabiei, SARS coronavirus, Schistosoma genus, Shigella genus, Sin Nombre virus, Hantavirus, Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Strongyloides stercoralis, Taenia genus, Taenia solium, Tick-borne encephalitis virus (TBEV), Toxocara canis or Toxocara cati, Toxoplasma gondii, Treponema pallidum, Trichinella spiralis, Trichomonas vaginalis, Trichophyton spp, Trichuris trichiura, Trypanosoma brucei, Trypanosoma cruzi, Ureaplasma urealyticum, Varicella zoster virus (VZV), Varicella zoster virus (VZV), Variola major or Variola minor, vCJD prion, Venezuelan equine encephalitis virus, Vibrio cholerae, West Nile virus, Western equine encephalitis virus, Wuchereria bancrofti, Yellow fever virus, Yersinia enterocolitica, Yersinia pestis, and Yersinia pseudotuberculosis.

[0367] In some embodiments, the caTCR effector cells are used for treating oncogenic infectious diseases, such as infection by oncogenic viruses. Oncogenic viruses include, but are not limited to, CMV, EBV, HBV, KSHV, HPV, MCV, HTLV-1, HIV-1, and HCV. The target antigen of the caTCR can be a viral oncoprotein including, but not limited to, Tax, E7, E6/E7, E6, HBx, EBNA proteins (*e.g.*, EBNA3 A, EBNA3 C, and EBNA 2), v-cyclin, LANA1, LANA2, LMP-1, k-bZIP, RTA, KSHV K8, and fragments thereof. *See* Ahuja, Richa, *et al.*, *Curr. Sci.*, 2014.

Articles of Manufacture and Kits

[0368] In some embodiments of the invention, there is provided an article of manufacture containing materials useful for the treatment of a target antigen-positive disease such as cancer (for example adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer or thyroid cancer) or viral infection (for example infection by CMV, EBV, HBV, KSHV, HPV, MCV, HTLV-1, HIV-1, or HCV). The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an effector cell presenting on its surface a caTCR of the invention. The label or package insert indicates that the composition is used for treating the particular condition. The label or package insert will further comprise instructions for administering the caTCR effector cell composition to the patient. Articles of manufacture and kits comprising combinatorial therapies described herein are also contemplated.

[0369] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. In some embodiments, the package insert indicates that the composition is used for treating a target antigen-positive cancer (such as adrenocortical carcinoma, bladder cancer, leukemia, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer or thyroid cancer). In other embodiments, the package insert indicates that the composition is used for treating a target antigen-positive viral infection (for example infection by CMV, EBV, HBV, KSHV, HPV, MCV, HTLV-1, HIV-1, or HCV).

[0370] Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0371] Kits are also provided that are useful for various purposes, *e.g.*, for treatment of a target antigen-positive disease or disorder described herein, optionally in combination with the articles of manufacture. Kits of the invention include one or more containers comprising a caTCR effector cell composition (or unit dosage form and/or article of manufacture), and in some embodiments, further comprise another agent (such as the agents described herein) and/or instructions for use in accordance with any of the methods described herein. The kit may further comprise a description of selection of individuals suitable for treatment. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

[0372] For example, in some embodiments, the kit comprises a composition comprising an effector cell presenting on its surface a caTCR. In some embodiments, the kit comprises a) a composition comprising an effector cell presenting on its surface a caTCR, and b) an effective amount of at least one other agent, wherein the other agent increases the expression of MHC proteins and/or enhances the surface presentation of peptides by MHC proteins (*e.g.*, IFNγ, IFNβ, IFNα, or Hsp90 inhibitor). In some embodiments, the kit comprises a) a composition comprising an effector cell presenting on its surface a caTCR, and b) instructions for administering the caTCR effector cell composition to an individual for treatment of a target antigen-positive disease (such as cancer or viral infection). In some embodiments, the kit comprises a) a composition comprising an effector cell presenting on its surface a caTCR, b) an effective amount of at least one other agent, wherein the other agent increases the expression of MHC proteins and/or enhances the surface presentation of peptides by MHC proteins (*e.g.*, IFNγ, IFNβ, IFNα, or Hsp90 inhibitor), and c) instructions for administering the caTCR effector cell composition and the other agent(s) to an individual for treatment of a target antigen-positive disease (such as cancer or viral infection). The caTCR effector cell composition and the other agent(s) can be present in separate containers or in a single container. For example, the kit may comprise one distinct composition or two or more compositions wherein one composition comprises the caTCR effector cell and another composition comprises the other agent.

[0373] In some embodiments, the kit comprises a) a composition comprising a caTCR, and b) instructions for combining the caTCR with effector cells (such as effector cells, *e.g.*, T cells or natural killer cells, derived from an individual) to form a composition comprising the effector cells presenting on their surface the caTCR and administering the caTCR effector cell composition to the individual for treatment of a target antigen-positive disease (such as cancer or viral infection). In some embodiments, the kit comprises a) a composition comprising a caTCR, and b) an effector cell (such as a cytotoxic cell). In some embodiments, the kit comprises a) a composition comprising a caTCR, b) an effector cell (such as a cytotoxic cell), and c) instructions for combining the caTCR with the effector cell to form a composition comprising the effector cell presenting on its surface the caTCR and administering the caTCR effector cell composition to an individual for the treatment of a target antigen-positive disease (such as cancer or viral infection).

[0374] In some embodiments, the kit comprises a nucleic acid (or set of nucleic acids) encoding a caTCR. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding a caTCR, and b) a host cell (such as an effector cell) for expressing the nucleic acid (or set of nucleic acids). In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding a caTCR, and b) instructions for i) expressing the caTCR in a host cell (such as an effector cell, *e.g.*, a T cell), ii) preparing a composition comprising the host cell expressing the caTCR, and iii) administering the composition comprising the host cell expressing the caTCR to an individual for the treatment of a target antigen-positive disease (such as cancer or viral infection). In some embodiments, the host cell is derived from the individual. In some embodiments, the kit comprises a) a nucleic acid (or set of nucleic acids) encoding a caTCR, b) a host cell (such as an effector cell) for expressing the nucleic acid (or set of nucleic acids), and c) instructions for i) expressing the caTCR in the host cell, ii) preparing a composition comprising the host cell expressing the caTCR, and iii) administering the composition comprising the host cell expressing the caTCR to an individual for the treatment of a target antigen-positive disease (such as cancer or viral infection).

[0375] The kits of the invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0376] The instructions relating to the use of the caTCR effector cell compositions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. For example, kits may be provided that contain sufficient dosages of a caTCR effector cell composition as disclosed herein to provide effective treatment of an individual for an extended period, such as any of a week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the caTCR and pharmaceutical compositions and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

[0377] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

Exemplary Embodiments

[0378] Embodiment 1.A chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein at least one of the TCR-TMs is non-naturally occurring, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule, and wherein the antigen-binding module is not a naturally occurring T cell receptor antigen-binding moiety.

[0379] Embodiment 2. The caTCR of embodiment 1, wherein both of the TCR-TMs are non-naturally occurring.

[0380] Embodiment 3. The caTCR of embodiment 1 or 2, wherein the first TCR-TM is derived from one of the transmembrane domains of a first naturally occurring T cell receptor and the second TCR-TM is derived from the other transmembrane domain of the first naturally occurring T cell receptor.

[0381] Embodiment 4. The caTCR of embodiment 3, wherein the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the first naturally occurring T cell receptor transmembrane domains.

[0382] Embodiment 5. The caTCR of embodiment 3 or 4, wherein the first TCR-TM comprises up to 5 amino acid substitutions compared to the transmembrane domain from which

it is derived and/or the second TCR-TM comprises up to 5 amino acid substitutions compared to the transmembrane domain from which it is derived.

[0383] Embodiment 6. The caTCR of embodiment 5, wherein the first TCR-TM comprises a single amino acid substitution and/or the second TCR-TM comprises a single amino acid substitution.

[0384] Embodiment 7. The caTCR of embodiment 5 or 6, wherein a substituted amino acid in the first TCR-TM is proximal to a substituted amino acid in the second TCR-TM.

[0385] Embodiment 8. The caTCR of any one of embodiments 5-7, wherein one or more substituted amino acids are proximal to an amino acid in the first or second TCR-TM involved in binding to CD3.

[0386] Embodiment 9. The caTCR of any one of embodiments 5-8, wherein one or more substituted amino acids are more hydrophobic than their corresponding unsubstituted amino acid.

[0387] Embodiment 10. The caTCR of embodiment 9, wherein each of the substituted amino acids are more hydrophobic than their corresponding unsubstituted amino acid.

[0388] Embodiment 11. The caTCR of embodiment 1, wherein the first TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 7 and 9-13, and wherein the second TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26.

[0389] Embodiment 12. The caTCR of embodiment 1, wherein the first and second TCR-TMs comprise the amino acids sequence of a) SEQ ID NOs: 9 and 8, respectively; b) SEQ ID NOs: 7 and 14, respectively; c) SEQ ID NOs: 7 and 15, respectively; d) SEQ ID NOs: 7 and 16, respectively; e) SEQ ID NOs: 10 and 16, respectively; f) SEQ ID NOs: 7 and 17, respectively; g) SEQ ID NOs: 7 and 18, respectively; h) SEQ ID NOs: 7 and 19, respectively; i) SEQ ID NOs: 7 and 20, respectively; j) SEQ ID NOs: 7 and 21, respectively; k) SEQ ID NOs: 7 and 22, respectively; l) SEQ ID NOs: 11 and 23, respectively; m) SEQ ID NOs: 12 and 24, respectively; n) SEQ ID NOs: 7 and 25, respectively; o) SEQ ID NOs: 13 and 26, respectively.

[0390] Embodiment 13. The caTCR of any one of embodiments 1-12, wherein the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR.

[0391] Embodiment 14. The caTCR of embodiment 13, wherein the stabilization module is selected from the group consisting of a C_H1-C_L module, a C_H2-C_H2 module, a C_H3-C_H3 module, and a C_H4-C_H4 module.

[0392] Embodiment 15. The caTCR of embodiment 13 or 14, wherein there is a covalent linkage between the first and second stabilization domains.

[0393] Embodiment 16. The caTCR of embodiment 15, wherein the covalent linkage is a disulfide linkage.

[0394] Embodiment 17. The caTCR of any one of embodiments 13-16, wherein the stabilization module is located between the antigen-binding module and the TCRM.

[0395] Embodiment 18. The caTCR of any one of embodiments 1-17, wherein the caTCR further comprises a spacer module between the antigen-binding module and the TCRM, and wherein the spacer module comprises one or more peptide linkers connecting the antigen-binding module to the TCRM.

[0396] Embodiment 19. The caTCR of embodiment 18, wherein the peptide linker is from about 5 to about 50 amino acids in length.

[0397] Embodiment 20. The caTCR of any one of embodiments 1-19, wherein the antigen-binding module is an antibody moiety selected from the group consisting of a Fab, a Fab', a (Fab')2, an Fv, and a single chain Fv (scFv).

[0398] Embodiment 21. The caTCR of any one of embodiments 1-20, wherein the caTCR comprises two or more antigen-binding modules.

[0399] Embodiment 22. The caTCR of embodiment 21, wherein the caTCR is multispecific.

[0400] Embodiment 23. The caTCR of any one of embodiments 1-22, wherein the antigen-binding module comprises at least one scFv linked to at least one of the TCRDs.

[0401] Embodiment 24. The caTCR of any one of embodiments 1-23, wherein the antigen-binding module comprises a V_H domain linked to one of the TCRDs and a V_L domain linked to the other TCRD.

[0402] Embodiment 25. The caTCR of any one of embodiments 1-24, wherein the caTCR is a heterodimer comprising a first polypeptide chain comprising the first TCRD and a second polypeptide chain comprising the second TCRD.

[0403] Embodiment 26. The caTCR of any one of embodiments 1-25, wherein the target antigen is a cell surface antigen.

[0404] Embodiment 27. The caTCR of embodiment 26, wherein the cell surface antigen is selected from the group consisting of protein, carbohydrate, and lipid.

[0405] Embodiment 28. The caTCR of embodiment 26, wherein the cell surface antigen is selected from the group consisting of CD19, CD20, CD22, CD47, GPC-3, ROR1, ROR2, BCMA, GPRC5D, and FCRL5.

[0406] Embodiment 29. The caTCR of any one of embodiments 1-25, wherein the target antigen is a complex comprising a peptide and a major histocompatibility complex (MHC) protein.

[0407] Embodiment 30. The caTCR of embodiment 29, wherein the peptide is derived from a protein selected from the group consisting of WT-1, AFP, HPV16-E7, NY-ESO-1, PRAME, EBV-LMP2A, HIV-1, KRAS, Histone H3.3, and PSA.

[0408] Embodiment 31. The caTCR of any one of embodiments 1-30, wherein the caTCR binds to the target antigen with an equilibrium dissociation constant (Kd) from about 0.1 pM to about 500 nM.

[0409] Embodiment 32. A caTCR comprising: a) a multispecific antigen-binding module comprising a first antigen-binding domain that specifically binds to a first target antigen and a second antigen-binding domain that specifically binds to a second target antigen; and b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM; wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

[0410] Embodiment 33. The caTCR of embodiment 32, wherein the first antigen-binding domain is a Fv comprising a first heavy chain variable domain (V_H1) and a first light chain variable domain (V_L1), and the second antigen-binding domain is a Fab comprising a second V_H (V_H2) fused to a heavy chain constant domain 1 (C_H1) and a second V_L (V_L2) fused to a light chain constant domain (C_L).

[0411] Embodiment 34. The caTCR of embodiment 33, comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_H2-C_H1-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_L2-C_L-TCRD2; (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_H2-C_H1-TCRD2; (iii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_H2-C_H1-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_L2-C_L-TCRD2; or (iv) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_H2-C_H1-TCRD2, wherein L1 and L2 are peptide linkers.

[0412] Embodiment 35. The caTCR of embodiment 32, wherein the first antigen-binding domain is a first Fv comprising a V_H1 and a V_L1, and the second antigen-binding domain is a

second Fv comprising a V_H2 and a V_L2, and wherein the caTCR further comprises a C_H1 and a C_L.

[0413] Embodiment 36. The caTCR of embodiment 35, comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_H2-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L2-L2-V_L1-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_L2-C_L-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H2-L2-V_H1-C_H1-TCRD2, wherein L1 and L2 are peptide linkers.

[0414] Embodiment 37. The caTCR of embodiment 32, wherein the first antigen-binding domain is a first scFv (scFv1) and the second antigen-binding domain is a second scFv (scFv2), and wherein the caTCR further comprises a C_H1 and a C_L.

[0415] Embodiment 38. The caTCR of embodiment 37, comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L2-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L2-C_L-TCRD2; wherein L1 and L2 are peptide linkers.

[0416] Embodiment 39. The caTCR of embodiment 32, wherein the first antigen-binding domain is an scFv and the second antigen-binding domain is a Fab comprising a V_H fused to a C_H1 and a V_L fused to a C_L.

[0417] Embodiment 40. The caTCR of embodiment 39, comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-TCRD2; or (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-V_L-C_L-TCRD2; wherein L1 and L2 are peptide linkers.

[0418] Embodiment 41. The caTCR of embodiment 39, comprising: (i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1, and a third polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-TCRD2; (ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L, and a third polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-TCRD2;

(iii) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1, and a third polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-L2-TCRD2; or (iv) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-TCRD1, a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L, and a third polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-L2-TCRD2; wherein L1 and L2 are peptide linkers.

[0419] Embodiment 42. The caTCR of embodiment 34, 36, 38, 40 or 41, wherein L1 and/or L2 are about 5 to about 50 amino acids long.

[0420] Embodiment 43. The caTCR of embodiment 42, wherein L1 and/or L2 comprises the amino acid sequence of any one of SEQ ID NOs: 82-85.

[0421] Embodiment 44. The caTCR of any one of embodiments 32-43, wherein the first target antigen is a first cell surface antigen, and the second target antigen is a second cell surface antigen.

[0422] Embodiment 45. The caTCR of embodiment 44, wherein the first target antigen is CD19 and the second target antigen is CD22, or the first target antigen is CD22 and the second target antigen is CD19.

[0423] Embodiment 46. The caTCR of embodiment 44, wherein the first target antigen is CD19 and the second target antigen is CD20, or the first target antigen is CD20 and the second target antigen is CD19.

[0424] Embodiment 47. The caTCR of embodiment 45 or 46, wherein the antigen-binding domain that specifically binds to CD22 comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 65, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 69.

[0425] Embodiment 48. The caTCR of embodiment 47, wherein the antigen-binding domain that specifically binds to CD22 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 65 and a V_L comprising the amino acid sequence of SEQ ID NO: 69.

[0426] Embodiment 49. The caTCR of any one of embodiments 45-48, wherein the antigen-binding domain that specifically binds to CD19 comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 74, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 78.

[0427] Embodiment 50. The caTCR of embodiment 49, wherein the antigen-binding domain that specifically binds to CD19 comprises a V_H comprising the amino acid sequence of SEQ ID NO: 74 and a V_L comprising the amino acid sequence of SEQ ID NO: 78.

[0428] Embodiment 51. The caTCR of any one of embodiments 32-50, wherein the first TCR-TM and the second TCR-TM are both naturally occurring.

[0429] Embodiment 52. The caTCR of any one of embodiments 32-50, wherein at least one of the TCR-TMs is non-naturally occurring.

[0430] Embodiment 53. The caTCR of any one of embodiments 32-52, wherein the multispecific antigen-binding module is bispecific.

[0431] Embodiment 54. The caTCR of any one of embodiments 1-53, wherein the first TCRD further comprises a first connecting peptide or fragment thereof of a TCR subunit.

[0432] Embodiment 55. The caTCR of any one of embodiments 1-54, wherein the second TCRD further comprises a second connecting peptide or fragment thereof of a TCR subunit.

[0433] Embodiment 56. The caTCR of embodiment 55, wherein the TCRM comprises a disulfide bond between a residue in the first connecting peptide and a residue in the second connecting peptide.

[0434] Embodiment 57. The caTCR of any one of embodiments 1-56, wherein the first TCRD further comprises a first TCR intracellular domain comprising a TCR intracellular sequence.

[0435] Embodiment 58. The caTCR of any one of embodiments 1-57, wherein the second TCRD further comprises a second TCR intracellular domain comprising a TCR intracellular sequence.

[0436] Embodiment 59. The caTCR of any one of embodiments 1-58, wherein the caTCR further comprises a first accessory intracellular domain comprising a co-stimulatory intracellular signaling sequence.

[0437] Embodiment 60. The caTCR of any one of embodiments 1-59, wherein the TCR-associated signaling molecule is selected from the group consisting of CD3δε, CD3γε, and ζζ.

[0438] Embodiment 61. The caTCR of any one of embodiments 3-60, wherein the first naturally occurring T cell receptor is a γ/δ T cell receptor.

[0439] Embodiment 62. The caTCR of any one of embodiments 3-60, wherein the first naturally occurring T cell receptor is an α/β T cell receptor.

[0440] Embodiment 63. One or more nucleic acids encoding the caTCR of any one of embodiments 1-62.

[0441] Embodiment 64. The one or more nucleic acids of embodiment 63, wherein the caTCR is encoded on the same nucleic acid molecule.

[0442] Embodiment 65. One or more vectors comprising the one or more nucleic acids of embodiment 63 or 64.

[0443] Embodiment 66. The one or more vectors of embodiment 65, comprising a single vector comprising the one or more nucleic acids.

[0444] Embodiment 67. A composition comprising the one or more nucleic acids of embodiment 63 or 64 or the one or more vectors of embodiment 65 or 66.

[0445] Embodiment 68. A complex comprising the caTCR of any one of embodiments 1-62 and at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ.

[0446] Embodiment 69. The complex of embodiment 68, wherein the complex is an octamer comprising the caTCR and CD3δε, CD3γε, and ζζ.

[0447] Embodiment 70. An effector cell presenting on its surface the caTCR of any one of embodiments 1-62 or the complex of embodiment 68 or 69.

[0448] Embodiment 71. An effector cell comprising the one or more nucleic acids of embodiment 63 or 64 or the one or more vectors of embodiment 65 or 66, wherein the effector cell expresses the caTCR.

[0449] Embodiment 72. The effector cell of embodiment 70 or 71, wherein the effector cell does not express an endogenous T cell receptor subunit capable of pairing with the first and/or second TCRD.

[0450] Embodiment 73. The effector cell of embodiment 72, wherein the effector cell naturally does not express an endogenous T cell receptor subunit capable of pairing with the first and/or second TCRD.

[0451] Embodiment 74. The effector cell of embodiment 72, wherein the effector cell is modified to block or decrease the expression of a first endogenous TCR subunit and/or a second endogenous TCR subunit.

[0452] Embodiment 75. The effector cell of any one of embodiments 70-74, wherein the effector cell is a CD3+ cell.

[0453] Embodiment 76. The effector cell of embodiment 75, wherein the CD3+ cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer T cell, and a suppressor T cell.

[0454] Embodiment 77. The effector cell of any one of embodiments 70-76, wherein the caTCR is a heterodimer, comprising a) a first vector comprising a first nucleic acid sequence encoding a first polypeptide chain of the caTCR under the control of a first promoter and b) a second vector comprising a second nucleic acid sequence encoding a second polypeptide chain of the caTCR under the control of a second promoter.

[0455] Embodiment 78. The effector cell of any one of embodiments 70-76, wherein the caTCR is a heterodimer, comprising a vector comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR under the control of a first promoter; and b) a second nucleic acid sequence encoding a second polypeptide chain of the caTCR under the control of a second promoter.

[0456] Embodiment 79. The effector cell of any one of embodiments 70-76, wherein the caTCR is a heterodimer, comprising a vector comprising a) a first nucleic acid sequence encoding a first polypeptide chain of the caTCR and a second nucleic acid sequence encoding a second polypeptide chain of the caTCR, wherein the first and second nucleic acid sequences are under the control of a single promoter.

[0457] Embodiment 80. The effector cell of any one of embodiments 70-78, wherein the expression of the first polypeptide chain of the caTCR is more than two-fold different than the expression of the second polypeptide chain of the caTCR.

[0458] Embodiment 81. A method of killing a target cell presenting a target antigen, comprising contacting the target cell with the effector cell of any one of embodiments 70-80, wherein the caTCR specifically binds to the target antigen.

[0459] Embodiment 82. The method of embodiment 81, wherein the contacting is in vivo.

[0460] Embodiment 83. The method of embodiment 81, wherein the contacting is in vitro.

[0461] Embodiment 84. A pharmaceutical composition comprising: (a) the caTCR of any one of embodiments 1-62, the one or more nucleic acids of embodiment 63 or 64 or the one or more vectors of embodiment 65 or 66, or the effector cell of any one of embodiments 70-80; and (b) a pharmaceutically acceptable carrier.

[0462] Embodiment 85. A method of treating a target antigen-associated disease in an individual in need thereof comprising administering to the individual an effective amount of the composition of embodiment 84.

[0463] Embodiment 86. The method of embodiment 85, wherein the target antigen-associated disease is cancer.

[0464] Embodiment 87. The method of embodiment 86, wherein the cancer is selected from the group consisting of adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancers, esophageal cancer, glioblastoma, glioma, hepatocellular carcinoma, head and neck cancer, kidney cancer, leukemia, lung cancer, lymphoma, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer and thyroid cancer.

[0465] Embodiment 88. The method of embodiment 85, wherein the target antigen-associated disease is viral infection.

[0466] Embodiment 89. The method of embodiment 88, wherein the viral infection is caused by a virus selected from the group consisting of Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), Hepatitis B Virus (HBV), Kaposi's Sarcoma associated herpesvirus (KSHV), Human papillomavirus (HPV), Molluscum contagiosum virus (MCV), Human T cell leukemia virus 1 (HTLV-1), Human immunodeficiency virus (HIV), and Hepatitis C Virus (HCV).

[0467] Embodiment 90. A method of enriching a heterogeneous cell population for the effector cell of any one of embodiments 70-80, comprising: a) contacting the heterogeneous cell population with a ligand comprising the target antigen or one or more epitopes contained therein to form complexes of the effector cell bound to the ligand; and b) separating the complexes from the heterogeneous cell population, thereby generating a cell population enriched for the effector cell.

[0468] Embodiment 91. A nucleic acid library comprising sequences encoding a plurality of caTCRs according to any one of embodiments 1-62.

[0469] Embodiment 92. A method of screening the library of embodiment 91 for caTCRs having high affinity for a target antigen, comprising: a) introducing the nucleic acid library into a plurality of cells, such that the caTCRs are expressed on the surface of the plurality of cells; b) incubating the plurality of cells with a ligand comprising the target antigen or one or more epitopes contained therein; c) collecting cells bound to the ligand; and d) isolating sequences encoding the caTCRs from cells collected in step c), thereby identifying caTCRs specific for the target antigen.

Examples

Materials and Methods

Cell Samples, Cell Lines, and Antibodies

[0470] The cell lines HepG2 (ATCC HB-8065; HLA-A2+, AFP^+), SK-HEP-1 (ATCC HTB-52; HLA-A2+, AFP^-), Raji (ATCC CCL-86; $CD19^+$), CA46 (ATCC CRL-1648; $CD19^+$), Jurkat (ATCC CRL-2899, $CD19^-$), J.RT3-T3.5 (ATCC TIB-153), Jeko-1 (ATCC CRL-3006; $CD19^+$), K562 (ATCC CC-243, $CD19^-$), THP-1 (ATCC TIB-202, $CD19^-$), Daudi (ATCC CCL-213; $CD19^+$), HeLa (ATCC CCL-2), MDA-MB-231 (ATCC HTB-26) and MCF-7 (ATCC HTB-22) were obtained from the American Type Culture Collection. Jurkat is a human T lymphocyte cell line derived from T cell leukemia. J.RT3-T3.5 is a mutant line derived from Jurkat cells that lacks the T cell receptor β chain. Raji is a Burkitt lymphoma cell line that expresses CD19. Raji-CD19 knockout (Raji-CD19KO) line was generated by CRISPR technology. Three different guide sequences were designed to target CD19 in Raji cells. CRISPR-Cas9 vector was purchased from Origene and each guide was cloned separately into the pCas-Guide vector. Three days after electroporation, efficiency of knock-out by each guide was evaluated by flow cytometry and the best CD19-knock-out pool was chosen for clonal selection by limiting dilution. The selected clone was confirmed as a complete CD19 knock-out by sequencing. Another control cell line, SK-HEP-1-AFP-MG was generated by transducing SK-HEP-1 cell line with a minigene cassette expressing an AFP peptide AFP158, which results in a high level of cell surface expression of AFP158/HLA-A*02:01 complex. K562 is a chronic myelogenous leukemia (CML) cell line. K562-CD22 was generated by transducing K562 cell line with a vector expressing CD22. K562-CD19 was generated by transducing K562 cell line with a vector expressing CD19. K562-CD19/CD22 was generated by transducing K562 cell line with a vector expressing CD19 and a vector expressing CD22. All cell lines were cultured in RPMI 1640 or DMEM supplemented with 10% FBS and 2 mM glutamine at 37°C/5% CO_2.

[0471] Monoclonal Ab against human HLA-A02 (clone BB7.2) conjugated to FITC or APC, and its isotype control mouse IgG 2b conjugated to FITC or APC, antibodies against human or mouse CD3, human T cell receptor various subunit, 3xFlag tag, HA tag, goat F(ab)2 anti-human IgG conjugated with PE or FITC, and fluorescence-conjugated goat F(ab')2 anti-mouse Ig's (Invitrogen) were purchased. The anti-idiotypic antibody against an AFP158/HLA-A*02:01-specific antibody was developed and produced in house at Eureka Therapeutics. Flow cytometry data were collected using BD FACSCanto II and analyzed using FlowJo software package.

[0472] All peptides were purchased and synthesized by Elim Biopharma. Peptides were >90% pure. The peptides were dissolved in DMSO or diluted in saline at 10 mg/mL and frozen at -80°C. Biotinylated single chain AFP158/HLA-A*02:01 and control peptides/HLA-A*02:01 complex monomers were generated by refolding the peptides with recombinant HLA-A*02:01 and beta-2 microglobulin (β2M). The monomers were biotinylated via the BSP peptide linked to the C-terminal end of HLA-A*02:01 extracellular domain (ECD) by the BirA enzyme. Fluorescence-labelled streptavidin was mixed with biotinylated peptide/HLA-A*02:01 complex monomer to form fluorescence-labelled peptide/HLA-A*02:01 tetramer.

[0473] Lentiviruses containing human CD19-specific or AFP158/HLA-A*02:01-specific CAR or caTCRs were produced, for example, by transfection of 293T cells with vectors encoding the chimeric constructs. Primary human T-cells were used for transduction after one-day stimulation with CD3/CD28 beads (DYNABEADS®, Invitrogen) in the presence of interleukin-2 (IL-2) at 100 U/ml. Concentrated lentiviruses were applied to T-cells in Retronectin- (Takara) coated 6-well plates for 96 hours. Transduction efficiencies of the anti-AFP and anti-CD19 chimeric constructs were assessed by flow cytometry, using biotinylated AFP158/HLA-A*02:01 tetramer ("AFP158 tetramer") with PE-conjugated streptavidin or anti-myc antibody respectively. Repeat flow cytometry analyses were done on day 5 and every 3-4 days thereafter.

[0474] Cell lines were transduced with either one or two vectors that encode the two subunits of caTCR construct. Five days post-transduction, cell lysates were generated for western blot using anti-HA (Anti-HA tag antibody - ChIP Grade, Abcam) or anti-Flag antibody (Anti-Flag Antibody Produced in Rabbit, Sigma).

[0475] Tumor cytotoxicities were assayed by Cytox 96 Non-radioactive LDH Cytotoxicity Assay (Promega). $CD3^+$ T cells were prepared from PBMC-enriched whole blood using EasySep Human T Cell Isolation Kit (StemCell Technologies) which negatively depletes CD14, CD16, CD19, CD20, CD36, CD56, CD66b, CD123, glycophorin A expressing cells. Human T cells were activated and expanded with, for example, CD3/CD28 Dynabeads (Invitrogen) according to manufacturer's protocol. Activated T cells (ATC) were cultured and maintained in RPMI1640 medium with 10% FBS plus 100 U/ml IL-2, and used at day 7-14. Activated T cells (effector cells) and target cells were co-cultured at various effector-to-target ratios (*e.g.*, 2:1, 2.5:1 or 5:1) for 16 hours and assayed for cytotoxicities.

Example 1. Chimeric antibody-T cell receptor (caTCR) designs

[0476] Various chimeric antibody-T cell receptor (caTCRs) designs are contemplated, and six different examples are shown in FIG. 1 (caTCR-1, caTCR-2, caTCR-3, caTCR-4, caTCR-5, and caTCR-6). In these designs, various antibody moieties (Fab, Fab', (Fab')2, Fv, or scFv) are fused to the amino terminus of T cell receptor α/β chains or γ/δ chains lacking variable and constant domains and including all or part of their connecting peptide (region after the constant domain), their transmembrane domain, or a variant thereof, and any intracellular domain to form caTCR heterodimers which can be expressed on the surface of T cells. In a native TCR, the Vα/Vβ or Vδ/Vγ domains form the antigen-binding domain of the TCR. Our designs replace the Vα-Cα/Vβ-Cβ or Vδ-Cδ/Vγ-Cγ regions with various antibody moieties, and introduce at least one variant TCR transmembrane domain, thus conferring an antibody's binding specificity to the construct, and resulting in an enhanced ability of the construct to recruit accessory molecules in a TCR complex, such as CD3δε, CD3γε and CD3ζζ, as compared to TCRs or related constructs with only naturally occurring TCR transmembrane domains. The caTCR constructs were named as follows: caTCR-[design#]-[variant location][#]. Design# 1 corresponds to caTCR with a Fab antibody moiety, design# 2 corresponds to caTCR with a Fab' antibody moiety, design# 3 corresponds to caTCR with a (Fab')2 antibody moiety, design# 4 corresponds to caTCR with an Fv antibody moiety, design# 5 corresponds to caTCR with a single scFv antibody moiety, and design# 6 corresponds to caTCR with two scFv antibody moieties (*see* FIG. 1). No variant location and # 0 (*e.g.*, caTCR-1-0) corresponds to a construct with naturally occurring TCR domains, and # ≥ 1 corresponds to caTCR with specific variants in the variant location (*e.g.*, caTCR-1-TM1 corresponds to one transmembrane domain variant, caTCR-1-EC1 corresponds to one extracellular domain variant; *see* Table 2).

[0477] In the caTCR-1 (IgV_H-IgC_H1-TCRδ/IgV_L-IgC_L-TCRγ) design, the variable domain and the first constant domain (IgV_H-IgC_H1) of an antibody heavy chain replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The variable domain and the constant domain (IgV_L-IgC_L) of the corresponding antibody light chain replaces the amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ chain after the Vγ-Cγ region, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids.

[0478] In one embodiment of caTCR-1, one chain includes the IgV_H domain of an anti-AFP158/HLA-A*02:01 antibody (SEQ ID NO: 52) fused to an IgC_H1 domain (SEQ ID NO: 37) fused to a carboxy-terminal portion of the TCRδ chain including the transmembrane domain and all or part of the connecting peptide of the TCRδ chain, and the other chain includes the IgV_L domain of the anti-AFP158/HLA-A*02:01 antibody (SEQ ID NO: 53) fused to an IgC_L domain (SEQ ID NO: 48) fused to a carboxy-terminal portion of the TCRγ chain including the transmembrane domain and all or part of the connecting peptide of the TCRγ chain, wherein at least one of the TCR domains (such as a TCR transmembrane domain) is a non-naturally occurring variant comprising one or more amino acid substitutions. In some embodiments, the carboxy terminal portion of the TCRδ chain includes a connecting peptide having the amino acid sequence of SEQ ID NO: 31 or 32. In some embodiments, the carboxy terminal portion of the TCRδ chain includes a transmembrane domain having the amino acid sequence of any one of SEQ ID NOs: 7, and 9-13. In some embodiments, the carboxy terminal portion of the TCRγ chain includes a connecting peptide having the amino acid sequence of SEQ ID NO: 33 or 34. In some embodiments, the carboxy terminal portion of the TCRγ chain includes a transmembrane domain having the amino acid sequence of any one of SEQ ID NOs: 8, and 14-26.

[0479] In the caTCR-2 (IgV_H-IgC_H1-hinge-TCRδ/IgV_L-IgC_L-linker-TCRγ) design, the variable domain, the first constant domain, and the hinge (IgV_H-IgC_H1-hinge) of an antibody heavy chain replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The variable domain and the constant domain of the corresponding antibody light chain fused to a linker (IgV_L-IgC_L-linker) replaces the amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ chain after the Vγ-Cγ region, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids.

[0480] In the caTCR-3 (IgV_H-IgC_H1-hinge-TCRδ/IgV_H-IgC_H1-hinge-TCRγ + IgV_L-IgC_L) design, the variable domain, the first constant domain, and the hinge (IgV_H-IgC_H1-hinge) of an antibody heavy chain replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The variable domain, the first constant

domain, and the hinge (IgV_H-IgC_H1-hinge) of the antibody heavy chain replaces the amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ chain after the Vγ-Cγ region, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids. The variable domain and the constant domain of the corresponding antibody light chain (IgV_L-IgC_L) are associated with the IgV_H-IgC_H1 domains.

[0481] In the caTCR-4 (IgV_H-TCRδ/IgV_L-TCRγ) design, the variable domain (IgV_H) of an antibody heavy chain replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The variable domain (IgV_L) of the corresponding antibody light chain replaces the amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ chain after the Vγ-Cγ region, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids.

[0482] In the caTCR-5 (IgV_H-IgV_L-TCRδ/TCRγ) design, the variable domain of an antibody heavy chain fused to the variable domain of the corresponding antibody light chain (IgV_H- IgV_L or IgV_L- IgV_H) replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ chain after the Vγ-Cγ region is deleted, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids.

[0483] In the caTCR-6 (IgV_H-IgV_L-TCRδ/IgV_H-IgV_L-TCRγ) design, the variable domain of an antibody heavy chain fused to the variable domain of the corresponding antibody light chain (IgV_H- IgV_L or IgV_L- IgV_H) replaces the amino terminal portion of the TCRδ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRδ chain after the Vδ-Cδ region, optionally wherein the transmembrane domain of the TCRδ chain is modified, such as by substitution of one or more amino acids. The variable domain of an antibody heavy chain fused to the variable domain of the corresponding antibody light chain (IgV_H- IgV_L or IgV_L- IgV_H) replaces the amino terminal portion of the TCRγ chain up to a position bordering or within the connecting peptide in the extracellular domain of the TCRγ

chain after the Vγ-Cγ region, optionally wherein the transmembrane domain of the TCRγ chain is modified, such as by substitution of one or more amino acids.

Example 2: Transmembrane amino acids that were substituted to make caTCR-1-TM variants

[0484] This example describes variants of naturally occurring TCR transmembrane domains used in generating caTCR-1-TM constructs. The caTCR-1 receptor contained transmembrane domains derived from δ and γ TCR subunits. A schematic representation of the caTCR-1 receptor is shown in FIG. 2 with highlights on the transmembrane domains. Substitutions to enhance the efficacy of caTCR therapy focused on the amino acids that make up the transmembrane domains of the two chains. Alpha helical wheel projections of the transmembrane regions that make up the caTCR-1-0 receptor are shown highlighting the positions that were mutated to form the caTCR-1-TM transmembrane mutants (FIG. 3).

[0485] The naturally occurring transmembrane domain of the δ TCR subunit has the amino acid sequence of SEQ ID NO: 7, and was modified by the following substitutions: L4C; M6V; V12F and N15S; F245S; or L25S. The naturally occurring transmembrane domain of the γ TCR subunit has the amino acid sequence of SEQ ID NO: 8, and was modified by the following substitutions: Y2L, M3V, A16V, and I18V; Y2I, M3I, A16I, and I18L; L8F, V12F, and F15S; Y1Q; Y2L; M3V; M3I; L5C; V13Y; A16V; I18V; C19M; or C21G. The caTCR-1-TM variants generated are summarized in Table 2.

Example 3. Detection of caTCR cell surface expression and TCR-CD3 complex formation by flow cytometry

[0486] The 3 transmembrane mutant receptors (caTCR-1-TM3, -TM4, -TM5) along with the caTCR-1-0 were individually transduced into J.RT3-T3.5 cells. The cells transduced with caTCR-1-0 and ca TCR-1-TM mutant constructs were assessed by anti-CD3ε antibody to assess the rescue of CD3ε expression on the TCR negative J.RT3-T3.5 cells and PE-labelled AFP158/HLA-A*02:01 tetramer to measure the expression of the caTCR construct on the cell surface. Flow cytometry analysis was used to measure the MFI for CD3 and caTCR on the surface of the cells. Expression of CD3 or caTCR is represented as a percentage of the MFI measure on caTCR-1-0 transduced cells.

[0487] Results are shown in FIG. 4. Mock transductions did not confer binding to AFP158/HLA-A*02:01 tetramers and did not result in CD3ε expression on the J.RT3-T3.5 cell

surface. JRT3-T3.5 cells individually transduced to express the mutant receptors and stained with fluorescently labelled AFP158/HLA-A*02:01 and anti-CD3ε displayed distinct peaks of fluorescence intensities with MFI values above those observed with caTCR-1-0. Remarkably, the mutant receptors were not only able to rescue cell surface expression of CD3ε in the TCR negative JRT3-T3.5 cell line, but were also able to increase expression of the CD3 signaling complex above that associated with the expression of caTCR-1-0 on the cell surface.

[0488] This demonstrated that the transmembrane mutants were able to improve upon the critical requirement of caTCR to recruit endogenous CD3 complex. This may be viewed as unexpected because the constant domains of the TCR have been attributed to the interaction with the CD3 chains (reviewed by Kuhns and Davis, *Front. Immunol.* 2012; 3: 159; and Wang and Reinherz, *Immunol. Rev.* 2012, 250:102), and the role of the TCR transmembrane region to the stability of the TCR-CD3 signaling complex has been underappreciated. Furthermore, our chimeras replaced the TCR constant domains with IgG C_H1, demonstrating that the TCR constant domain is not necessary for CD3 assembly with any of the caTCR receptors assayed. This indicates that all of the exogenous caTCR chimeras tested formed functional receptors that can bind their cognate antigens, and rescued the cell surface expression of CD3 complex on J.RT3-T3.5 cells.

Example 4. Co-IP pull down of CD3ζ with caTCR-1-TM mutants

[0489] Anti-FLAG immune-precipitation was performed from cell lysates of JRT3-T3.5 cells transduced as in (FIG. 4) to pull down caTCR complexes through the FLAG tag fused to chain II of all of the caTCR receptors. Transduced JRT3-T3.5 cells were lysed and caTCR complexes were washed with non-ionic detergent and purified as in (Cohen et al, 2006 *Cancer Res.*). Expression of caTCR chain II was detected in western blots using anti-Flag antibodies (FIG. 5). Association of the FLAG-tagged caTCR with CD3 complex was visualized by Western blot with anti-CD3ζ antibody (FIG. 5). caTCR-1-TM4 and caTCR-1-TM5 had a higher affinity for the CD3 complex as can be seen by the increased amount of CD3ζ pulled down by the two receptors (FIG 5. arrows). caTCR-1-TM4 has mutations on chain II while caTCR-1-TM5 has the same mutations on chain II along with additional mutations on chain 1.

Example 5. Characterizing *in vitro* killing activity of AFP positive tumors with T cells transduced with caTCR-1 transmembrane mutants

[0490] Primary T cells were mock-transduced or transduced with caTCR-1-0 or caTCR-1 transmembrane mutants (-TM1 to -TM5, and -TM14 to -TM16) encoding an anti-AFP158/HLA-A*02:01 binding moiety (V_H and V_L domains of SEQ ID NOs: 52 and 53, respectively). The transduction efficiency was determined by staining with PE-labeled AFP158/HLA-A*02:01 tetramers. All caTCR-1 T-cells, were matched at 40% receptor positivity by mixing with mock T-cells. Two cell lines were used: SK-HEP-1 (AFP-/HLA-A2+) and SK-HEP-1-AFP-MG (SK-HEP-1 transduced with an AFP minigene) at an effector-to-target ratio of 2.5:1. Specific T-cell lysis was measured after 16 hours incubation using the Cytox 96 Non-radioactive Cytotoxicity Assay (Promega). T cells transduced with all caTCR-1-TM mutants bearing the anti-AFP158/HLA-A*02:01 binding moiety directed killing of antigen-positive cell line SK-HEP-1-AFP-MG, but did not lead to killing of antigen-negative cell line SK-HEP-1 (FIG. 6). The level of specific lysis observed in all caTCR-1-TM mutant transduced cells was comparable to that of caTCR-1-0 transduced T-cells, demonstrating that the TM mutants (TM1-TM5) retain their functional ability to signal T-cell cytotoxicity towards antigen-positive tumor cells.

Example 6. Cytokines released during *in vitro* killing of SK-HEP-1-AFP-MG tumor cell line with caTCR-1-TM mutant T-cells

[0491] Primary T cells were mock-transduced or transduced with caTCR-1-0 or caTCR-1 transmembrane mutants (-TM1 to -TM5) encoding an anti-AFP158/HLA-A*02:01 binding moiety (V_H and V_L domains of SEQ ID NOs: 52 and 53, respectively). The transduction efficiency was determined by staining with PE-labeled AFP158/HLA-A*02:01 tetramers. All caTCR-1 T cells were matched at 40% receptor positivity by mixing with mock T-cells. SK-HEP-1-AFP-MG (SK-HEP-1 transduced with an AFP minigene) was used at an effector-to-target ratio of 2.5:1. Cytokine Release of IL-2, IL-10, GM-CSF, IFN-γ and TNF-α into the media after 16 hours SK-HEP1-AFP-MG *in vitro* killing assay (FIG. 6) was measured using the Magpix multiplex system (Luminex) with the Bio-plex Pro Human Cytokine 8-plex Assay (BioRad). Assay supernatants from SK-HEP-1-AFP-MG target reactions were diluted 10-fold. Cytokine concentrations were determined with the standard curve supplied with the BioRad Bio-plex kit (FIG. 7). Slight increases in cytokine release with the transmembrane mutants suggest that specific transmembrane mutants can increase antigen induced T-cell signaling strength while maintaining regulation mechanisms that prevent toxic levels of cytokine release.

Example 7. Characterizing *in vitro* killing activity of AFP positive tumors with T cells transduced with caTCR-1 transmembrane mutants

[0492] Primary T cells were mock-transduced or transduced with caTCR-1-0 or caTCR-1 transmembrane mutants (-TM5 to -TM13) encoding an anti-AFP158/HLA-A*02:01 binding moiety (V_H and V_L domains of SEQ ID NOs: 52 and 53, respectively). The transduction efficiency was determined by staining with PE-labeled AFP158/HLA-A*02:01 tetramers. All caTCR-1 T-cells were matched at 40% receptor positivity by mixing with mock T-cells. Three cell lines were used: SK-HEP-1 (AFP-/HLA-A2+), SK-HEP-1-AFP-MG (SK-HEP-1 transduced with an AFP minigene), and the endogenously AFP expressing HepG2 (AFP+/HLA-A2+) at an effector-to-target ratio of 2.5:1. Specific T-cell lysis was measured after 16 hours incubation using the Cytox 96 Non-radioactive Cytotoxicity Assay (Promega). T cells transduced with all caTCR-1-TM mutants bearing the anti-AFP158/HLA-A*02:01 binding moiety directed killing of antigen-positive cell lines HepG2 and SK-HEP-1-AFP-MG, but did not lead to killing of antigen-negative cell line SK-HEP-1 (FIG. 8). Specific lysis of both AFP-positive tumor cells, SK-HEP1-AFP-MG and HepG2, was comparable between all caTCR-1-TM mutant transduced T-cells, demonstrating that the TM mutants (-TM5 to -TM13) retained their functional ability to signal T-cell cytotoxicity towards antigen positive tumor cells.

Example 8. Cytokines released during *in vitro* killing of HepG2 tumor cell line with caTCR-1-TM mutant T-cells

[0493] Primary T cells were mock-transduced or transduced with caTCR-1-0 or caTCR-1 transmembrane mutants (-TM5 to -TM13) encoding an anti-AFP158/HLA-A*02:01 binding moiety (V_H and V_L domains of SEQ ID NOs: 52 and 53, respectively). The transduction efficiency was determined by staining with PE-labeled AFP158/HLA-A*02:01 tetramers. All caTCR-1 T-cells were matched at 40% receptor positivity by mixing with mock T-cells. HepG2 cells were used at an effector-to-target ratio of 2.5:1. Cytokine Release of GM-CSF, IFN-γ and TNF-α into the media after 16 hours HepG2 *in vitro* killing assay (FIG. 8) was measured using the Magpix multiplex system (Luminex) with the Bio-plex Pro Human Cytokine 8-plex Assay (BioRad). Assay supernatants from HepG2 target reactions were diluted 4-fold. Cytokine concentrations were determined with the standard curve supplied with the BioRad Bio-plex kit (FIG. 9). The notable increase in cytokine release with caTCR-1-TM5 when killing HepG2

demonstrates that the transmembrane mutations within the caTCR-1-TM5 receptor confer an increased cytotoxic signaling potential.

Example 9. *in vitro* killing of CD19-positive NALM6 tumors with T cells transduced with caTCR-1-TM5

[0494] Primary T cells were transduced with caTCR-1-0 or caTCR-1-TM5 encoding an anti-CD19 binding moiety (V_H and V_L domains of SEQ ID NOs: 54 and 55, respectively). The transduction efficiency was determined and T-cells were matched at 40% receptor positivity by mixing with mock T-cells. NALM6 target cells were labeled with CFSE for 5 min prior to being incubated with appropriate T-cells for 4 hours at effector-to-target ratios of 1:1, 1:2, or 1:4. The number of CFSE positive cells was counted by FACS analysis and the percent killing was determined by dividing by the number of CFSE-positive cells in wells with no T-cells (FIG. 10). The *in vitro* killing efficiency of CD19-positive NALM6 tumors remained comparable between the parental caTCR-1-0 and the mutant caTCR-1-TM5 transduced T-cells, demonstrating that the caTCR-1-TM5 mutant retains its cytotoxic signaling function in an anti-CD19 model.

Example 10. Cytokines released during *in vitro* killing of NALM6 tumor cell line with caTCR-1-TM5 T-cells

[0495] Primary T cells were transduced with caTCR-1-0 or caTCR-1-TM5 encoding an anti-CD19 binding moiety (V_H and V_L domains of SEQ ID NOs: 54 and 55, respectively). The transduction efficiency was determined and T-cells were matched at 40% receptor positivity by mixing with mock T-cells. NALM6 was used at an effector-to-target ratio of 2.5:1. Cytokine Release of IL-2, GM-CSF, IFN-γ and TNF-α into the media after 16 hours NALM6 *in vitro* killing assay (FIG. 10) was measured using the Magpix multiplex system (Luminex) with the Bio-plex Pro Human Cytokine 8-plex Assay (BioRad). Assay supernatants from NALM6 target reactions were diluted 4-fold. Cytokine concentrations were determined with the standard curve supplied with the BioRad Bio-plex kit (FIG. 11). The increase in cytokine release with caTCR-1-TM5 when killing NALM6 demonstrates that the caTCR-1-TM5 mutant receptor retains its increased cytotoxic signaling potential in an anti-CD19 model.

Example 11. Intracellular cytokine production in response to CD19 positive target cells

[0496] To more directly determine the result of caTCR-1 signaling on cytokine expression we measured intracellular cytokine production in response to stimulation with antigen-positive

tumor cells. Primary T cells were transduced with caTCR-1-0 or caTCR-1-TM5 encoding an anti-CD19 binding moiety (V_H and V_L domains of SEQ ID NOs: 54 and 55, respectively). The transduction efficiency was determined and T-cells were matched at 40% receptor positivity by mixing with mock T-cells. Mock-transduced T cells or T-cells transduced with caTCR-1-0 or caTCR-1-TM5 were co-cultured with NALM6 or Raji target cells at a ratio of 2.5:1 with a protein transport inhibitor cocktail (eBioscience Cat#00498003) to prevent cytokine secretion. After 4 hours of treatment, T cells were stained for caTCR-1 receptor and anti-CD4 antibody along with anti-TNF-α, anti-IFN-γ, or anti-IL-2 antibodies. Using flow cytometry, gating on caTCR-1 receptor positive cells, we calculated the percent of cells positive for intracellular TNF-α, IFN-γ, and IL-2 (Table 4). Stimulation through the caTCR-1-TM5 mutant receptor led to an increase in cells with intracellular cytokine expression, further demonstrating the ability of the transmembrane mutations in caTCR-1-TM5 to confer increased T-cell cytotoxic signaling potential.

Table 4

	Raji	NALM6	T cell alone
	Intracellular TNFα Expression in CD8+ T-cells (% positive)		
Mock	0.5055	1.09	0.94
-0	11.5	12.7	0.73
-TM5	15.3	17.85	0.91
	Intracellular IL-2 Expression in CD4+ T-cells (% positive)		
Mock	2.935	0.21	0.28
-0	5.51	4.74	0.33
-TM5	8.555	7.46	0.22
	Intracellular IFNγ Expression in CD8+ T-cells (% positive)		
Mock	0.545	0.21	0.39
-0	6.2	6.8	1.01
-TM5	5.96	7.32	1.08

Example 12. Proliferation and Persistence after stimulation through the caTCR-1 receptors

[0497] Primary T cells were transduced with caTCR-1-0 or caTCR-1-TM5 encoding an anti-CD19 binding moiety (V_H and V_L domains of SEQ ID NOs: 54 and 55, respectively). The transduction efficiency was determined and T-cells were matched at 40% receptor positivity by mixing with mock T-cells. T-cells were labeled with CFSE and stimulated with Raji or NALM6 target cells. 5×10^4 NALM6 cells were used at an effector-to-target ratio of 2:1. CFSE

fluorescence was measured with FACS analysis (FIGS. 12A and 12B). CFSE fluorescent intensity decreases with each cell division and thus can be used to assay the proliferation potential of the T-cell in response to antigen stimulation. To observe the persistence of T-cells to survive after stimulation we counted the total number of viable T-cells that remained after 3 days post target cell exposure (Table 5). Stimulation through the caTCR-1-TM5 receptor showed an increase in both proliferation potential as seen with the leftward shift in CFSE peaks on day 3 and persistence from total number of viable T-cells. These are important attributes as both persistence and proliferation of T-cells during adoptive T-cell therapy are associated with better clinical outcomes.

Table 5

	# of T cells persisting after engagement			
	BV173		NALM6	
	-0	-TM5	-0	-TM5
	Protocol A			
Day 3	10354	17726	6591	8067
Day 5	2945	3590	1240	3190
Day 7	253	460	329	534
	Protocol B			
Day 3	16057	39189	18457	32564
Day 5	2945	31236	3190	12712
Day 7	253	7323	534	3021

Example 13. Construction and characterization of bispecific anti-CD19 anti-CD22 caTCRs

[0498] Therapies with chimeric antigen receptors targeting a single target antigen may be vulnerable to antigen escape due to loss of the target antigen gene, alternative splicing and epitope loss. Bispecific caTCRs were designed to provide therapies with improved efficacy by reducing the potential for antigen escape.

[0499] Bispecific caTCRs with one antigen-binding domain derived from an anti-CD19 antibody and one antigen-binding domain derived from an anti-CD22 antibody were constructed (referred herein as "anti-CD19 anti-CD22 caTCRs" or abbreviated as "CD19 CD22 caTCRs"). The anti-CD19 antibody has previously been described in WO2017066136A2. The anti-CD22 antibody is known as "anti-CD22 clone 2," described in USSN 62/650,955 filed March 30, 2018. Each anti-CD19 anti-CD22 caTCR comprises two polypeptides, one containing a TCR delta domain and one containing a TCR gamma domain. Different structural formats and linkers (different lengths and sequences) were used in the constructs. In anti-CD19 anti-CD22 caTCR-9-2, mutations were introduced to the C_H1 (S64E and S66V mutations) and C_L (S69L and T71S mutations) domains to stabilize the dimerization. Other anti-CD19 anti-CD22 caTCR constructs

can be generated by changing the linkers, stabilization domains and/or transmembrane domains (*e.g.*, using mutant transmembrane domains), and/or by swapping the antigen-binding domains.

[0500] Table 6 below shows the structural features from the N-terminus to the C-terminus of each polypeptide in each anti-CD19 anti-CD22 caTCR construct. Lentiviral vectors encoding each pair of polypeptides of the anti-CD19 anti-CD22 caTCR construct were prepared.

Table 6.

Construct	Polypeptide chain 1	Polypeptide chain 2
caTCR-7-1a	anti-CD22 V_H-linker 1-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:88)	anti-CD22 V_L-linker 1-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:89)
caTCR-7-2a	anti-CD22 V_L-linker 1-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:90)	anti-CD22 V_H-linker 1-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:91)
caTCR-7-3a	anti-CD19 V_H-linker 1-anti-CD22 V_H-C_H1-TCRδ (SEQ ID NO:92)	anti-CD19 V_L-linker 1-anti-CD22 V_L-C_L-TCRγ (SEQ ID NO:93)
caTCR-7-4	anti-CD19 V_H-linker 1-anti-CD22 V_L-C_H1-TCRδ (SEQ ID NO:94)	anti-CD19 V_L-linker 1-anti-CD22 V_H-C_L-TCRγ (SEQ ID NO:95)
caTCR-7-1b	anti-CD22V_H-linker 2-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:96)	anti-CD22 V_L-linker 2-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:97)
caTCR-7-2b	anti-CD22 V_L-linker 2-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:98)	anti-CD22 V_H-linker 2-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:99)
caTCR-7-1c	anti-CD22 V_H-linker 3-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:100)	anti-CD22 V_L-linker 3-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:101)
caTCR-7-2b	anti-CD22 V_L-linker 3-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:102)	anti-CD22 V_H-linker 3-anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:103)
caTCR-8-1	anti-CD22 scFv-linker 1-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:104)	anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:105)
caTCR-8-2	anti-CD22 scFv-linker 2-anti-CD19 V_H-C_H1-TCRδ (SEQ ID NO:106)	anti-CD19 V_L-C_L-TCRγ (SEQ ID NO:107)
caTCR-9-1	anti-CD22 scFv-linker 3-C_H1-TCRδ (SEQ ID NO:108)	anti-CD19 scFv-linker 3-C_L-TCRγ (SEQ ID NO:109)
caTCR-9-2	anti-CD22 scFv-linker 3-C_H1 (S64E/S66V)-TCRδ (SEQ ID NO:110)	anti-CD19 scFv-linker 3-C_L (S69L/T71S)-TCRγ (SEQ ID NO:111)
caTCR-9-3	anti-CD22 scFv-linker 4-C_H1-TCRδ (SEQ ID NO:112)	anti-CD19 scFv-linker 4-C_L (S69LT71S)-TCRγ (SEQ ID NO:113)

Cytokine secretion

[0501] Primary T cells were mock-transduced or transduced with a lentiviral vector encoding the anti-CD19 anti-CD22 caTCR-7-2a construct. The transduced T cells were mixed with K562 (CD19 CD22 double negative), K562-CD22 (K562 transduced with CD22), K562-CD19 (K562 transduced with CD19), Raji (which expresses both CD19 and CD22), or Raji CD19 K/O (Raji with CD19 knocked out) cells at an effector-to-target ratio of 2.5:1. T-cells secrete IFN-γ when activated by TCR engagement with target ligand. The cytokine release levels of IFN-γ were

measured and used as an indicator of the receptor's signaling potential (the higher levels, the higher the receptor's signaling potential and cytotoxic T cell activity). Cytokine concentrations were measured after 16 hours of co-culture using the Magpix multiplex system (Luminex) with the Bio-plex Pro Human Cytokine 8-plex Assay (BioRad).

[0502] As shown in FIG. 14, antigen-specific IFN-γ release was observed across a panel of $CD19^+$ and/or $CD22^+$ cell lines. T cells expressing the anti-CD19 anti-CD22 caTCR-7-2a construct specifically activated IFN-γ secretion when incubated with K562 expressing either CD19 or CD22 antigens. The caTCR-7-2a T cells also released IFN-γ in a model of antigen escape in which the CD19 gene was knocked out of the $CD19^+CD22^+$ Raji lymphoma cell line (Raji CD19 K/O), thus demonstrating the ability of the single caTCR-7 to bind two independent antigens.

In vitro killing

[0503] The *in vitro* cell killing activity of the various anti-CD19 anti-CD22 caTCR-7 constructs was assessed by an LDH cytotoxicity assay as described in Methods. Briefly, human primary T cells from a donor (R177) were mock-transduced, or transduced with a lentivirus encoding anti-CD19 caTCR-1, or an anti-CD19 anti-CD22 caTCR construct (caTCR-7-1b, caTCR-7-2b, caTCR-7-1c or caTCR-7-2c). The transduced T cells were mixed with K562, K562-CD22 (K562 expressing CD22), K562-CD19 (K562 expressing CD19), or K562-CD19/CD22 (K562 cells expressing CD19 and CD22 antigens) cells at an effector-to-target ratio of 2:1. Specific T-cell lysis was measured after 16hr incubation using the Cytox 96 Non-radioactive Cytotoxicity Assay (Promega).

[0504] As shown in FIG. 15, T cells expressing the various bispecific anti-CD19 anti-CD22 caTCRs were capable of lysing K562 target cells expressing CD19, CD22, or both CD19 and CD22. However, T cells expressing the monospecific CD19 caTCR-1 was only capable of lysing K562 target cells expressing CD19.

Sequence Listing

SEQ ID NO	Description	Sequence
1	TCRα constant domain	PNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRSMDFKSNS AVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFRILLLK VAGFNLLMTLRLWSS
2	TCRβ constant domain	EDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWWVNGKEVHSGVSTDPQP LKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAE AWGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDF
3	TCRδ constant domain	SQPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDPAIVISPSGKYNAVKLGKYE DSNSVTCSVQHDNKTVHSTDFEVKTDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMM SLTVLGLRMLFAKTVAVNFLLTAKLFFL
4	TCRγ constant domain	DKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWQEKKSNTILGSQEGNTMK TNDTYMKFSWLTVPEKSLDKEHRCIVRHENNKNGVDQEIIFPPIKTDVITMDPKDNCSKDAN DTLLLQLTNTSAYYMYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
5	TCRα transmembrane domain	ILLLKVAGFNLLMTLRLWSS
6	TCRβ transmembrane domain	TILYEILLGKATLYAVLVSALVL
7	TCRδ transmembrane domain	VLGLRMLFAKTVAVNFLLTAKLFFL
8	TCRγ transmembrane domain (same as uniprot)	YYMYLLLLLKSVVYFAIITCCLL
9	TCRδ transmembrane domain F24S	VLGLRMLFAKTVAVNFLLTAKLF**S**L
10	TCRδ transmembrane domain M6V	VLGLR**V**LFAKTVAVNFLLTAKLFFL
11	TCRδ transmembrane domain L4C	VLGCRMLFAKTVAVNFLLTAKLFFL
12	TCRδ transmembrane domain V12F, N15S	VLGLRMLFAKTFAVSFLLTAKLFFL
13	TCRδ transmembrane domain L25S	VLGLRMLFAKTVAVNFLLTAKLFF**S**
14	TCRγ transmembrane domain V13Y	YYMYLLLLLKSV**Y**YFAIITCCLLRRTAF
15	TCRγ	YYMYLLLLLKSVVYFAIITC**G**LLRRTAF

	transmembrane domain C21G	
16	TCRγ transmembrane domain Y2L, M3V, A16V, I18V	Y**LV**YLLLLLKSVVYF**VI****V**TCCLLRRTAF
17	TCRγ transmembrane domain Y2L	YLMYLLLLLKSVVYFAIITCCLLRRTAF
18	TCRγ transmembrane domain M3V	YYVYLLLLLKSVVYFAIITCCLLRRTAF
19	TCRγ transmembrane domain A16V	YYMYLLLLLKSVVYFVIITCCLLRRTAF
20	TCRγ transmembrane domain I18V	YYMYLLLLLKSVVYFAIVTCCLLRRTAF
21	TCRγ transmembrane domain M3I	YYIYLLLLLKSVVYFAIITCCLLRRTAF
22	TCRγ transmembrane domain Y2I, M3I, A16I, I18L	YIIYLLLLLKSVVYFIILTCCLLRRTAF
23	TCRγ transmembrane domain L5C	YYMYCLLLLKSVVYFAIITCCLLRRTAF
24	TCRγ transmembrane domain L8F, V12F, F15S	YYMYLLLFLKSFVYSAIITCCLLRRTAF
25	TCRγ transmembrane domain C19M	YYMYLLLLLKSVVYFAIIT**M**CLLRRTAF
26	TCRγ transmembrane domain Y1Q	**Q**YMYLLLLLKSVVYFAIITCCLLRRTAF
27	TCRα connecting peptide	ESSCDVKLVEKSFETDTNLNFQNLSVIGFR
28	TCRα connecting peptide MD	IPEDTFFPSPESSCDVKLVEKSFETDTNLNFQNLSVIGFR
29	TCRβ connecting peptide	ADCGFTSVSYQQGVLSA
30	TCRβ	GRADCGFTSVSYQQGVLSA

	connecting peptide MD	
31	TCRδ connecting peptide	DHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLR
32	TCRδ connecting peptide MD	EVKTDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLR
33	TCRγ connecting peptide	MDPKDNCSKDANDTLLLQLTNTSA
34	TCRγ connecting peptide MD	PIKTDVITMDPKDNCSKDANDTLLLQLTNTSA
35	TCRβ intracellular domain	MAMVKRKDF
36	TCRγ intracellular domain	RRTAFCCNGEKS
37	IgG1 C_H1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
38	IgG2-0C C_H1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERK
39	IgG2-1C C_H1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKC
40	IgG2-2C C_H1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCC
41	IgG3 C_H1	ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTP
42	IgG4 C_H1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYG
43	IgA1 C_H1	ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQDASGDL YTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCPVPSTPPTPSPSTPPTPSPS
44	IgA2 C_H1	ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVTARNFPPSQDASGDL YTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPCPVPPPPP
45	IgD C_H1	APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTQSQPQRTFPEIQRRDSY YMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVPTAQPQAEGSLAK ATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTP
46	IgE C_H1	ASTQSPSVFPLTRCCKNIPSNATSVTLGCLATGYFPEPVMVTWDTGSLNGTTMTLPATTLTLS GHYATISLLTVSGAWAKQMFTCRVAHTPSSTDWVDNKTFS
47	IgM C_H1	GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPSVLRGGK YAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLP
48	IgCL domain	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQS NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
49	CD28 co-stimulatory fragment 2	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
50	4-1BB co-stimulatory	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

	fragment 2	
51	OX40 co-stimulatory fragment 2	ALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI
52	IgV_H domain of anti-AFP158/HLA-A*02:01 antibody	EVQLVQSGAEVKKPGESLTISCKASGYSFPNYWITWVRQMSGGGLEWMGRIDPGDSYTTYN PSFQGHVTISIDKSTNTAYLHWNSLKASDTAMYYCARYYVSLVDIWGQGTLVTVSS
53	IgV_L domain of anti-AFP158/HLA-A*02:01 antibody	QSVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVNNRPSEVSN RFSGSKSGNTASLTISGLQAEDEADYYCSSYTTGSRAVFGGGTKLTVL
54	IgV_H domain of anti-CD19 antibody	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWG QGTLVTVSS
55	IgV_L domain of anti-CD19 antibody	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLG
56	IgV_H domain of anti-CD20 antibody	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSY NQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVS S
57	IgV_L domain of anti-CD20 antibody	QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGS GSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKR
58	IgV_H domain of anti-GPC3 antibody	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFS ASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLG
59	IgV_L domain of anti-GPC3 antibody	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSGGSSTYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVTVSS
60	IgV_H domain of anti-CD47	QVQLQESGPGLVKPSQTLSLTCTVSGYTFTNYYVFWVRQARGQRLEWIGDINPVNGDTNFNE KFKNRVTISADKSISTAYLQWSSLKASDTAMYYCARGGYTMDYWGQGTLVTVSS
61	IgV_L domain of anti-CD47	DIVMTQTPLSLPVTPGEPASISCRSSQSLVHSNGNTYLHWYQQKPGKAPKLLIYKVSYRFSGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHVPRTFGQGTKVEIKR
62	anti-CD22 HC-CDR1	GFTFSNYA
63	anti-CD22 HC-CDR2	ISGSGGST
64	anti-CD22 HC-CDR3	ARYGSAAWMDS
65	IgV_H domain of anti-CD22 antibody	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS
66	anti-CD22 LC-CDR1	HDIRNY
67	anti-CD22 LC-CDR2	AAS
68	anti-CD22 LC-	QQYDGLPLT

	CDR3	
69	IgV_L domain of anti-CD22 antibody	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKR
70	anti-CD22 scFv	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS
71	anti-CD19 HC-CDR1	GYSFTSY
72	anti-CD19 HC-CDR2	IYPGDSDT
73	anti-CD19 HC-CDR3	ARQVWGWQGGMYPRSNWWYNLDS
74	IgV_H domain of anti-CD19 antibody	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTIADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWGQ GTLVTVSS
75	anti-CD19 LC-CDR1	NIGSKS
76	anti-CD19 LC-CDR2	DDS
77	anti-CD19 LC-CDR3	QVWDSSSDYVV
78	IgV_L domain of anti-CD19 antibody	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVL
79	anti-CD19 scFv	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGSRGGGGSGGGGSGGG GSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSS
80	TCR delta domain	EVKTDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLL TAKLFFL
81	TCR gamma domain	PIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAIITCCLLRRTAFCCN GEKS
82	Linker 1	GGGGS
83	Linker 2	GGGGSGGGGS
84	Linker 3	GGGGSGGGGSGGGGS
85	Linker 4	TPLGDTTH
86	C_H1, S64E S66V mutant	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY ELVSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC
87	Kappa C_L, S69L T71S mutant	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
88	anti-CD19 anti-CD22 caTCR-7-1a chain 1	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRY SPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDS WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH

		TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVK PKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
89	anti-CD19 anti-CD22 caTCR-7-1a chain 2	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSLPVLTQPPSVSVAP GKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTISRV EAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPSSEELQANKATLVCLIS DFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHE GSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAI ITCCLLRRTAFCCNGEKS
90	anti-CD19 anti-CD22 caTCR-7-2a chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSEVQLVQSGAEVKK PGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTISADKSI STAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVKPKETENTKQPSKSCH KPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
91	anti-CD19 anti-CD22 caTCR-7-2a chain 2	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSLPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERF SGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPS SEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPE QWKSHRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAY YMYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
92	anti-CD19 anti-CD22 caTCR-7-3 chain 1	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWG QGTLVTVSSGGGGSQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLE WVSSISGSGGSTYYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDS WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVK PKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
93	anti-CD19 anti-CD22 caTCR-7-3 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGGGGGSDIQLTQSPSSL STSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFSGRGSGTDFTLT ISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG LSSPVTKSFNRGECPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAI ITCCLLRRTAFCCNGEKS
94	anti-CD19 anti-CD22 caTCR-7-4 chain 1	EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSP SFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWG QGTLVTVSSGGGGSDIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIY AASNLQTGVPSRFSGRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECPIKTDVITMDPKDNCSKDANDTLL LQLTNTSAYYMYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
95	anti-CD19 anti-CD22 caTCR-7-4 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGGGGGSQVQLVESGGG LVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYADSVKGRFTISR DTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT QTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKV

		NMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
96	anti-CD19 anti-CD22 caTCR-7-1b chain 1	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSGGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPG DSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWW YNLDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDS TDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFF L
97	anti-CD19 anti-CD22 caTCR-7-1b chain 2	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSGGGGSLPVLTQPPS VSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTAT LTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPSSEELQANKAT LVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSC QVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKS VVYFAIITCCLLRRTAFCCNGEKS
98	anti-CD19 anti-CD22 caTCR-7-2b chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSGGGGSEVQLVQSG AEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQGQVTIS ADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWGQGTLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVKPKETENTKQPS KSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
99	anti-CD19 anti-CD22 caTCR-7-2b chain 2	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSGGGGSLPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPS GIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTV TLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSY LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLT NTSAYYMYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
100	anti-CD19 anti-CD22 caTCR-7-1c chain 1	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSGGGGSGGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWM GIIYPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYP RSNWWYNLDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC EVKTDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLL TAKLFFL
101	anti-CD19 anti-CD22 caTCR-7-1c chain 2	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSGGGGSGGGGSLPV LTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNS GNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPSSEELQ ANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKS HRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYL LLLLKSVVYFAIITCCLLRRTAFCCNGEKS
102	anti-CD19 anti-CD22 caTCR-7-2c chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRGGGGSGGGGSGGGGSEVQ LVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDSDTRYSPSFQ GQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWYNLDSWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL

		QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVKPKETEN TKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
103	anti-CD19 anti-CD22 caTCR-7-2c chain 2	QVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGSTYYA DSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSSGGG GSGGGGSGGGGSLPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYD DSDRPSGIPERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQP KANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDAND TLLLQLTNTSAYYMYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
104	anti-CD19 anti-CD22 caTCR-8-1 chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS GGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDST DHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
105	anti-CD19 anti-CD22 caTCR-8-1 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPSSE ELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYY MYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
106	anti-CD19 anti-CD22 caTCR-8-2 chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS GGGGSGGGGSEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGII YPGDSDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSN WWYNLDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVK TDSTDHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAK LFFL
107	anti-CD19 anti-CD22 caTCR-8-2 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGQPKANPTVTLFPPSSE ELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQ WKSHRSYSCQVTHEGSTVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYY MYLLLLLKSVVYFAIITCCLLRRTAFCCNGEKS
108	anti-CD19 anti-CD22 caTCR-9-1 chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS GGGGSGGGGSGGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDST DHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
109	anti-CD19 anti-CD22 caTCR-9-1 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGSRGGGGSGGGGSGGG GSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSSGGGGSGGGGSGGGGSQPKANPTVTLFPPSSEELQANKATLVCLISDF

		YPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGS TVEKTVAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAIIT CCLLRRTAFCCNGEKS
110	anti-CD19 anti-CD22 caTCR-9-2 chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS GGGGSGGGGSGGGGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYELVSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDST DHVKPKETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
111	anti-CD19 anti-CD22 caTCR-9-2 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGSRGGGGSGGGGSGGG GSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSSGGGGSGGGGSGGGGSTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLLSSLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGECPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAII TCCLLRRTAFCCNGEKS
112	anti-CD19 anti-CD22 caTCR-9-3 chain 1	DIQLTQSPSSLSTSVGDRVTITCQASHDIRNYLNWYQQKPGKAPNLLIYAASNLQTGVPSRFS GRGSGTDFTLTISSLQPEDIATYYCQQYDGLPLTFGQGTRLEIKRSRGGGGSGGGGSGGGGSL EMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGGST YYADSVKGRFTISRDTSKNTLYLQMNSLRAEDTAVYYCARYGSAAWMDSWGQGTLVTVSS TPLGDTTHTSGASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCEVKTDSTDHVKP KETENTKQPSKSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL
113	anti-CD19 anti-CD22 caTCR-9-3 chain 2	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGSRGGGGSGGGGSGGG GSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSSTPLGDTTHTSGQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAV TVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKT VAPTECSPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYYMYLLLLLKSVVYFAIITCCLLR RTAFCCNGEKS
114	6NFAT response element	GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCG TGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGC GTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGG CGT
115	TA promoter	GCCGCCCCGACTGCATCTGCGTGTTCCAATTCGCCAATGACAAGACGCTGGGCGGGGTTT GTGTCATCATAGAACTAAAGACATGCAAATATATTTCTTCCGGGGACACCGCCAGCAAAC GCGAGCAACGGGCCACGGGGATGAAGCAG
116	NFAT-derived promoter	GGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGCG TGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGGC GTGGAGGAAAAACTGTTTCATACAGAAGGCGTGGAGGAAAAACTGTTTCATACAGAAGG CGTCTCGAGGCCGCCCCGACTGCATCTGCGTGTTCCAATTCGCCAATGACAAGACGCTGG GCGGGGTTTGTGTCATCATAGAACTAAAGACATGCAAATATATTTCTTCCGGGGACACCG CCAGCAAACGCGAGCAACGGGCCACGGGGATGAAGCAG
117	anti-GPC3 #37 scFv	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFS ASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRGGGGSGGGGSGG GGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSG GSSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVT VSS
118	CD28 co-stimulatory	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIF WVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

	fragment 1	
119	4-1BB co-stimulatory fragment 1	PADLSPGASSVTPPAPAREPGHSPQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEGGCEL
120	OX40 co-stimulatory fragment 1	DPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAI LLALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI
121	CD8 TM fragment	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLV ITLYC
122	CD27 co-stimulatory fragment 1	PTHLPYVSEMLEARTAGHMQTLADFRQLPARTLSTHWPPQRSLCSSDFIRILVIFSGMFLVFTL AGALFLHQRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP
123	CD27 co-stimulatory fragment 2	QRRKYRSNKGESPVEPAEPCRYSCPREEEGSTIPIQEDYRKPEPACSP
124	CD30 co-stimulatory fragment 1	APPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKPVLDAGPVLF WVILVLVVVVGSSAFLLCHRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGAS VTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIE KIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLS VEEEGKEDPLPTAASGK
125	CD30 co-stimulatory fragment 2	HRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLM ETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAE LPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
126	CSR1	AAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVA FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
127	anti-CD19 CSR	LPVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSG SNSGNTATLTISRVEAGDEADYYCQVWDSSSDYVVFGGGTKLTVLGSRGGGGSGGGGSGGG GSLEMAEVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGD SDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCARQVWGWQGGMYPRSNWWY NLDSWGQGTLVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVV VGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
128	anti-GPC3 CSR	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFS ASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRGGGGSGGGGSGG GGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSG GSSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVT VSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLV TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
129	GPC3-37/CD3 BsAb	QSVLTQPPSVSAAPGQRVTISCSGTRSNIGSDYVSWYQHLPGTAPKLLVYGDNLRPSGIPDRFS ASKSGTSATLGITGLQTGDEADYYCGTWDYTLNGVVFGGGTKLTVLGSRGGGGSGGGGSGG GGSLEMAQVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSVIYSG GSSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTSYLNHGDYWGQGTLVT VSSTSGGGGSDVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYI NPSRGYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQG TTVTVSSGEGTSTGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQ KPGKAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGG GTKVEIK

CLAIMS

What is claimed is:

1. A chimeric antibody-T cell receptor (TCR) construct (caTCR) comprising an antigen-binding module that specifically binds to a target antigen and a T cell receptor module (TCRM) comprising a first TCR domain (TCRD) comprising a first TCR transmembrane domain (TCR-TM) and a second TCRD comprising a second TCR-TM, wherein at least one of the TCR-TMs is non-naturally occurring, wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule, and wherein the antigen-binding module is not a naturally occurring T cell receptor antigen-binding moiety.

2. The caTCR of claim 1, wherein both of the TCR-TMs are non-naturally occurring.

3. The caTCR of claim 1 or 2, wherein the first TCR-TM is derived from one of the transmembrane domains of a first naturally occurring T cell receptor and the second TCR-TM is derived from the other transmembrane domain of the first naturally occurring T cell receptor.

4. The caTCR of claim 3, wherein the first naturally occurring T cell receptor is a γ/δ T cell receptor.

5. The caTCR of claim 3 or 4, wherein the TCRM allows for enhanced recruitment of the at least one TCR-associated signaling molecule as compared to a TCRM comprising the first naturally occurring T cell receptor transmembrane domains.

6. The caTCR of any one of claims 3-5, wherein the first TCR-TM comprises up to 5 amino acid substitutions compared to the transmembrane domain from which it is derived and/or the second TCR-TM comprises up to 5 amino acid substitutions compared to the transmembrane domain from which it is derived.

7. The caTCR of claim 6, wherein a substituted amino acid in the first TCR-TM is proximal to a substituted amino acid in the second TCR-TM.

8. The caTCR of claim 6 or 7, wherein one or more substituted amino acids are proximal to an amino acid in the first or second TCR-TM involved in binding to CD3.

9. The caTCR of any one of claims 6-8, wherein one or more substituted amino acids are more hydrophobic than their corresponding unsubstituted amino acid.

10. The caTCR of claim 1, wherein the first TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 7 and 9-13, and wherein the second TCR-TM comprises the amino acid sequence of any one of SEQ ID NOs: 8 and 14-26.

11. The caTCR of claim 1, wherein the first and second TCR-TMs comprise the amino acids sequence of a) SEQ ID NOs: 9 and 8, respectively; b) SEQ ID NOs: 7 and 14, respectively; c)

SEQ ID NOs: 7 and 15, respectively; d) SEQ ID NOs: 7 and 16, respectively; e) SEQ ID NOs: 10 and 16, respectively; f) SEQ ID NOs: 7 and 17, respectively; g) SEQ ID NOs: 7 and 18, respectively; h) SEQ ID NOs: 7 and 19, respectively; i) SEQ ID NOs: 7 and 20, respectively; j) SEQ ID NOs: 7 and 21, respectively; k) SEQ ID NOs: 7 and 22, respectively; l) SEQ ID NOs: 11 and 23, respectively; m) SEQ ID NOs: 12 and 24, respectively; n) SEQ ID NOs: 7 and 25, respectively; o) SEQ ID NOs: 13 and 26, respectively.

12. The caTCR of any one of claims 1-11, wherein the caTCR further comprises a stabilization module comprising a first stabilization domain and a second stabilization domain, wherein the first and second stabilization domains have a binding affinity for each other that stabilizes the caTCR.

13. The caTCR of embodiment 12, wherein the stabilization module is selected from the group consisting of a C_H1-C_L module, a C_H2-C_H2 module, a C_H3-C_H3 module, and a C_H4-C_H4 module.

14. The caTCR of any one of claims 1-13, wherein the caTCR further comprises a spacer module between the antigen-binding module and the TCRM, and wherein the spacer module comprises one or more peptide linkers connecting the antigen-binding module to the TCRM.

15. The caTCR of any one of claims 1-14, wherein the caTCR is multispecific.

16. The caTCR of any one of claims 1-15, wherein the antigen-binding module comprises a V_H domain linked to one of the TCRDs and a V_L domain linked to the other TCRD.

17. The caTCR of any one of claims 1-16, wherein the target antigen is a cell surface antigen.

18. The caTCR of any one of claims 1-16, wherein the target antigen is a complex comprising a peptide and a major histocompatibility complex (MHC) protein.

19. A caTCR comprising:

a) a multispecific antigen-binding module comprising a first antigen-binding domain that specifically binds to a first target antigen and a second antigen-binding domain that specifically binds to a second target antigen; and

b) a TCRM comprising a first TCRD (TCRD1) comprising a first TCR-TM and a second TCRD (TCRD2) comprising a second TCR-TM;

wherein the TCRM facilitates recruitment of at least one TCR-associated signaling molecule.

20. The caTCR of claim 19, wherein the first antigen-binding domain is a Fv comprising a first heavy chain variable domain (V_H1) and a first light chain variable domain (V_L1),

and the second antigen-binding domain is a Fab comprising a second V_H (V_H2) fused to a heavy chain constant domain 1 (C_H1) and a second V_L (V_L2) fused to a light chain constant domain (C_L).

21. The caTCR of claim 20, comprising:

(i) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_H2-C_H1-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_L2-C_L-TCRD2;

(ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L2-V_H2- C_H1-TCRD2;

(iii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_H2-C_H1-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_L2-C_L-TCRD2; or

(iv) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_L1-L1-V_L2-C_L-TCRD1; and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_H1-L2-V_H2-C_H1-TCRD2,

wherein L1 and L2 are peptide linkers.

22. The caTCR of claim 19, wherein the first antigen-binding domain is a first scFv (scFv1) and the second antigen-binding domain is a second scFv (scFv2), and wherein the caTCR further comprises a C_H1 and a C_L.

23. The caTCR of claim 22, comprising:

(i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L2-C_L-TCRD2; or

(ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv2-L1-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv1-L2-C_L-TCRD2;

wherein L1 and L2 are peptide linkers.

24. The caTCR of claim 19, wherein the first antigen-binding domain is an scFv and the second antigen-binding domain is a Fab comprising a V_H fused to a C_H1 and a V_L fused to a C_L.

25. The caTCR of claim 24, comprising:

(i) a first polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L1-V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: V_L-C_L-TCRD2; or

(ii) a first polypeptide chain comprising from the N-terminus to the C-terminus: V_H-C_H1-TCRD1, and a second polypeptide chain comprising from the N-terminus to the C-terminus: scFv-L2-V_L-C_L-TCRD2;

wherein L1 and L2 are peptide linkers.

26. The caTCR of claim 21, 23 or 25, wherein L1 and/or L2 are about 5 to about 50 amino acids long.

27. The caTCR of any one of claims 19-26, wherein the first target antigen is a first cell surface antigen, and the second target antigen is a second cell surface antigen.

28. The caTCR of claim 27, wherein the first target antigen is CD19 and the second target antigen is CD22, or the first target antigen is CD22 and the second target antigen is CD19.

29. The caTCR of claim 28, wherein the antigen-binding domain that specifically binds to CD22 comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 65, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 69.

30. The caTCR of claim 28 or 29, wherein the antigen-binding domain that specifically binds to CD19 comprises a V_H comprising HC-CDR1, HC-CDR2 and HC-CDR3 of the V_H comprising the amino acid sequence of SEQ ID NO: 74, and a V_L comprising LC-CDR1, LC-CDR2 and LC-CDR3 of the V_L comprising the amino acid sequence of SEQ ID NO: 78.

31. The caTCR of any one of claims 19-30, wherein at least one of the TCR-TMs is non-naturally occurring.

32. The caTCR of any one of claims 1-31, wherein the first TCRD further comprises a first connecting peptide or fragment thereof of a TCR subunit; and/or the second TCRD further comprises a second connecting peptide or fragment thereof of a TCR subunit.

33. The caTCR of any one of claims 1-32, wherein the first TCRD further comprises a first TCR intracellular domain comprising a TCR intracellular sequence; and/or the second TCRD further comprises a second TCR intracellular domain comprising a TCR intracellular sequence.

34. One or more nucleic acids encoding the caTCR of any one of claims 1-33.

35. One or more vectors comprising the one or more nucleic acids of claim 34.

36. A complex comprising the caTCR of any one of claims 1-33 and at least one TCR-associated signaling molecule selected from the group consisting of CD3δε, CD3γε, and ζζ.

37. An effector cell presenting on its surface the caTCR of any one of claims 1-33 or the complex of claim 36.

38. A method of killing a target cell presenting a target antigen, comprising contacting the target cell with the effector cell of claim 37, wherein the caTCR specifically binds to the target antigen.

39. A pharmaceutical composition comprising: (a) the caTCR of any one of claims 1-33, the one or more nucleic acids of claim 34 or the one or more vectors of claim 35, or the effector cell of claim 37; and (b) a pharmaceutically acceptable carrier.

40. A method of treating a target antigen-associated disease in an individual in need thereof comprising administering to the individual an effective amount of the composition of claim 39.

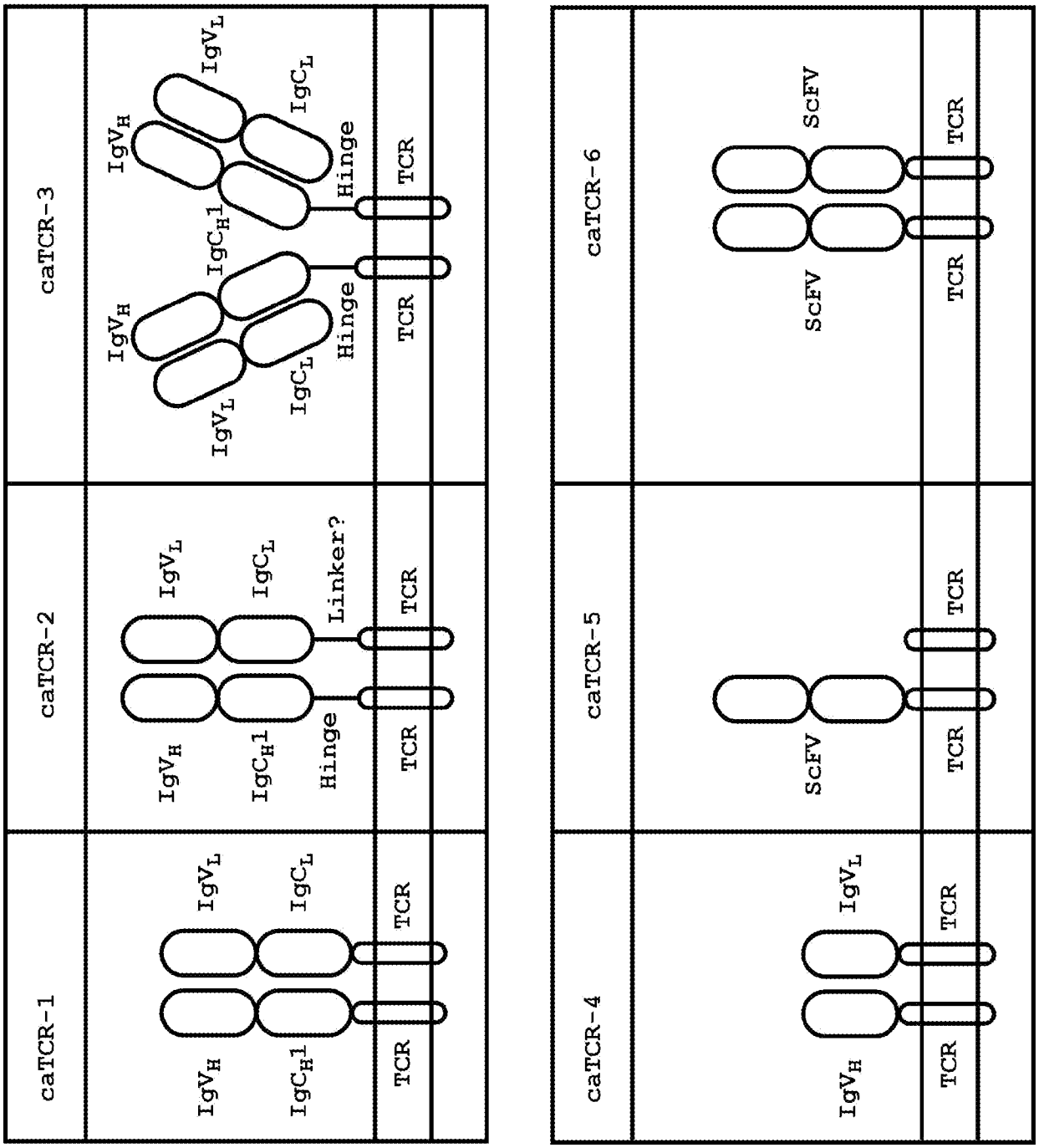

caTCR-1
caTCR-2
caTCR-3
caTCR-4
caTCR-5
caTCR-6
IgV$_H$
IgV$_L$
IgC$_H$1
IgC$_L$
Hinge
Linker?
ScFV
TCR

FIG. 1

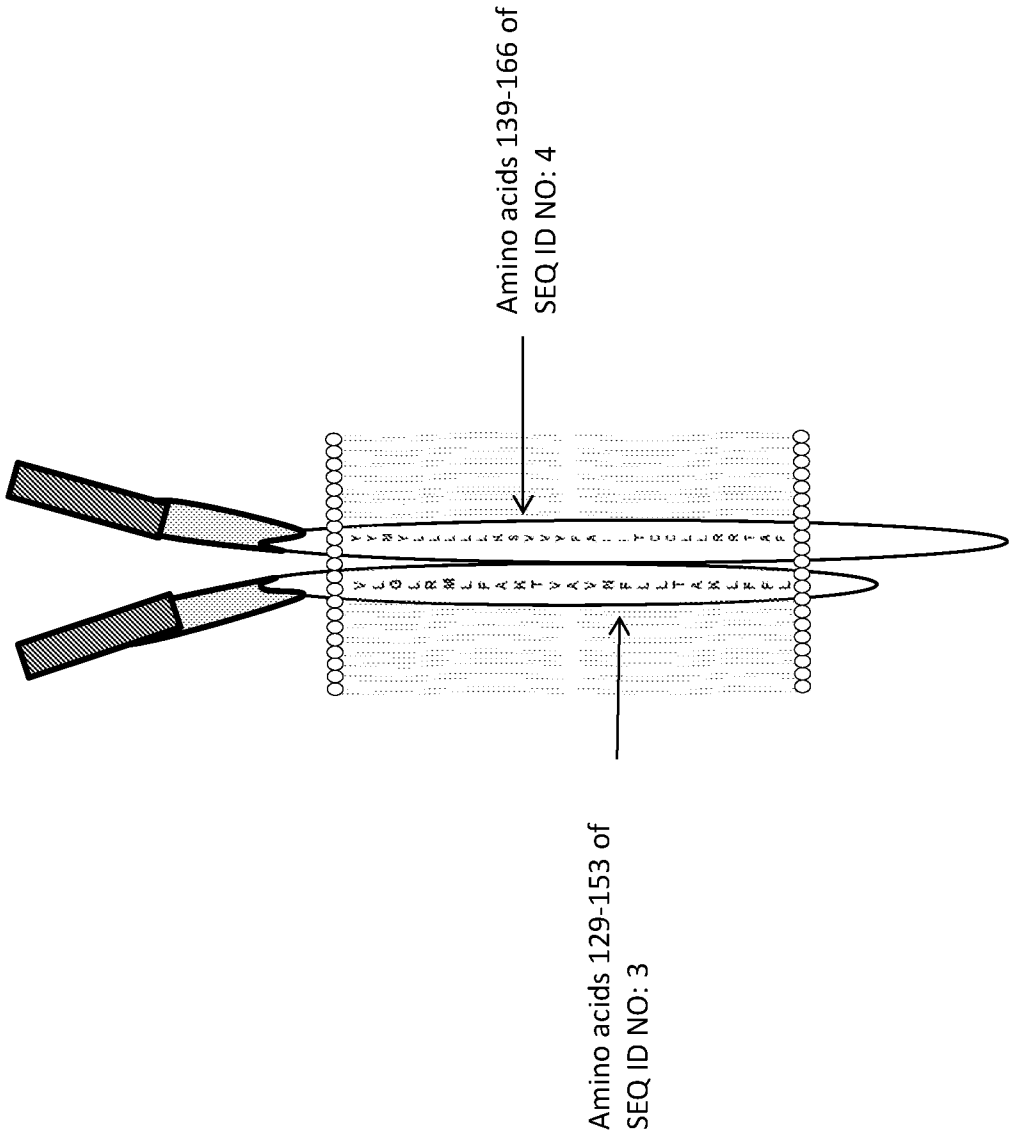
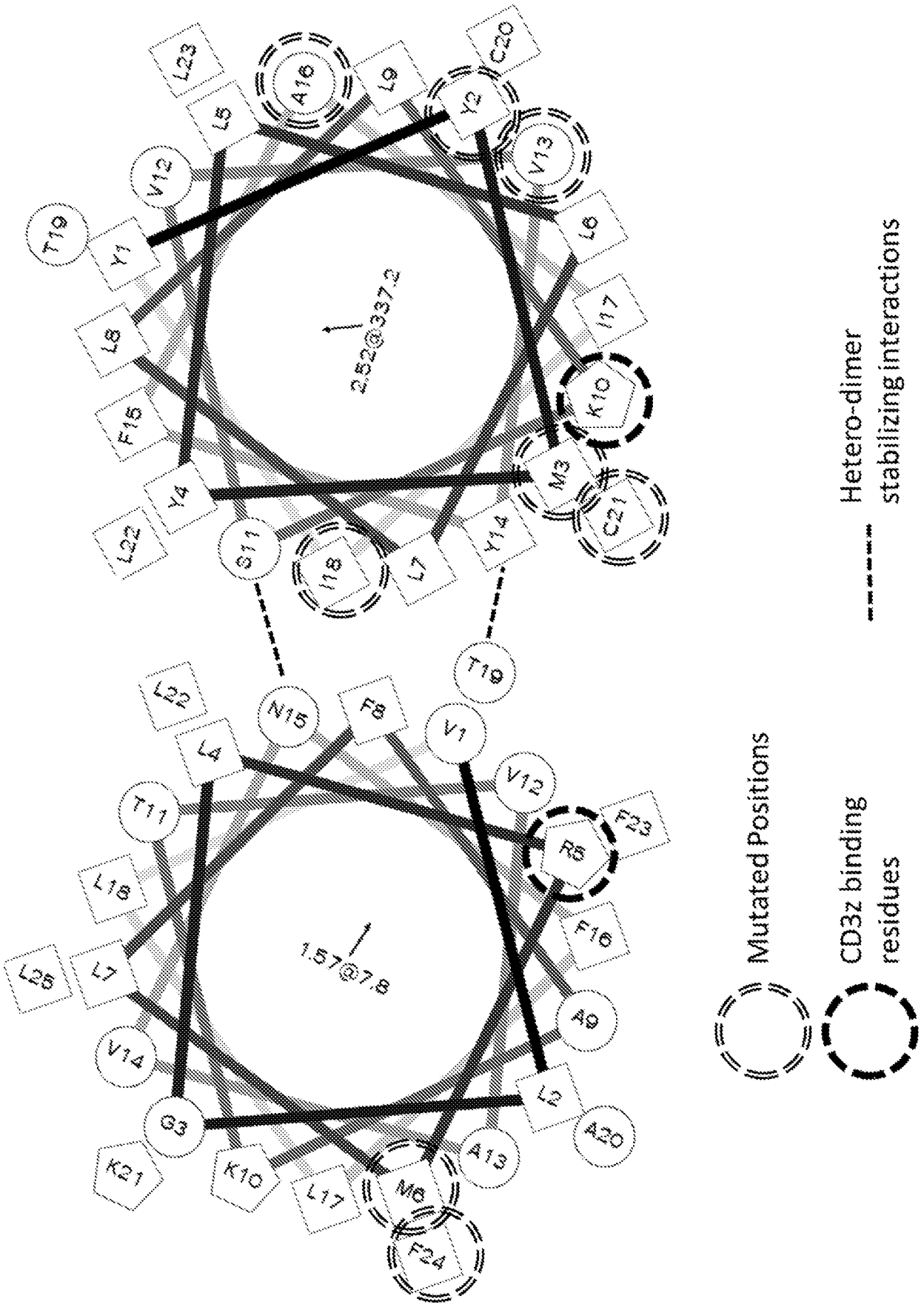

Amino acids 139-166 of SEQ ID NO: 4
Amino acids 129-153 of SEQ ID NO: 3

FIG. 2

FIG. 3


252@337.2
157@7.8
Hetero-dimer stabilizing interactions
Mutated Positions
CD3z binding residues

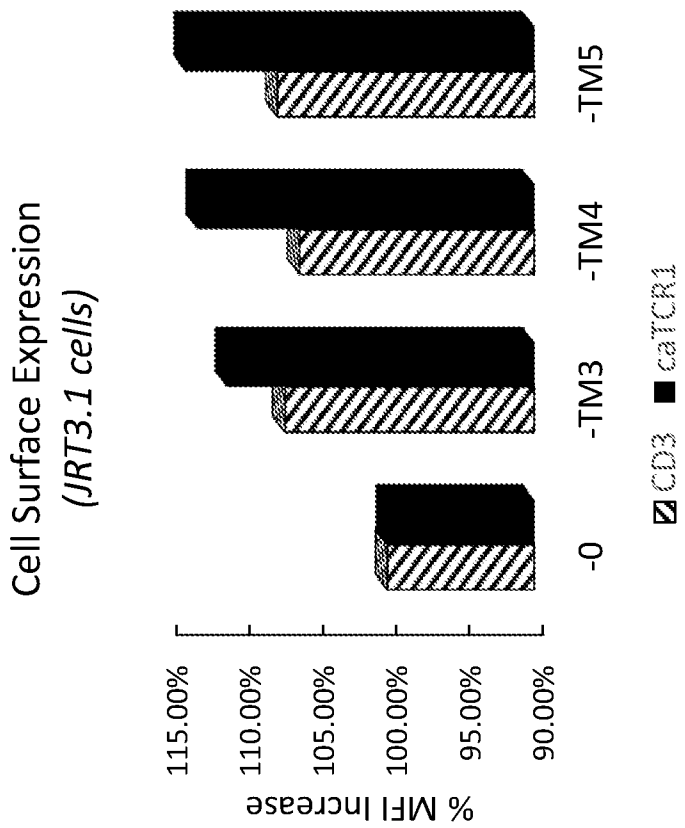
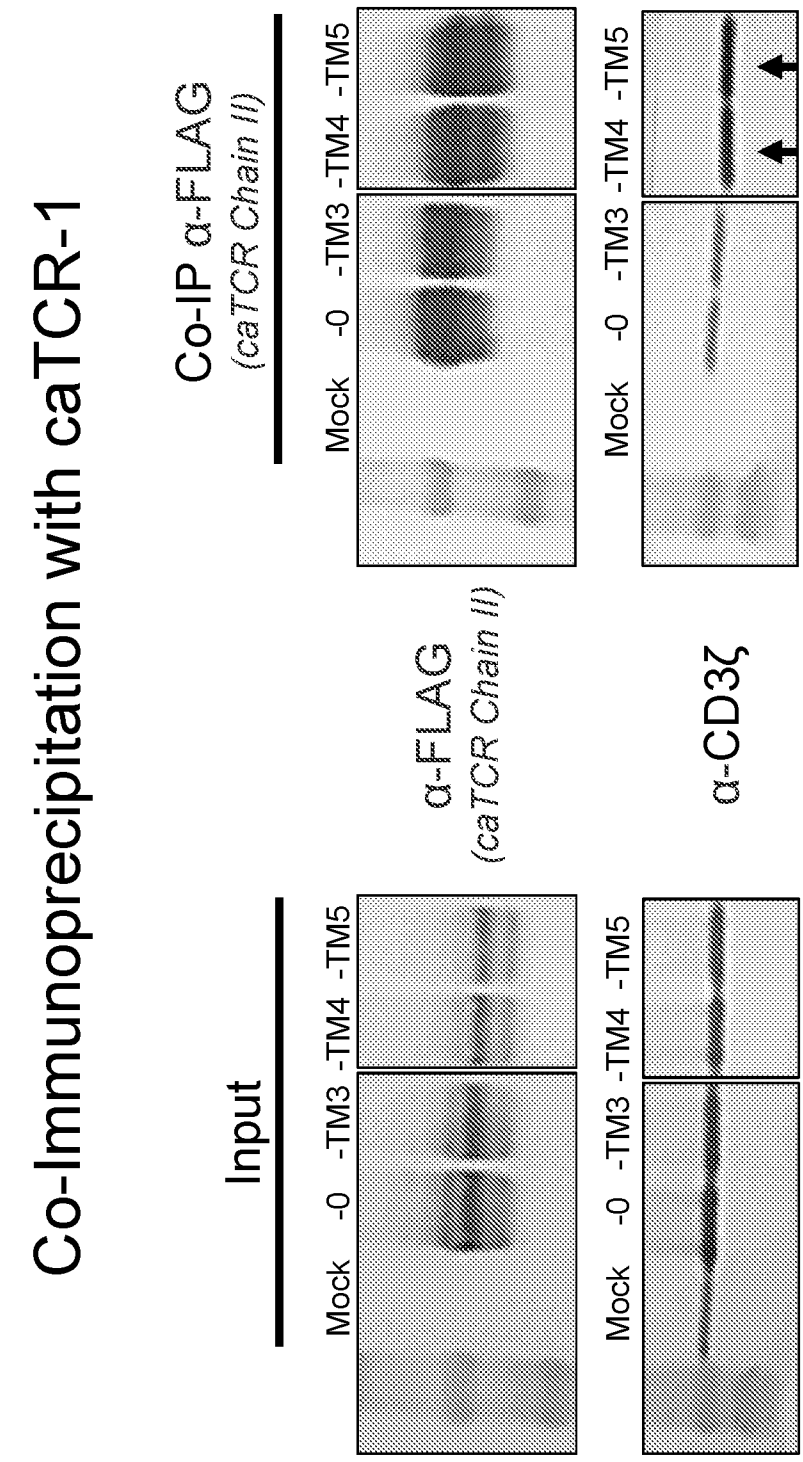

Cell Surface Expression
(JRT3.1 cells)
% MFI Increase
115.00%
110.00%
105.00%
100.00%
95.00%
90.00%
-0
-TM3
-TM4
-TM5
CD3
caTCR1

FIG. 4

FIG. 5


Co-Immunoprecipitation with caTCR-1
Input
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(caTCR Chain II)
Mock -0 -TM3 -TM4 -TM5
α-FLAG
(caTCR Chain II)
α-CD3ζ

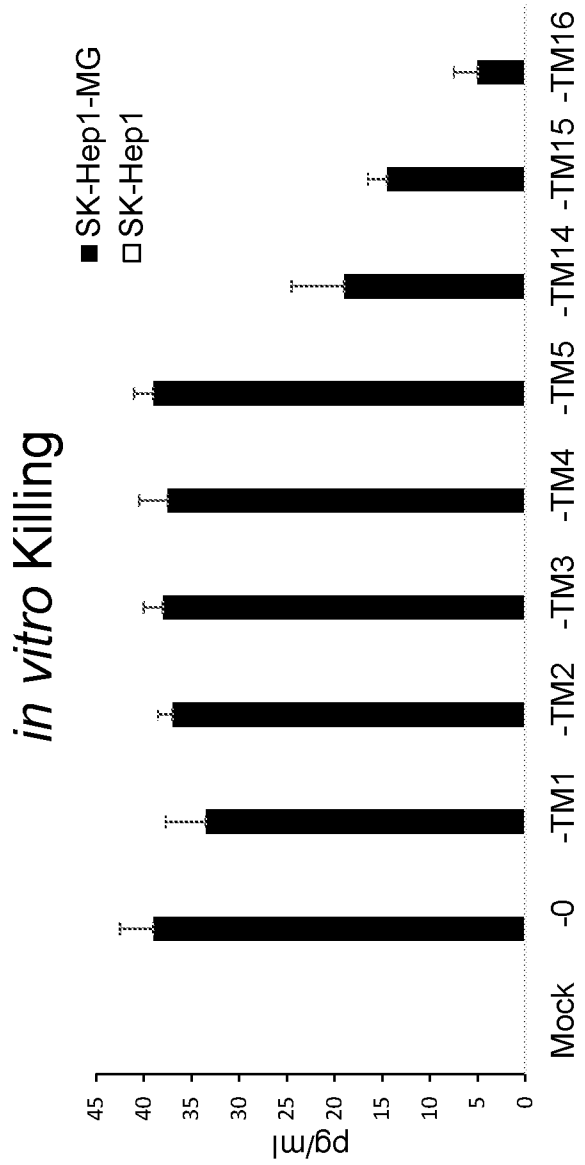

in vitro Killing
SK-Hep1-MG
SK-Hep1
pg/ml
0
5
10
15
20
25
30
35
40
45
Mock
-0
-TM1
-TM2
-TM3
-TM4
-TM5
-TM14
-TM15
-TM16

FIG. 6

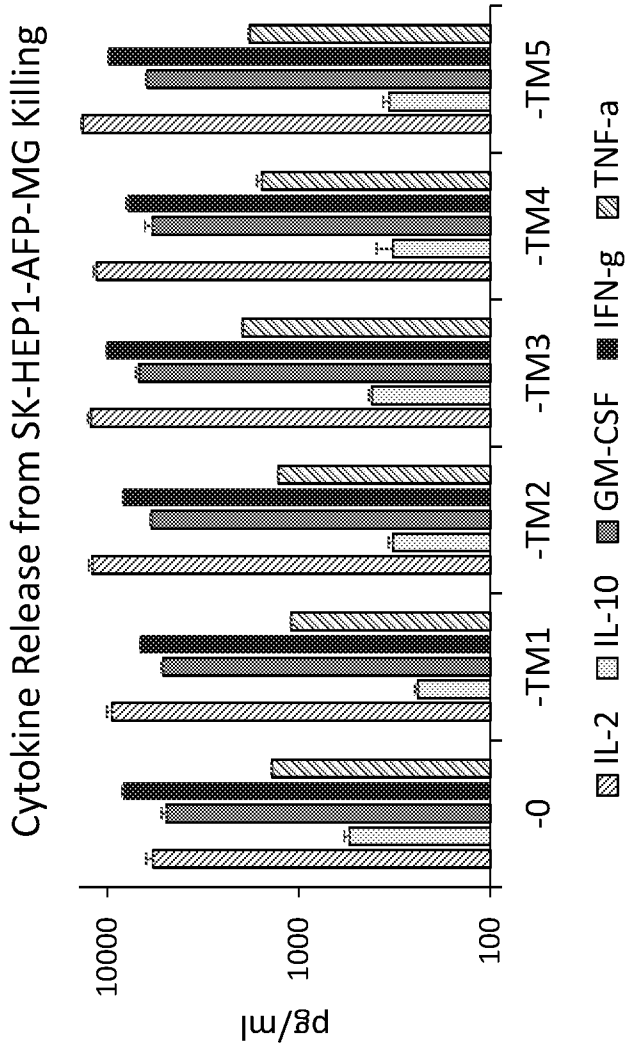

Cytokine Release from SK-HEP1-AFP-MG Killing
pg/ml
10000
1000
100
-0
-TM1
-TM2
-TM3
-TM4
-TM5
IL-2
IL-10
GM-CSF
IFN-g
TNF-a

FIG. 7

in vitro Killing

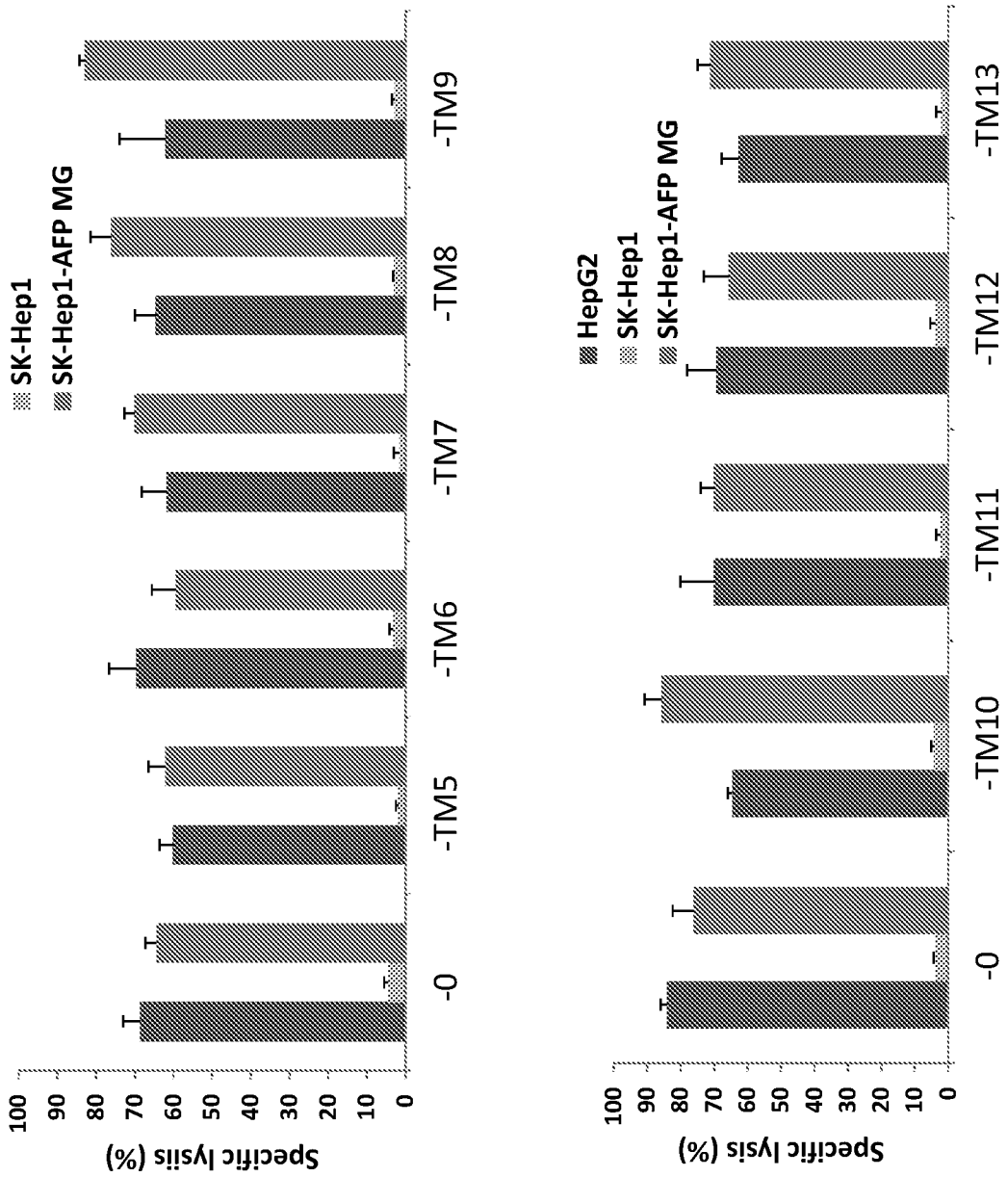
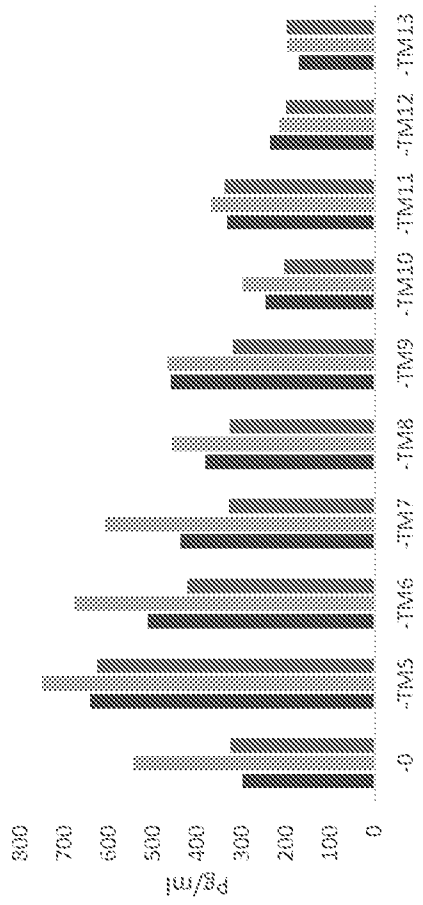


FIG. 8


Cytokine Release from HEPG2 Killing
pg/ml
800
700
600
500
400
300
200
100
0
-0
-TM5
-TM6
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-TM9
-TM10
-TM11
-TM12
-TM13
GM-CSF
IFN-g
TNF-a

FIG. 9

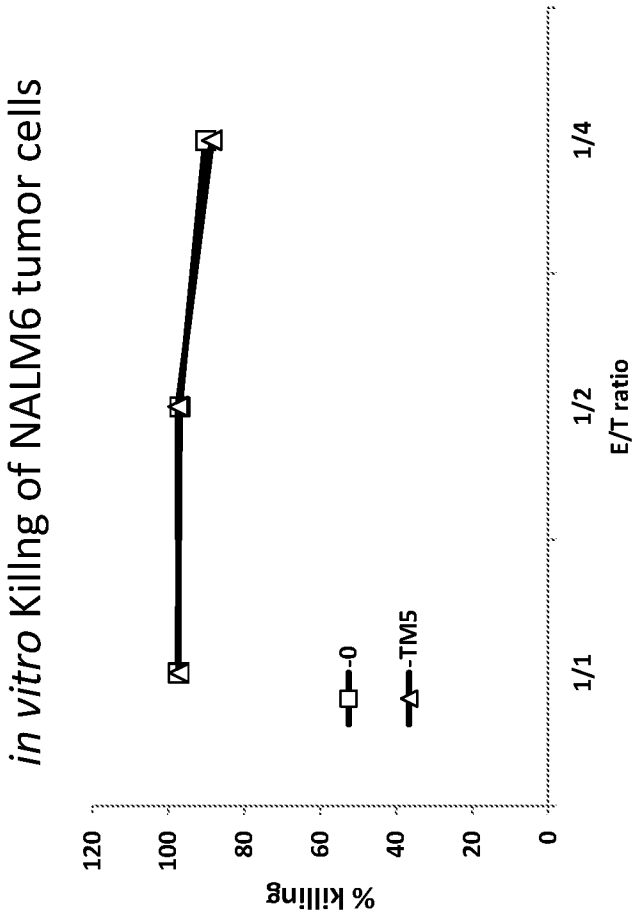

in vitro Killng of NALM6 tumor cells
% killing
120
100
80
60
40
20
0
1/1
1/2
1/4
E/T ratio
-0
-TM5

FIG. 10

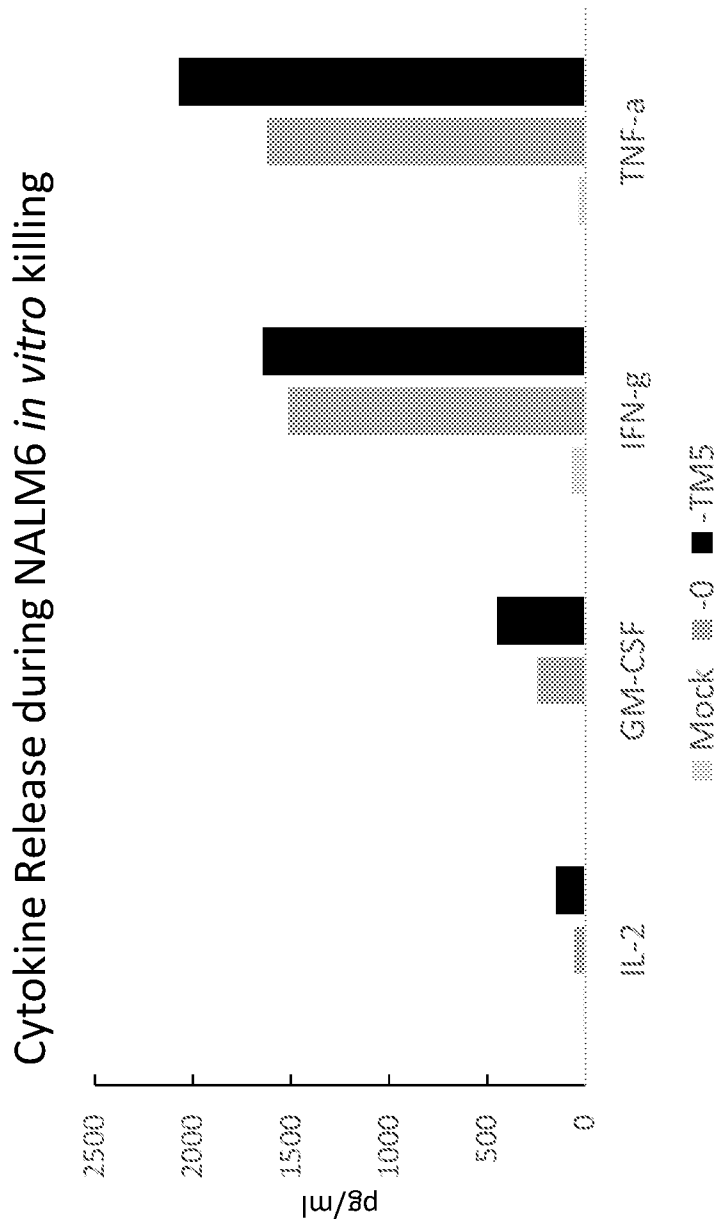

Cytokine Release during NALM6 *in vitro* killing
pg/ml
2500
2000
1500
1000
500
0
IL-2
GM-CSF
IFN-g
TNF-a
Mock
-0
-TM5

FIG. 11

FIG. 12A

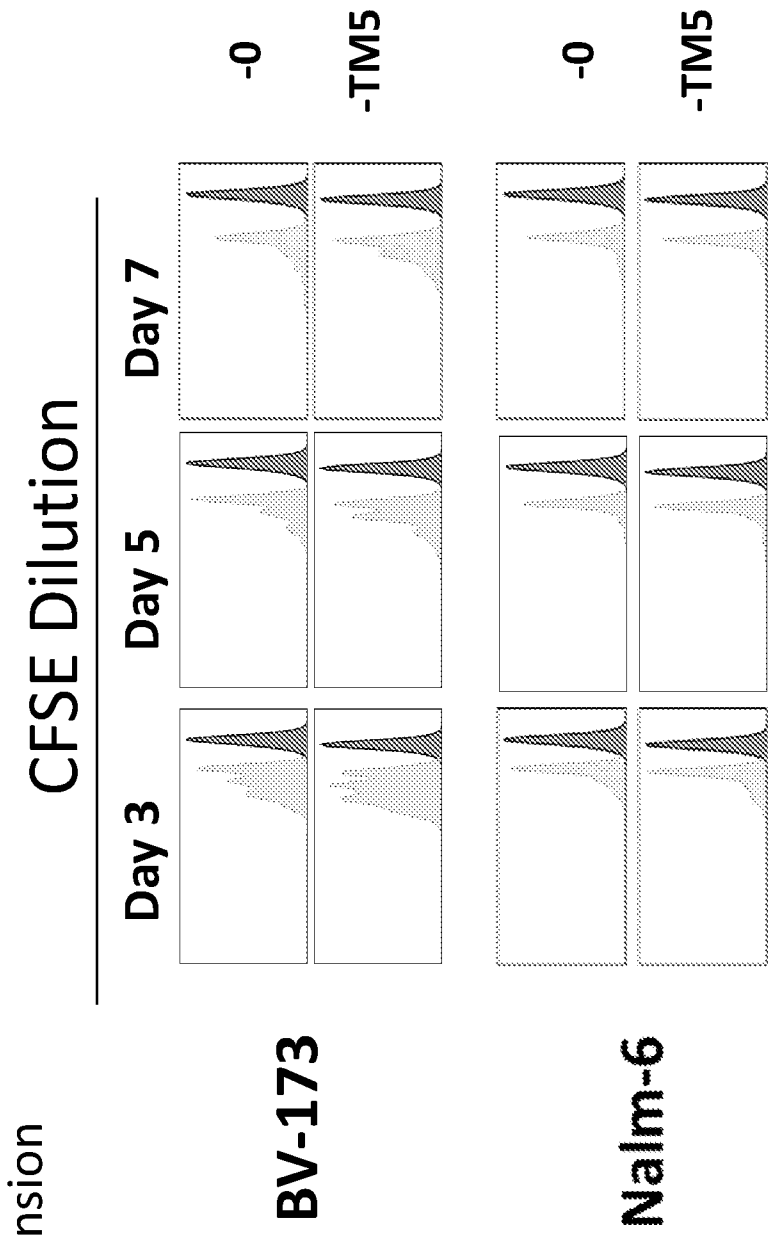

caTCR-1 T-cell Expansion
Protocol A
CFSE Dilution
Day 3
Day 5
Day 7
-0
-TM5
-0
-TM5
BV-173
Nalm-6

FIG. 12B

caTCR-1 T-cell Expansion

Protocol B

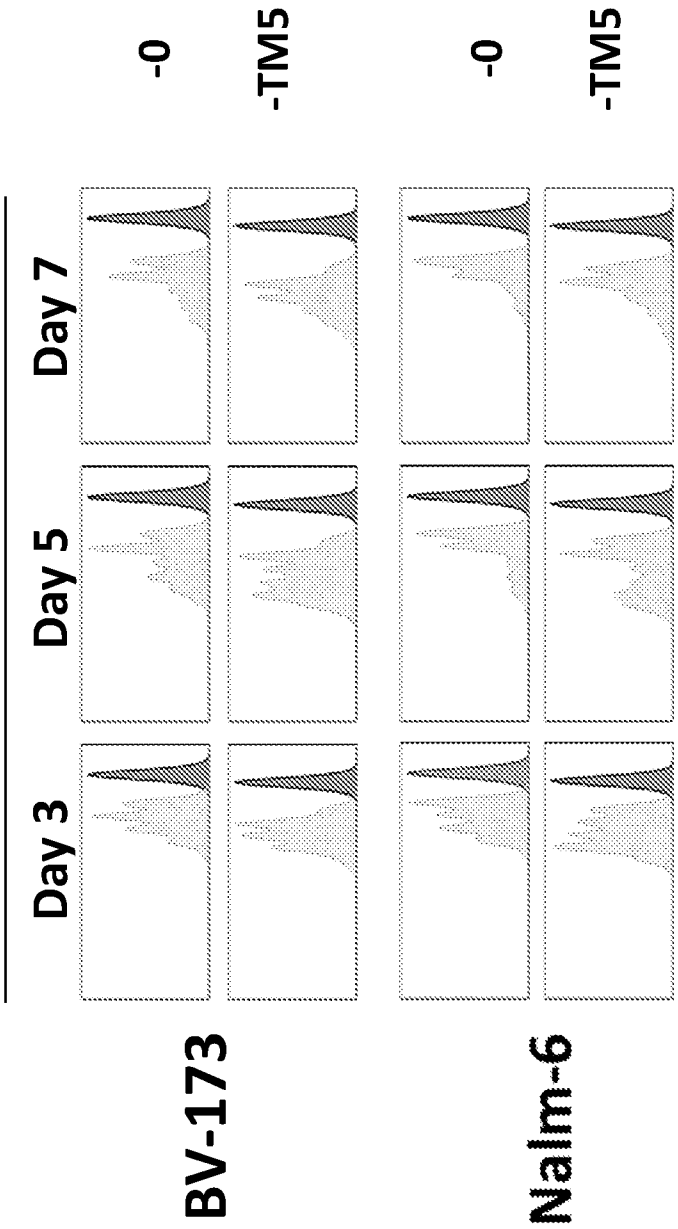


FIG. 13A

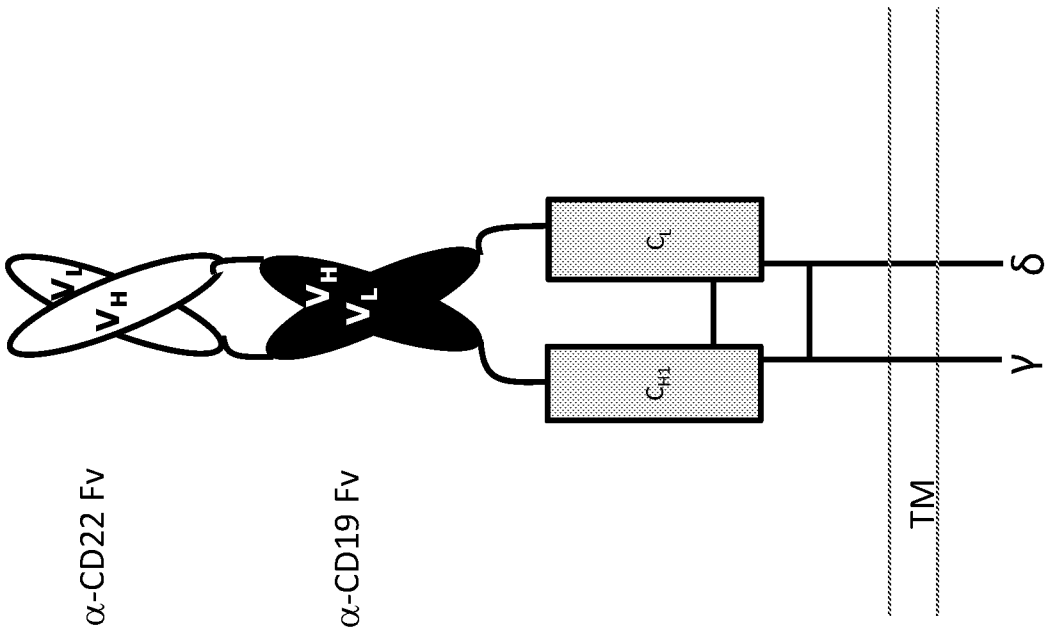


FIG. 13B

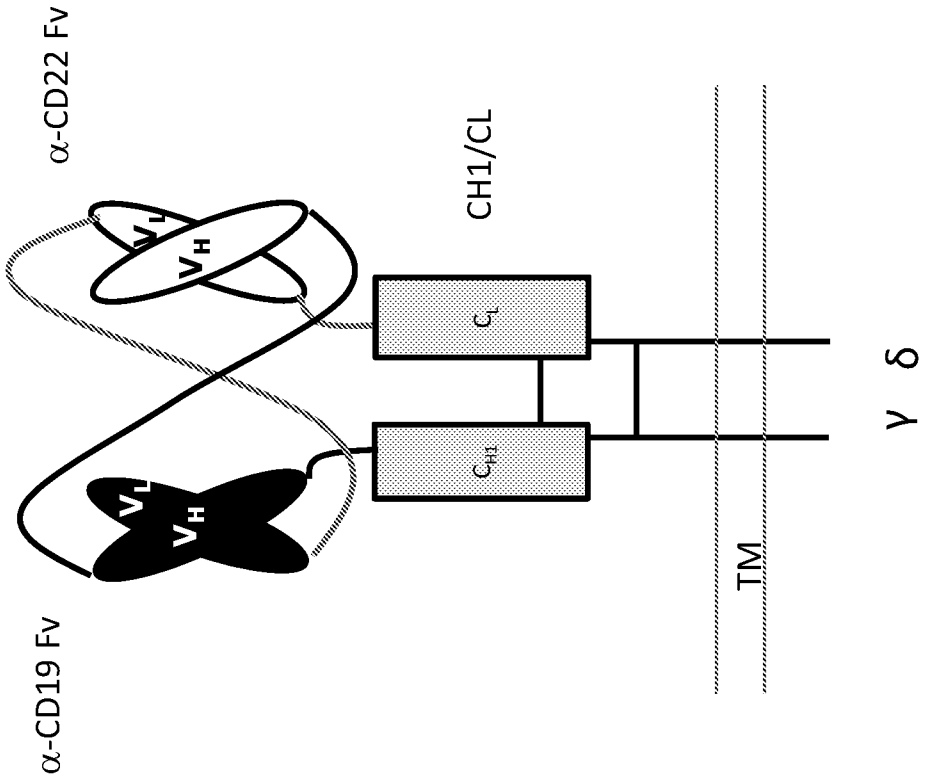


FIG. 13C

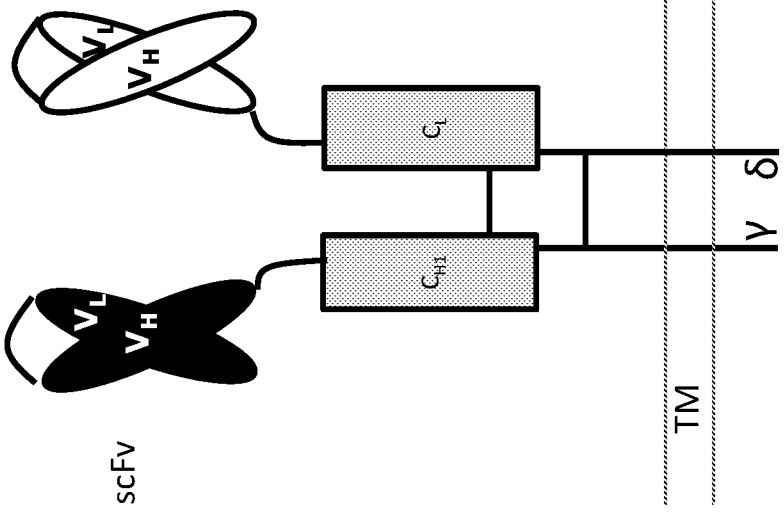


FIG. 13D

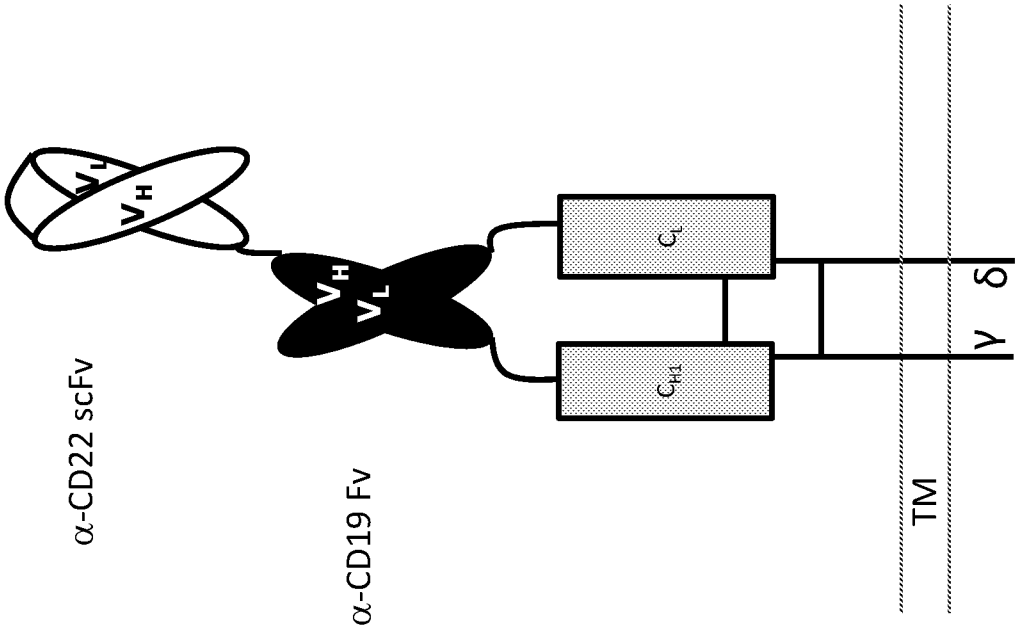
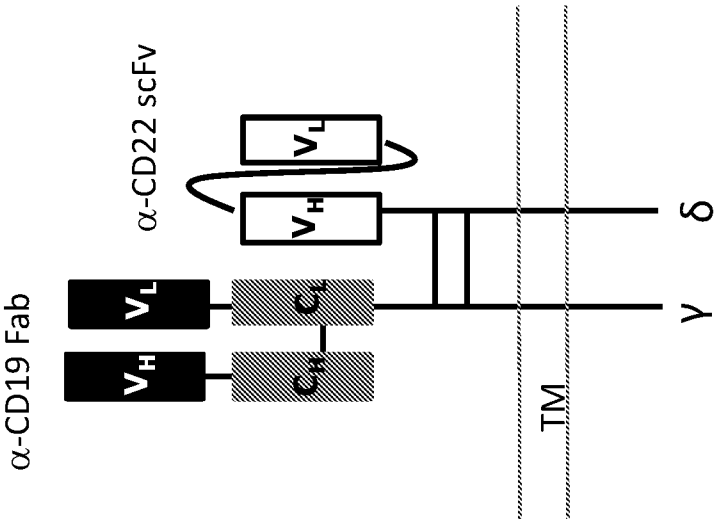


FIG. 13E



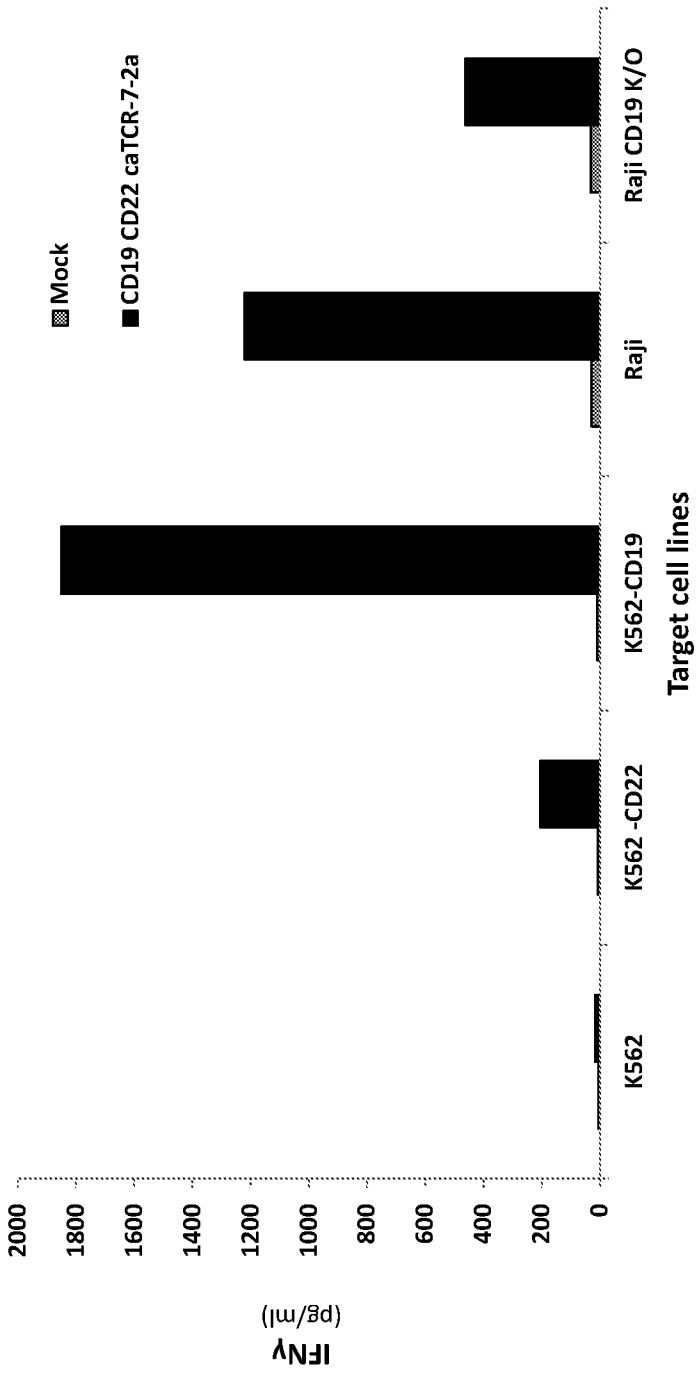

Mock
CD19 CD22 caTCR-7-2a
IFNγ (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
K562
K562 -CD22
K562-CD19
Raji
Raji CD19 K/O
Target cell lines

FIG. 14

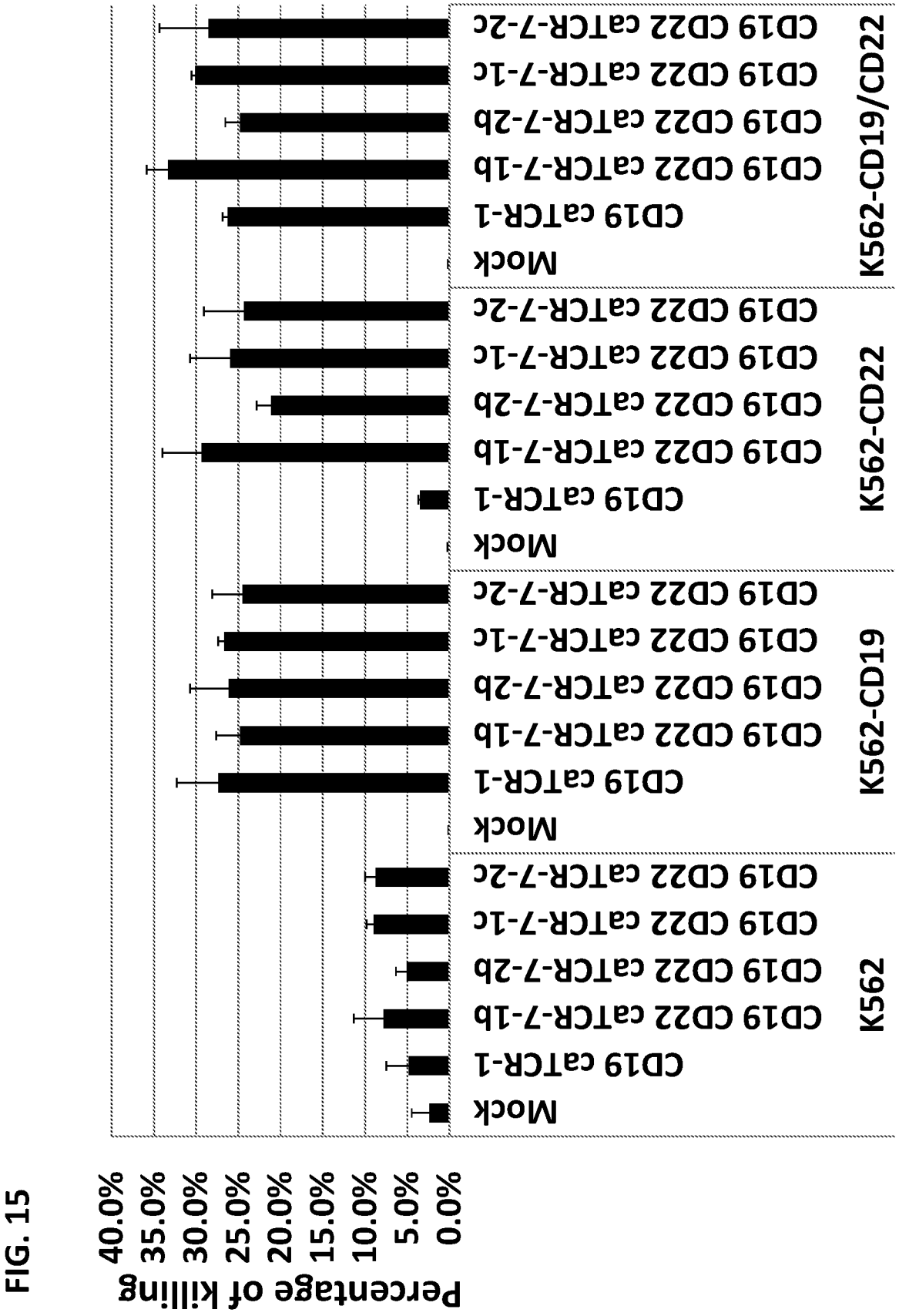

Percentage of killing
40.0%
35.0%
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
K562
Mock
CD19 caTCR-1
CD19 CD22 caTCR-7-1b
CD19 CD22 caTCR-7-2b
CD19 CD22 caTCR-7-1c
CD19 CD22 caTCR-7-2c
K562-CD19
Mock
CD19 caTCR-1
CD19 CD22 caTCR-7-1b
CD19 CD22 caTCR-7-2b
CD19 CD22 caTCR-7-1c
CD19 CD22 caTCR-7-2c
K562-CD22
Mock
CD19 caTCR-1
CD19 CD22 caTCR-7-1b
CD19 CD22 caTCR-7-2b
CD19 CD22 caTCR-7-1c
CD19 CD22 caTCR-7-2c
K562-CD19/CD22
Mock
CD19 caTCR-1
CD19 CD22 caTCR-7-1b
CD19 CD22 caTCR-7-2b
CD19 CD22 caTCR-7-1c
CD19 CD22 caTCR-7-2c

FIG. 15

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

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

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/029217

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

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

1. ☐ Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:

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

3. ☐ Claims Nos:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

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

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/029217

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/725 (2006.01) ***A61K 35/17 (2015.01)*** ***A61P 35/00 (2006.01)*** ***C07K 16/30 (2006.01)*** ***C07K 19/00 (2006.01)*** ***C12N 5/0783 (2010.01)***

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

PATENW, CAPLUS, BIOSIS, EMBASE, MEDLINE & keywords: T cell receptor, antibody, chimeric, chimeric antigen receptor, dimer, transmembrane, multichain and like terms and inventor names; CPC/IPC: C07K14/7051, C07K16, C07K2319 OR C07K19/00.

GENOMEQUST: SEQ ID NOs: 9-26, 65, 69, 74, 78

GOOGLE PATENTS: chimeric antigen receptor, bispecific

ESPACENET, Internal databases: Applicant and Inventor names

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 2018	18 July 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Richard Filmer AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832735

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/US2018/029217**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/039523 A1 (CELLECTIS) 13 March 2014 Abstract; pp. 1 para. 1; pp. 14 para. 4 - pp 5 para. 1; pp 15 para. 3; pp. 19 para. 4; pp. 36 para. 5 - pp. 37 para. 1; pp. 42 para. 4; pp. 50 para. 4 - pp. 51 para. 1; Fig. 3	1-4, 6, 9, 10, 12, 14, 15, 17-19, 27, 28, 31-40
X Y	EP 0340793 B1 (YEDA RESEARCH AND DEVELOPMENT COMPANY LIMITED) 30 August 1995 col. 1 line 37 - col. 3 line 24 col. 1 line 37 - col. 3 line 24	1-4, 6, 9, 10, 12, 14, 17, 18, 32-40 15, 19, 27, 28, 31
Y	US 2013/0280220 A1 (AHMED et al.) 24 October 2013 Abstract; [0007]-[0028], [0048]; Fig. 1	15, 19, 27, 28, 31
P,X	WO 2017/070608 A1 (EUREKA THERAPEUTICS, INC.) 27 April 2017 [0016]-[0041], [0113]; Fig. 1	1-4, 6, 9, 10, 12-40

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Invention 1 is defined by claims 1-18 (completely) and claims 32-40 (in part). The feature of a T cell receptor module comprising a non-naturally occurring TCR transmembrane domain is specific to this group of claims.
- Invention 2 is defined by claims 19-31 (completely) and claims 32-40 (in part). The feature of a chimeric antibody T cell receptor comprising a multispecific antigen-binding module is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is a chimeric antibody T cell receptor comprising an antigen-binding module and two TCR transmembrane domains. However this feature does not make a contribution over the prior art because it is disclosed in:

EP 0340793 B1 (YEDA RESEARCH AND DEVELOPMENT COMPANY LIMITED) 30 August 1995

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/029217**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2014/039523 A1	13 March 2014	WO 2014039523 A1	13 Mar 2014
		AR 096274 A1	16 Dec 2015
		AU 2013266733 A1	15 Jan 2015
		AU 2013266733 B2	05 Jul 2018
		AU 2013266734 A1	15 Jan 2015
		AU 2013312838 A1	12 Mar 2015
		AU 2014266830 A1	26 Nov 2015
		AU 2014266833 A1	24 Dec 2015
		AU 2014267436 A1	12 Nov 2015
		AU 2014273085 A1	10 Dec 2015
		AU 2014273490 A1	17 Dec 2015
		AU 2014351797 A1	09 Jun 2016
		BR 112015004522 A2	21 Nov 2017
		CA 2874609 A1	28 Nov 2013
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		CA 2883502 A1	13 Mar 2014
		CA 2911292 A1	20 Nov 2014
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		CA 2931267 A1	28 May 2015
		CN 104718284 A	17 Jun 2015
		CN 104769103 A	08 Jul 2015
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		CN 105378067 A	02 Mar 2016
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		CN 105431532 A	23 Mar 2016
		CN 105765061 A	13 Jul 2016
		EA 201492222 A1	29 May 2015
		EA 201501109 A1	30 Jun 2016
		EP 2855666 A1	08 Apr 2015
		EP 2855667 A1	08 Apr 2015
		EP 2893004 A1	15 Jul 2015
		EP 2997132 A1	23 Mar 2016
		EP 2997133 A1	23 Mar 2016

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/029217**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		EP 2997141 A1	23 Mar 2016
		EP 3004337 A1	13 Apr 2016
		EP 3004337 B1	02 Aug 2017
		EP 3004339 A2	13 Apr 2016
		EP 3071687 A1	28 Sep 2016
		EP 3276000 A2	31 Jan 2018
		EP 3279315 A2	07 Feb 2018
		EP 3309248 A1	18 Apr 2018
		HK 1208879 A1	18 Mar 2016
		HK 1212728 A1	17 Jun 2016
		HK 1222678 A1	07 Jul 2017
		HK 1222679 A1	07 Jul 2017
		HK 1223399 A1	28 Jul 2017
		IN 10991DEN2014 A	25 Sep 2015
		JP 2015528298 A	28 Sep 2015
		JP 6352920 B2	04 Jul 2018
		JP 2015523064 A	13 Aug 2015
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		JP 2016520074 A	11 Jul 2016
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		JP 2016525888 A	01 Sep 2016
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		KR 20150029651 A	18 Mar 2015
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		MA 37681 A2	30 Jun 2016
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		MX 2014014364 A	22 Sep 2015
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/029217**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
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		MX 2016006177 A	19 Aug 2016
		NZ 703281 A	27 Jan 2017
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		SG 10201708896W A	29 Nov 2017
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		US 2016361359 A1	15 Dec 2016
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		WO 2013176915 A1	28 Nov 2013
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2018/029217**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
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		WO 2015075195 A1	28 May 2015
EP 0340793 B1	30 August 1995	EP 0340793 A2	08 Nov 1989
		EP 0340793 B1	30 Aug 1995
		AU 3401289 A	09 Nov 1989
		IL 86278 A	24 Jun 2003
		JP H02174681 A	06 Jul 1990
		JP 3001208 B2	24 Jan 2000
		US 5906936 A	25 May 1999
		US 5912172 A	15 Jun 1999
US 2013/0280220 A1	24 October 2013	US 2013280220 A1	24 Oct 2013
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		KR 20180063325 A	11 Jun 2018
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		US 2018085457 A1	29 Mar 2018

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.