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*).

[0013] 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).

[0014] 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; 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.

[0015] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[0016] The present application in one example provides a construct (such as an isolated construct) comprising an antibody moiety (such as a Fab-like antigen-binding module) fused to a T cell receptor module (said construct also referred to herein as an "antibody-TCR chimeric molecule," or "abTCR"). In some examples, the abTCR comprises a Fab-like 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 module. In some examples, 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 examples, the target antigen is a cell-surface antigen.

[0016a] In one aspect, the present disclosure refers to an antibody-T cell receptor (TCR) chimeric molecule (abTCR) that specifically binds to a target antigen, comprising:

a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H and C_H1 antibody domains and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit, wherein the first polypeptide chain lacks variable and constant domains of the first TCR subunit; and

b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the second polypeptide chain lacks variable and constant domains of the second TCR subunit,

wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form an antigen-binding module that specifically binds to the target antigen,

wherein the target antigen is a cell surface antigen or a complex comprising a peptide and a major histocompatibility complex (MHC) protein, wherein

(i) the first TCR subunit is a TCR α chain, and the second TCR subunit is a TCR β chain;

(ii) the first TCR subunit is a TCR β chain, and the second TCR subunit is a TCR α chain;

(iii) the first TCR subunit is a TCR γ chain, and the second TCR subunit is a TCR δ chain; or

(iv) the first TCR subunit is a TCR δ chain, and the second TCR subunit is a TCR γ chain,

and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module;

wherein optionally the antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain and a residue in the C_L domain.

[0017] In some examples, there is provided an abTCR (such as an isolated abTCR) that specifically binds to a target antigen, wherein the abTCR comprises: a) a first polypeptide chain comprising a first antigen-binding domain comprising V_H and C_H1 antibody domains

and a first T cell receptor domain (TCRD) comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising V_L and C_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H and C_H1 domains of the first antigen-binding domain and the V_L and C_L domains of the second antigen-binding domain form a Fab-like antigen-binding module that specifically binds to the target antigen, and wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module. In some examples, the first polypeptide chain and the second polypeptide chain are linked via one or more disulfide bonds. In some examples, the Fab-like antigen-binding module comprises a disulfide bond between a residue in the C_H1 domain in the first polypeptide chain and a residue in the C_L domain in the second polypeptide chain. In some examples, the first polypeptide chain further comprises a first peptide linker between the first antigen-binding domain and the first TCRD. In some examples, the second polypeptide chain further comprises a second peptide linker between the second antigen-binding domain and the second TCRD. In some examples, the first peptide linker and/or the second peptide linker are, individually, from about 5 to about 50 amino acids in length. In some examples, the target antigen is a cell surface antigen. In some examples, the cell surface antigen is selected from the group consisting of protein, carbohydrate, and lipid. In some examples, the cell surface antigen is CD19, ROR1, ROR2, BCMA, GPRC5D, or FCRL5. In some examples, the target antigen is a complex comprising a peptide and a major histocompatibility complex (MHC) protein.

[0018] In some examples, there is provided an abTCR that specifically binds to a target antigen, comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to the target antigen, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module, and wherein the target antigen is a complex

comprising a peptide and an MHC protein. In some examples, the first polypeptide chain further comprises a first peptide linker between the first antigen-binding domain and the first TCRD and the second polypeptide chain further comprises a second peptide linker between the second antigen-binding domain and the second TCRD. In some examples, the first and/or second peptide linkers comprise, individually, a constant domain or fragment thereof from an immunoglobulin or T cell receptor subunit. In some examples, the first and/or second peptide linkers comprise, individually, a CH1, CH2, CH3, CH4 or CL antibody domain, or a fragment thereof. In some examples, the first and/or second peptide linkers comprise, individually, a Cα, Cβ, Cγ, or Cδ TCR domain, or a fragment thereof.

[0019] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, the first TCRD further comprises a first connecting peptide or fragment thereof of a TCR subunit N-terminal to the first transmembrane domain. the second TCRD further comprises a second connecting peptide or fragment thereof of a TCR subunit N-terminal to the second transmembrane domain. In some examples, the TCRM comprises a disulfide bond between a residue in the first connecting peptide and a residue in the second connecting peptide. In some examples, the first TCRD further comprises a first TCR intracellular domain comprising a TCR intracellular sequence C-terminal to the first transmembrane domain. In some examples, the second TCRD further comprises a second TCR intracellular domain comprising a TCR intracellular sequence C-terminal to the second transmembrane domain. In some examples, the abTCR binds to the target antigen with an equilibrium dissociation constant (K_d) from about 0.1 pM to about 500 nM. In some examples, the TCR-associated signaling module is selected from the group consisting of CD3δε, CD3γε, and ζζ.

[0020] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, the first polypeptide chain further comprises a first accessory intracellular domain comprising a co-stimulatory intracellular signaling sequence C-terminal to the first transmembrane domain. In some examples, the second polypeptide chain further comprises a second accessory intracellular domain comprising a co-stimulatory intracellular signaling sequence C-terminal to the second transmembrane domain. In some examples, the first polypeptide chain further comprises a first signaling peptide N-terminal to the first antigen-binding domain. In some examples, the second polypeptide chain further comprises a second signaling peptide N-terminal to the second antigen-binding domain.

[0021] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above where the target antigen is a complex comprising a peptide and a major histocompatibility complex (MHC) protein, 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, and PSA.

[0022] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, a) the first TCR subunit is a TCR α chain, and the second TCR subunit is a TCR β chain; b) the first TCR subunit is a TCR β chain, and the second TCR subunit is a TCR α chain; c) the first TCR subunit is a TCR γ chain, and the second TCR subunit is a TCR δ chain; or d) the first TCR subunit is a TCR δ chain, and the second TCR subunit is a TCR γ chain.

[0023] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided a nucleic acid encoding the first and second polypeptide chains of the abTCR. In one aspect, the present disclosure refers to nucleic acid(s) encoding the first and second polypeptide chains of the abTCR described herein.

[0024] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided complex comprising the abTCR and at least one TCR-associated signaling module selected from the group consisting of CD3δε, CD3γε, and ζζ. In some examples, the complex is an octamer comprising the abTCR and CD3δε, CD3γε, and ζζ. In one aspect, the present disclosure refers to a complex comprising the abTCR described herein and at least one TCR-associated signaling module selected from the group consisting of CD3δε, CD3γε, and ζζ, wherein optionally the complex is an octamer comprising the abTCR and CD3δε, CD3γε, and ζζ.

[0025] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR. In some examples, the effector cell comprises a nucleic acid encoding the abTCR. In some examples, the effector cell does not express the first TCR subunit and/or the second TCR subunit. For example, in some examples, a) the first TCR subunit is TCRγ and the second TCR subunit is TCRδ; or b) the first TCR subunit is TCRδ and the second TCR subunit is TCRγ; and the effector cell is an αβ T cell. In some examples, a) the first TCR subunit is TCRγ and the second TCR subunit is TCRδ; or b) the first TCR subunit is TCRδ and the second TCR subunit is TCRγ; and the effector cell is an αβ T cell. In some examples, the effector cell is modified to block or decrease the expression of a first endogenous TCR subunit and/or a second endogenous TCR subunit. For example, in some examples, the first TCR subunit is TCRα and the second TCR subunit is TCRβ; or b) the first TCR subunit is TCRβ and the second TCR subunit is TCRα; and the effector cell is an αβ T cell modified

to block or decrease the expression of TCRα and/or TCRβ. In some examples, a) the first TCR subunit is TCRγ and second TCR subunit is TCRδ; or b) the first TCR subunit is TCRδ and the second TCR subunit is TCRγ; and the effector cell is a γδ T cell modified to block or decrease the expression of TCRγ and/or TCRδ.

[0025a] In one aspect, the present disclosure refers to an effector cell presenting on its surface the abTCR described herein or the complex described herein, or comprising the nucleic acid(s) described herein, 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, optionally:

(i) wherein:

(1) the first TCR subunit is TCRα and second TCR subunit is TCRβ; or

(2) the first TCR subunit is TCRβ and the second TCR subunit is TCRα; and wherein the effector cell is an αβ T cell modified to block or decrease the expression of TCRα and/or TCRβ; or

(ii) wherein:

(1) the first TCR subunit is TCRγ and second TCR subunit is TCRδ; or

(2) the first TCR subunit is TCRδ and the second TCR subunit is TCRγ; and wherein the effector cell is a γδ T cell modified to block or decrease the expression of TCRγ and/or TCRδ.

[0025b] In one aspect, the present disclosure refers to an effector cell presenting on its surface the abTCR described herein or the complex described herein, or comprising the nucleic acid(s) described herein, and wherein the effector cell does not express the first TCR subunit and/or the second TCR subunit, optionally:

(i) wherein:

(1) the first TCR subunit is TCRα and the second TCR subunit is TCRβ; or

(2) the first TCR subunit is TCRβ and the second TCR subunit is TCRα; and wherein the effector cell is a γδ T cell; or

(ii) wherein:

(1) the first TCR subunit is TCRγ and the second TCR subunit is TCRδ; or

(2) the first TCR subunit is TCRδ and the second TCR subunit is TCRγ; and

wherein the effector cell is an αβ T cell.

[0026] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR, wherein the effector cell is a T cell. In some examples, the T 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.

[0027] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR, wherein the effector cell comprises a) a first vector comprising a first nucleic acid sequence encoding the first polypeptide chain of the abTCR under the control of a first promoter and b) a second vector comprising a second nucleic acid sequence encoding the second polypeptide chain of the abTCR under the control of a second promoter.

[0028] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR, wherein the effector cell comprises a vector comprising a) a first nucleic acid sequence encoding the first polypeptide chain of the abTCR under the control of a first promoter; and b) a second nucleic acid sequence encoding the second polypeptide chain of the abTCR under the control of a second promoter.

[0029] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR, wherein the effector cell comprises a vector comprising a) a first nucleic acid sequence encoding the first polypeptide chain of the abTCR and a second nucleic acid sequence encoding the second polypeptide chain of the abTCR, wherein the first and second nucleic acid sequences are under the control of a single promoter.

[0030] In some examples, according to any of the abTCRs (such as isolated abTCRs) described above, there is provided an effector cell presenting on its surface the abTCR, wherein the expression of the first polypeptide chain of the abTCR is more than two-fold different than the expression of the second polypeptide chain of the abTCR.

[0031] In some examples, there is provided a method of killing a target cell presenting a target antigen, comprising contacting the target cell with an effector cell expressing an abTCR according to any of the abTCRs (such as isolated abTCRs) described above, wherein the abTCR specifically binds to the target antigen.

[0032] In some examples, there is provided a method of killing a target cell presenting a target antigen, comprising contacting the target cell with an effector αβ T cell comprising an abTCR that specifically binds to the target antigen comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to the target antigen, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module, and wherein the first TCR subunit is TCRγ and the second TCR subunit is TCRδ, or the first TCR subunit is TCRδ and the second TCR subunit is TCRγ. In some examples, the first polypeptide chain further comprises a first peptide linker between the first antigen-binding domain and the first TCRD and the second polypeptide chain further comprises a second peptide linker between the second antigen-binding domain and the second TCRD. In some examples, the first and/or second peptide linkers comprise, individually, a constant domain or fragment thereof from an immunoglobulin or T cell receptor subunit. In some examples, the first and/or second peptide linkers comprise, individually, a CH1, CH2, CH3, CH4 or CL antibody domain, or a fragment thereof. In some examples, the first and/or second peptide linkers comprise, individually, a Cα, Cβ, Cγ, or Cδ TCR domain, or a fragment thereof.

[0033] In some examples, according to any of the methods of killing a target cell described aboved, the contacting is *in vivo*. In some examples, the contacting is *in vitro*.

[0033a] In one aspect, the present disclosure refers to a method of killing a target cell presenting a target antigen, wherein the method comprises contacting the target cell *in vitro* with the effector cell described herein, wherein the abTCR specifically binds to the target antigen.

[0034] In some examples, there is provided a pharmaceutical composition comprising an abTCR according to any of the abTCRs (such as isolated abTCRs) described above and a pharmaceutically acceptable carrier. In some examples, there is provided a pharmaceutical composition comprising a nucleic acid encoding an abTCR according to any

of the examples described above and a pharmaceutically acceptable carrier. In some examples, there is provided a pharmaceutical composition comprising an effector cell expressing an abTCR according to any of the abTCRs (such as isolated abTCRs) described above and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the abTCR described herein, the nucleic acid(s) described herein, or the effector cell described herein, and a pharmaceutically acceptable carrier.

[0035] In some examples, 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 comprising an effector cell expressing an abTCR according to any of the abTCRs (such as isolated abTCRs) described above.

[0036] In some examples, 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 composition comprising an effector αβ T cell comprising an abTCR that specifically binds to the target antigen comprising: a) a first polypeptide chain comprising a first antigen-binding domain comprising a V_H antibody domain and a first TCRD comprising a first transmembrane domain of a first TCR subunit; and b) a second polypeptide chain comprising a second antigen-binding domain comprising a V_L antibody domains and a second TCRD comprising a second transmembrane domain of a second TCR subunit, wherein the V_H domain of the first antigen-binding domain and the V_L domain of the second antigen-binding domain form an antigen-binding module that specifically binds to the target antigen, wherein the first TCRD and the second TCRD form a T cell receptor module (TCRM) that is capable of recruiting at least one TCR-associated signaling module, and wherein the first TCR subunit is TCRγ and the second TCR subunit is TCRδ, or the first TCR subunit is TCRδ and the second TCR subunit is TCRγ. In some examples, the wherein the first polypeptide chain further comprises a first peptide linker between the first antigen-binding domain and the first TCRD and the second polypeptide chain further comprises a second peptide linker between the second antigen-binding domain and the second TCRD. In some examples, the first and/or second peptide linkers comprise, individually, a constant domain or fragment thereof from an immunoglobulin or T cell receptor subunit. In some examples, the first and/or second peptide linkers comprise, individually, a CH1, CH2, CH3, CH4 or CL antibody domain, or a fragment thereof. In some examples, the first and/or second peptide linkers comprise, individually, a Cα, Cβ, Cγ, or Cδ TCR domain, or a fragment thereof.

[0037] In some examples, according to any of the methods of treating a target antigen-associated disease described above, the target antigen-associated disease is cancer. In some examples, 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, lymphoma, leukemia, 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 examples, the target antigen-associated disease is viral infection. In some examples, 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), HIV (Human immunodeficiency virus), and Hepatitis C Virus (HCV).

[0037a] In one aspect, the present disclosure refers to use of the pharmaceutical composition described herein in the manufacture of a medicament for:

(i) treating cancer in an individual in need thereof, wherein optionally 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, lymphoma, lung cancer, melanoma, mesothelioma, multiple myeloma, pancreatic cancer, pheochromocytoma, plasmacytoma, neuroblastoma, ovarian cancer, prostate cancer, sarcoma, stomach cancer, uterine cancer and thyroid cancer; or

(ii) treating a viral infection in an individual in need thereof, wherein optionally 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), HIV (Human immunodeficiency virus), and Hepatitis C Virus (HCV).

[0038] In some examples, 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 comprising a nucleic acid encoding an abTCR according to any of the abTCRs (such as isolated abTCRs) described above.

[0039] In some examples, there is provided a method of enriching a heterogeneous cell population for an effector cell expressing an abTCR according to any of the abTCRs (such as isolated abTCRs) described above, wherein the method comprises 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.

[0040] In some examples, there is provided a nucleic acid library comprising sequences encoding a plurality of abTCRs according to any of the abTCRs (such as isolated abTCRs) described above.

[0041] In some examples, there is provided a method of screening a nucleic acid library according to any of the examples described above for sequences encoding abTCRs specific for a target antigen, comprising: a) introducing the nucleic acid library into a plurality of cells, such that the abTCRs are expressed on the surface of the plurality of cells;