

BI-SPECIFIC ANTIBODIES FOR USE IN PRODUCING ARMED IMMUNE CELLS**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of the filing date of U.S. Provisional Application
5 No. 62/993,080, filed March 23, 2020, the entire contents of which is incorporated by
reference herein.

BACKGROUND OF THE INVENTION

Cancer is a disease characterized by abnormal cells that divide uncontrollably and have
the ability to infiltrate and destroy normal tissue and/or organ of a subject. Cancer is the second
10 leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018,
in which the most common cancers include, lung cancer (about 2.09 million cases), breast
cancer (about 2.09 million cases), colorectal cancer (about 1.80 million cases), prostate cancer
(about 1.28 million cases), skin cancer (about 1.04 million cases), and gastric cancer (about
1.03 million cases).

15 Treatments for cancers may vary with the type of cancer and how advanced it is.
Conventional treatments for cancers include surgery, radiation therapy, and chemotherapy.
Such treatments usually cause a variety of complications or side effects, such as infection,
blood clot, bleeding, nausea and vomiting, diarrhea, nerve or muscle damage, incontinence,
and sex and fertility issues. Immunotherapy provides an alternative strategy for cancer
20 treatment that aims at specifically stimulating immune responses of a subject against cancer
cells via, for example, blocking immune checkpoints, or enhancing the ability of immune cells
(e.g., T cells or B cells) to target and destroy cancer cells. Serious adverse effects associated
with immunotherapy-mediated overstimulation or non-specific toxicity have been reported in
cancer patients, including neurotoxicity, cytokine release syndrome (CRS), allergy, organ
25 inflammation, and autoimmune disorders.

It is therefore of great importance to develop efficient cancer treatment specifically
targeting cancer cells without affecting normal cells and/or tissues.

SUMMARY OF THE INVENTION

30 The present disclosure is based on the development of bispecific antibodies (BsAbs)
capable of binding to CD3 (*e.g.*, human CD3) and a tumor associated antigen (TAA). Such
BsAbs are capable of attaching to the surface of CD3-positive immune cells *via* binding of the
anti-CD3 moiety in the BsAb to the cell surface CD3 to produce armed immune cells.

Accordingly, the present disclosure features, in some aspects, a bi-specific antibody,

comprising: (a) a first antigen binding fragment that binds human CD3, and (b) a second antigen binding fragment that binds a tumor associated antigen (TAA). The first antigen binding fragment comprises (i) a first heavy chain comprising a first heavy chain variable region (V_H) and (ii) a first light chain comprising a first light chain variable region (V_L). In some embodiments, the first V_H comprises the same heavy chain complementary determining regions (CDRs) as a first reference antibody. In other embodiments, the first V_H comprises or no more than 5 amino acid variations in CDRs relative to the first reference antibody. Alternatively or in addition, the first V_L may comprise the same light chain CDRs as the first reference antibody. In other embodiments, the first V_L may comprise no more than 5 amino acid variations in the CDRs relative to the first reference antibody. In some examples, the first reference antibody is CTA.02. In some examples, the first reference antibody is CTA.03. In other examples, the first reference antibody is CTA.04. In yet other examples, the first reference antibody is CTA.05. Structural information of these exemplary reference antibodies are provided in **Table 1** below. In some examples, the first heavy chain and the first light chain comprise the same V_H and V_L as the first reference antibody.

The second antigen binding fragment comprises a second heavy chain comprising (i) a second heavy chain variable region (V_H), and (ii) a second light chain comprising a second light chain variable region (V_L). The second antigen binding fragment binds a TAA. Examples include CD20, CD19, EGFR, HER2, PSMA, CEA, EpCAM, FAP, PD-L1, CD38, CD33, cMET, CD47, TRAIL-R2, mesothelin, or GD2. In some instances, the second V_H comprises the same heavy chain complementary determining regions (CDRs) as a second reference antibody. Alternatively, the second V_H may comprise no more than five amino acid variations in the CDRs relative to the second reference antibody. Alternatively or in addition, the second V_L may comprise the same light chain CDRs. In other examples, the second V_L may comprise no more than 5 amino acid variations in the CDRs relative to the second reference antibody. In some instances, the second reference antibody is CTAT.01, CTAT.02, CTAT.03, CTAT.04, CTAT.05, CTAT.06, CTAT.07, CTAT.08, CTAT.09, CTAT.10, CTAT.11, CTAT.12, CTAT.13, CTAT.14, CTAT.15, or CTAT.16. See **Table 2** below. In some examples, the second antigen binding fragment comprises the same V_H and same V_L as the second reference antibody.

In some embodiments, the first antigen binding fragment is a Fab fragment and the second antigen binding fragment is a single chain variable fragment (scFv). In some examples, the Fab fragment comprises the first heavy chain, which comprises the first V_H and a CH1 fragment, and the first light chain, which comprises the first V_L and a light chain constant

region. In specific examples, the Fab fragment may comprise the first heavy chain and the first light chain, which respectively comprise the amino acid sequences of (a) SEQ ID NO:10 and SEQ ID NO: 11, (b) SEQ ID NO: 23 and SEQ ID NO: 24, 25, or 228, (c) SEQ ID NO: 35 and SEQ ID NO: 36, or (d) SEQ ID NO: 46 and SEQ ID NO: 47. In some examples, the scFv of
 5 the second antigen binding fragment comprises the amino acid sequence of any one of SEQ ID NOs: 254-271.

In some instances, the scFv is linked to the CH1 fragment, w optionally is via a peptide linker. Alternatively, the scFv is linked to the light chain constant region, optionally via a peptide linker. For example, the bi-specific antibody may comprise a first polypeptide
 10 comprising the first light chain and a second polypeptide comprising, from N-terminus to C-terminus, the first heavy chain, the peptide linker, and the scFv. Examples include any one of SEQ ID NOs: 229-248. Such a bi-specific antibody may comprise a second polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, and 228. See **Table 3** below.

In other instances, the first antigen binding fragment is a single chain variable fragment (scFv) and the second antigen binding fragment is a Fab fragment. The scFv may comprise the amino acid sequence of any one of SEQ ID NOs: 250-253. In some examples, the Fab fragment comprises the second heavy chain, which comprises the second V_H and a CH1 fragment, and the second light chain, which comprises the second V_L and a light chain constant
 20 region. In specific examples, the Fab fragment comprises the first heavy chain and the first light chain, which respectively comprise the amino acid sequences of (1) SEQ ID NO:57 and SEQ ID NO: 58, (2) SEQ ID NO: 72 and SEQ ID NO: 73, (3) SEQ ID NO: 83 and SEQ ID NO: 84, (4) SEQ ID NO: 94 and SEQ ID NO: 95, (5) SEQ ID NO:105 and SEQ ID NO:106, (6) SEQ ID NO: 116 and SEQ ID NO:117, (7) SEQ ID NO: 127 and SEQ ID NO:128, (8) SEQ
 25 ID NO:138 and SEQ ID NO:139, (9) SEQ ID NO:149 and SEQ ID NO:150, (10) SEQ ID NO:160 and SEQ ID NO:161, (11) SEQ ID NO: 171 and SEQ ID NO:172, (12) SEQ ID NO:182 and SEQ ID NO:183, (13) SEQ ID NO:193 and SEQ ID NO:194, (14) SEQ ID NO:204 and SEQ ID NO:205, (15) SEQ ID NO:215 and SEQ ID NO:216, or (16) SEQ ID NO:226 and SEQ ID NO:227.

In some examples, the scFv is linked to the CH1 fragment, optionally via a peptide linker. Alternatively, the scFv is linked to the light chain constant region, optionally via a peptide linker. Any of the peptide linker may be at least 5 amino acids in length.

In yet other instances, both the first antigen binding fragment and the second antigen binding fragment are scFv antibodies. In some examples, the bi-specific antibody comprises a

polypeptide comprising the two scFv antibodies.

In other aspects, the present disclosure provides an armed immune cell, comprising an immune cell that expresses surface CD3, and any of the bi-specific antibodies disclosed herein (e.g., those exemplified in **Tables 1-3**). The armed immune cell displays the bi-specific
5 antibody on the surface via interaction between the first antigen binding fragment in the bi-specific antibody and the CD3 expressed by the immune cell. In some embodiments, the immune cell is a T cell, a B cell, a monocyte, a macrophage, or a combination thereof. In some instances, the T cell can be a CD4+ T cell, a CD8+ T cell, a regulatory T cell, or a natural killer T cell. In some examples, the immune cell is a human immune cell, for example, immune
10 cells derived from a human donor.

In addition, provided herein is a method of producing the armed immune cell as disclosed herein. The method may comprise cultivating a cell population comprising the immune cells in the presence of the bi-specific antibody as disclosed herein to allow for binding of the bi-specific antibody to the immune cells, thereby producing the armed immune
15 cell. The armed immune cells produced by any of the methods disclosed herein are also within the scope of the present disclosure.

In some embodiments, the cell population comprises T cells, B cells, monocytes, macrophages, or a combination thereof. In some examples, the cell population comprises peripheral blood mononuclear cells (PBMCs) or immune cells derived from stem cells *in vitro*.
20 The stem cells may be hematopoietic stem cells, umbilical cord blood stem cells, or induced pluripotent stem (iPS) cells.

In some embodiments, the cultivating step is performed in a culture medium comprising a cytokine, which optionally comprises interleukin 2 (IL-2), interleukin 7 (IL-7), transforming growth factor-beta (TGF- β), or a combination thereof.

Further, provided herein is a method for treating cancer, comprising administering to a
25 subject in need thereof an effective amount of a population of any of the armed immune cells disclosed herein. The subject has or suspected of having a cancer that is positive with the TAA, to which the second antigen binding fragment of the bi-specific antibody binds. In some embodiments, the subject is a human cancer patient. In some embodiments, the armed immune
30 cells are autologous to the subject. Alternatively, the armed immune cells are allogenic to the subject. Exemplary cancers include, but are not limited to, melanoma, esophageal carcinoma, gastric carcinoma, brain tumor, small cell lung cancer, non-small cell lung cancer, bladder cancer, breast cancer, pancreatic cancer, colon cancer, rectal cancer, colorectal cancer, renal cancer, hepatocellular carcinoma, ovary cancer, prostate cancer, thyroid cancer, testis cancer,

head and neck squamous cell carcinoma, leukemia, lymphoma, and myeloma.

In other aspects, the present disclosure features a nucleic acid or a set of nucleic acids (two nucleic acid molecules), which encodes or collectively encodes any of the bi-specific antibodies disclosed herein. In some examples, the nucleic acid or set of nucleic acids is a
5 vector or a set of vectors, for examples, expression vector(s). Host cells (e.g., a bacterial cell, a yeast cell, or a mammalian cell) comprising any of the nucleic acid or set of nucleic acids disclosed herein are also within the scope of the present disclosure.

In addition, the present disclosure features a method for producing a bi-specific antibody, comprising: (i) culturing a host cell as disclosed herein under conditions allowing for
10 expressing of the bi-specific antibody; and (ii) harvesting the bi-specific antibody.

Also within the scope of the present disclosure are armed immune cells as disclosed herein for use in cancer treatment or use of any of the armed immune cells for manufacturing a medicament for use in treating a target cancer.

The details of one or more embodiments of the invention are set forth in the description
15 below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to
20 further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific embodiments presented herein.

FIGs 1A-1N are schematic diagrams of exemplary bi-specific antibody formats. FIGs. 1A-1D: structures of anti-CD3 Fab/anti-TAA scFv bi-specific formats. FIGs. 1E-1H: structures
25 of anti-CD3 scFv/anti-TAA Fab bi-specific formats. FIGs. 1I-1L: structures of anti-CD3 scFv/anti-TAA scFv bi-specific formats. FIGs. 1M: structure of anti-CD3 knob/anti-TAA hole bi-specific antibody formats, which comprises a monovalent anti-CD3 antibody and a monovalent anti-TAA antibody. FIG. 1N: anti-CD3 knob/anti-TAA scFv hole antibody, which comprises a monovalent anti-CD3 antibody and a monovalent anti-TAA scFv-Fc fusion
30 protein.

FIGs. 2A-2E include schematic diagrams of DNA constructs for expressing the illustrated recombinant bi-specific antibodies. FIG. 2A: exemplary constructs for expressing anti-CD3Fab/anti-TAA scFv bi-specific antibodies. FIG. 2B: exemplary constructions for expressing anti-CD3scFv/anti-TAA Fab bi-specific antibodies. FIG. 2C: exemplary constructs

for expressing anti-CD3 scFv/anti-TAA scFv bi-specific antibodies. FIG. 2D: exemplary constructs for expressing anti-CD3 knob/anti-TAA hole bi-specific antibodies. FIG. 2E: exemplary constructs for expressing anti-CD3 knob/anti-TAA scFv hole antibody.

FIG. 3 is a chart showing binding affinity of exemplary bi-specific antibodies to T cells as measured by flow cytometry.

FIG. 4 is a chart depicting the cytotoxic effect of T cells armed with exemplary bi-specific antibodies or activated by OKT3 antibody against HT-29 cancer cells.

FIG. 5 is a chart depicting a time course of the levels of exemplary bi-specific antibodies on the surface of T cells.

FIGs 6A-6B include photos showing expression and assembly of the exemplary bi-specific antibodies as indicated by SDS-PAGE under non-reducing conditions (FIG. 6A) and reducing conditions (FIG. 6B). Estimated molecular weight: intact BsAb = 95 kDa, heavy chain = 60 kDa, and light chain = 25 kDa. Lane 1: protein marker, Lane 2: CTA02Fab/CTAT02scFv, Lane 3: CTA04Fab/CTAT02scFv. Lane 4: CTA03Fab/CTAT02scFv, and Lane 5: CTA05Fab/CTAT02scFv.

FIGs. 7A-7F include photos showing expression and assembly of the exemplary anti-CD3 Fab/anti-tumor bi-specific antibodies as indicated via SDS-PAGE under reducing or non-reducing conditions. Estimated molecular weight: intact BsAb = 95 kDa. FIGs. 7A-7B: CTA03Fab/anti-TAA scFv bi-specific antibodies under non-reducing conditions. FIGs. 7C-7D: CTA03Fab/anti-TAA scFv bi-specific antibodies under reducing conditions. FIGs. 7E-7F: anti-CD3 scFv/CTAT03Fab bispecific antibodies under non-reducing and reducing conditions, respectively.

FIG. 8 is a diagram showing the binding activity of various bi-specific antibodies as indicated to T cells and to tumor cells. CD3⁺ T cells (Jurkat) and CD19⁺ B cell lymphoma (Raji) were incubated separately with exemplary bi-specific antibodies CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv, CTA04Fab/CTAT02scFv, and CTA05Fab/CTAT02scFv BsAbs, and then analyzed with FITC conjugated Goat anti-Human IgG Fab antibody and flow cytometer.

FIGs. 9A-9L include diagrams showing binding activity of exemplary anti-CD3 Fab/anti-tumor scFv bi-specific antibodies as indicated to T cells and to tumor cells. FIG. 9A: CTA03Fab/CTAT02scFv binding to CD3⁺ T cells (Jurkat) and CD19⁺ B cell lymphoma (Raji). FIG. 9B: CTA03Fab/CTAT03scFv binding to CD3⁺ T cells (Jurkat) and EGFR⁺ triple negative breast cancer (MDA-MB-231). FIG. 9C: CTA03Fab/CTAT04scFv binding to CD3⁺ T cells (Jurkat) and HER2⁺ breast cancer (MCF7/HER2). FIG. 9D: CTA03Fab/CTAT05scFv binding

to CD3⁺ T cells (Jurkat) and PSMA⁺ Prostate cancer (LNCaP). FIG. 9E:

CTA03Fab/CTAT07scFv binding to CD3⁺ T cells (Jurkat) and EpCAM⁺ Prostate cancer

(LNCaP). FIG. 9F: CTA03Fab/CTAT08scFv binding to CD3⁺ T cells (Jurkat) and FAP⁺

mouse fibroblasts cell(3T3/FAP). FIG. 9G: CTA03Fab/CTAT09scFv binding to CD3⁺ T cells

5 (Jurkat) and PDL1⁺ triple negative breast cancer (MDA-MB-231). FIG. 9H:

CTA03Fab/CTAT10scFv binding to CD3⁺ T cells (Jurkat) and CD38⁺ B cell lymphoma (Raji).

FIG. 9I: CTA03Fab/CTAT11scFv binding to CD3⁺ T cells (Jurkat) and CD33⁺ human acute

myeloid leukemia (HL-60). FIG. 9J: CTA03Fab/CTAT12scFv binding to CD3⁺ T cells (Jurkat)

and HGFR⁺ human lung carcinoma (A549). FIG. 9K: CTA03Fab/CTAT13scFv binding to

10 CD3⁺ T cells (Jurkat) and CD47⁺ breast cancer (MCF7/HER2). FIG. 9L: binding of BsAbs

CTA02scFv/CTAT03Fab CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and

CTA05scFv/CTAT03Fab to CD3⁺ T cells (Jurkat) (upper panel) and EGFR⁺ colon cancer

(HT-29) (bottom panel). Samples were analyzed with FITC conjugated Goat anti-Human IgG

Fab antibody and flow cytometry.

15 FIG. 10 is a chart showing the retention ability of exemplary BsAbs on T cell surface.

Human T cells were incubated with variant Anti-CD3Fab/anti-CD19scFv BsAbs with a Fab of
4 different anti-CD3 antibody (CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv,

CTA03Fab/CTAT02scFv, and CTA05Fab/CTAT02scFv) for 1hr, and then were cultured in
medium for 5 min, 24, 48, and 72 hr. After the culture, the cells were stained with FITC

20 conjugated Goat anti-Human IgG Fab antibody and the retention of BsAb on T cell surface was
analyzed using flow cytometry.

FIGs. 11A and 11B include diagrams showing formation of T cells armed with

exemplary BsAbs disclosed herein. FIG. 11A: OKT3, CTA01Fab/CTAT02scFv, and

CTA02Fab/CTAT02scFv, from left to right. FIG. 11B: CTA03Fab/CTAT02scFv (left) and

25 CTA05Fab/CTAT02scFv (right). PBMCs were cultured in the presence of OKT3 or the

various BsAbs. The cell cultures were then stained with FITC conjugated CD8 antibody and

PE conjugated goat anti-Human IgGFab, and then analyzed using flow cytometry.

FIGs. 12A-12D include diagrams showing formation of T cells armed with exemplary
BsAbs as indicated. FIG. 12A: OKT3, CTA03Fab/CTAT03scFv, and

30 CTA03Fab/CTAT04scFv (top panel, left to right), and CTA03Fab/CTAT09scFv,

CTA03Fab/CTAT010scFv, and CTA03Fab/CTAT11scFv (bottom panel, left to right). FIG.

12B: CTA03Fab/CTAT05scFv, CTA03Fab/CTAT07scFv, and CTA03Fab/CTAT08scFv (top

panel, left to right), and CTA03Fab/CTAT12scFv and CTA03Fab/CTAT13scFv (bottom, left

to right). FIG. 12C: OKT3, CTA01scFv/CTAT03Fab, and CTA02scFv/CTA03Fab (left to

right). FIG. 12D: CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab (left to right). The cell cultures were stained with anti-CD8 antibody and goat anti-Human IgG Fab, and then analyzed using flow cytometry.

FIGs. 13A-13B include charts showing cytotoxicity activity of T cells induced by OKT3 antibody or armed with exemplary bi-specific antibodies as indicated against tumor cells. FIG. 13A: anti-CD3Fab/anti-CD19scFv BsAbs against B cell lymphoma. FIG. 13B: anti-CD3scFv/CTAT03Fab BsAbs against HT29 cells. Cells were cultured with OKT3 or the exemplary BsAbs as indicated. The cell cultures were then incubated with CD19⁺ B cell lymphoma (Raji) at several effector cell: target cell ratios (3:1, 5:1 and 10:1) for 18 hr. Tumor cell death was determined with CytoTox 96[®] Non-Radioactive Cytotoxicity Assay (Promega, G1780).

FIGs. 14A-14G include charts showing *in vitro* cytotoxic activity of anti-CD3Fab/anti-EGFRscFv BsAb armed T cells against cancer cells. FIG. 14A: CTA01Fab/CTAT03scFv or CTA03Fab/CTAT03scFv armed T cells against HT29 cells (EGFR⁺ colon cancer cells). FIG. 14B: CTA01Fab/CTAT03scFv or CTA03Fab/CTAT03scFv armed T cells against HCT-116 cells (EGFR⁺ colon cancer cells). FIG. 14C: antiCD3Fab/anti-HER2scFv (CTA03Fab/CTAT04scFv) armed T cells against HER2⁺ breast cancer cells (MCF-7/HER2). FIG. 14D: antiCD3Fab/anti-PSMAscFv (CTA03Fab/CTAT05scFv) armed T cells against PSMA⁺ prostate cancer cells (LNCaP). FIG. 14E: antiCD3Fab/anti-EpCAMscFv (CTA03Fab/CTAT07scFv) armed T cells against EpCAM⁺ prostate cancer cells (LNCaP). FIGs. 14F-14G: antiCD3Fab/anti-FAPscFv (CTA03Fab/CTAT08scFv) armed T cells against FAP⁻ mouse fibroblasts cells (3T3) (FIG. 14F) or FAP⁺ mouse fibroblasts cells (3T3/FAP) (FIG. 14G). OKT3-cultured T cells or armed T cells were cocultured with the cancer cells at several effector cell: target cell ratios (3:1, 5:1, and 10:1) for 18 hr. Tumor cell death was determined with CytoTox 96[®] Non-Radioactive Cytotoxicity Assay (Promega, G1780).

FIGs. 15A-15E include charts showing *in vitro* cytotoxic activities of exemplary antiCD3Fab/anti-TAA scFv BsAbs against corresponding cancer cells. FIG. 15A: antiCD3Fab/anti-PDL1scFv (CTA03Fab/CTAT09scFv) armed T cells against triple negative breast cancer cells (MDA-MB-231). FIG. 15B: antiCD3Fab/anti-CD38scFv (CTA03Fab/CTAT10scFv) armed T cells against CD38⁺ B cell lymphoma cells (Raji). FIG. 15C: antiCD3Fab/anti-CD33scFv (CTA03Fab/CTAT11scFv) armed T cells against CD33⁺ human acute myeloid leukemia cells (HL-60). FIG. 15D: antiCD3Fab/anti-HGFRscFv (CTA03Fab/CTAT12scFv) armed T cells against HGFR⁺ human lung carcinoma cells (A549).

FIG. 15E: antiCD3Fab/anti-CD47scFv (CTA03Fab/CTAT13scFv) armed T cells against CD47⁺ breast cancer cells (MCF7/HER2). OKT3-cultured T cells or armed T cells were cocultured with the cancer cells at several effector cell: target cell ratios (3:1, 5:1, and 10:1) for 18 hr. Tumor cell death was determined with CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, G1780).

FIGs 16A-16C include diagrams showing *in vivo* therapeutic efficacy of exemplary anti-CD3Fab/anti-CD19scFv armed-T cell against lymphoma. SCID mice were i.v. inoculated with CD19⁺ B cell lymphoma cells (2.5×10^6 cells/mice). After 3 days, T cell, CTA01Fab/CTAT02scFv armed-T cells and CTA03Fab/CTAT02scFv armed-T cells were i.v. injected into the mice (5×10^6 cells/mice, once a week for 4 times). FIG. 16A: Body weight. FIG. 16B: survival rate. FIG. 16C: incidence of hindlimb paralysis.

FIGs. 17A-17B include diagrams showing *in vivo* therapeutic efficacy of exemplary CTA03Fab/CTAT03scFv armed-T cells and CTA03Fab/CTAT04scFv armed-T cells against human triple-negative breast cancer. ASID mice were s.c. inoculated with clinical human breast tumor tissues. After 19 days, T cell, CTA03Fab/CTAT03scFv armed-T cells and CTA03Fab/CTAT04scFv armed-T cells were i.v. injected into the mice (5×10^6 cells/mice, once a week for 4 times). FIG. 17A: Body weight. FIG. 17B: tumor size.

FIGs. 18A-18D include diagrams showing formation of BsAb Armed-NKT cells with various anti-CD3/anti-TAA BsAbs. FIG. 18A: OKT3 (left) and CTA03Fab/CTAT03scFv (right). FIG. 18B: CTA03Fab/CTAT04scFv (left) and CTA03Fab/CTAT05scFv (right). FIG. 18C: OKT3, CTA01scFv/CTAT03Fab, and CTA02scFv/CTAT03Fab (left to right). FIG. 18D: CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab (left to right). NKT cells (CD8⁺ CD25⁺) were cultured and differentiated in the presence of the OKT3 antibody or the BsAbs as indicated. The cells were then stained with anti-CD8 antibody, anti-CD56 antibody and FITC-conjugated anti-Human IgG Fab antibody. BsAbs on cell surface were analyzed using flow cytometry.

FIGs 19A-19C include charts showing binding activity and toxicity of point-mutant BsAbs CTA03Fab/CTAT02scFv. FIG. 19A: binding activity to CD3⁺ T cells (Jurkat). FIG. 19B: binding activity to CD19⁺ B cells lymphoma (Raji). FIG. 19C: cytotoxicity of armed T cells relative to T cells cultured with OKT3. The cells were included with CTA03Fab/CTAT02scFv, CTA03-01Fab/CTAT02-01scFv, CTA03-01Fab/CTAT02-02scFv, CTA03-02Fab/CTAT02-02scFv and CTA03-02Fab/CTAT02-01scFv BsAbs, and then stained with FITC conjugated Goat anti-Human IgG Fab antibody. Fluorescent signal on cell surface was detected using flow cytometry. For cytotoxicity analysis, T cells were cultured with OKT3

or with the various BsAbs to form armed T cells. The cells were then cocultured with CD19⁺ B cell lymphoma (Raji) at several effector cell: target cell ratios (3:1, 5:1 and 10:1) for 18 hr. Tumor cell death was determined with CytoTox 96[®] Non-Radioactive Cytotoxicity Assay (Promega, G1780).

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DETAILED DESCRIPTION OF THE INVENTION

The present disclosure is based on the development of bispecific antibodies (BsAbs) capable of binding to CD3 (*e.g.*, human CD3) and a tumor associated antigen (TAA). Such BsAbs are capable of attaching to the surface of CD3-positive immune cells via binding of the anti-CD3 moiety in the BsAb to the cell surface CD3 to produce armed immune cells. As used
10 anti-CD3 moiety in the BsAb to the cell surface CD3 to produce armed immune cells. As used herein, the term “an armed immune cell” refers to an immune cell that displays a bispecific antibody as disclosed herein via binding of the anti-CD3 moiety in the bispecific antibody to a cell surface CD3 molecule. Via the anti-TAA moiety in the bispecific antibody on the cell surface, an armed immune cell is capable of targeting disease cells (*e.g.*, cancer cells) that
15 express the TAA, thereby eliciting immune responses against the disease cells.

It is reported herein that the BsAbs disclosed herein show high binding activities to both CD3⁺ immune cells and TAA⁺ cancer cells and high retention levels on CD3⁺ immune cells for at least 72 hours. Immune cells armed with the BsAbs disclosed herein exhibited high cytotoxicity against cancer cells expressing the corresponding TAA both *in vitro* and *in vivo*.
20 Thus, the BsAbs and the armed immune cells disclosed herein would be expected to have high anti-cancer effects.

Accordingly, provided herein are bispecific antibodies capable of binding to CD3 and an TAA, armed immune cells displaying such, methods of using the bispecific antibodies for producing armed immune cells, and methods of treating cancer using the armed immune cells.

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I. Bispecific Antibodies That Bind CD3 and a Tumor Associated Antigen

In some aspects, the present disclosure provides bispecific antibodies capable of binding to CD3 (*e.g.*, CD3⁺ cells) and a tumor associated antigen (TAA) (*e.g.*, cancer cells expressing the TAA on cell surface). An antibody (interchangeably used in plural form) is an
30 immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. A bispecific antibody as disclosed herein comprises two antigen-binding moieties, one of which binds CD3 such as human CD3 and the other one of which binds a tumor associated antigen such as those disclosed herein.

A typical antibody molecule comprises a heavy chain variable region (V_H) and a light chain variable region (V_L), which are usually involved in antigen binding. The V_H and V_L regions can be further subdivided into regions of hypervariability, also known as “complementarity determining regions” (“CDR”), interspersed with regions that are more conserved, which are known as “framework regions” (“FR”). Each V_H and V_L is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the Chothia definition, the AbM definition, and/or the contact definition, all of which are well known in the art. See, *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia *et al.*, (1989) *Nature* 342:877; Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917, Al-lazikani *et al.* (1997) *J. Molec. Biol.* 273:927-948; and Almagro, J. *Mol. Recognit.* 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs.

In some embodiments, an antibody moiety disclosed herein may share the same heavy chain and/or light chain complementary determining regions (CDRs) or the same V_H and/or V_L chains as a reference antibody. Two antibodies having the same V_H and/or V_L CDRs means that their CDRs are identical when determined by the same approach (*e.g.*, the Kabat approach, the Chothia approach, the AbM approach, the Contact approach, or the IMGT approach as known in the art. See, *e.g.*, bioinf.org.uk/abs/). Such anti-CD19 antibodies may have the same V_H , the same V_L , or both as compared to an exemplary antibody described herein.

In some embodiments, an antibody moiety disclosed herein may share a certain level of sequence identity as compared with a reference sequence. The “percent identity” of two amino acid sequences is determined using the algorithm of Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 87:2264-68, 1990, modified as in Karlin and Altschul *Proc. Natl. Acad. Sci. USA* 90:5873-77, 1993. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* *J. Mol. Biol.* 215:403-10, 1990. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of interest. Where gaps exist between two sequences, Gapped BLAST can be utilized as described in Altschul *et al.*, *Nucleic Acids Res.* 25(17):3389-3402, 1997. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

In some embodiments, an antibody moiety disclosed herein may have one or more

amino acid variations relative to a reference antibody. The amino acid residue variations as disclosed in the present disclosure (*e.g.*, in framework regions and/or in CDRs) can be conservative amino acid residue substitutions. As used herein, a “conservative amino acid substitution” refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, *e.g.*, Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

15 **A. Bispecific Antibodies**

The bispecific antibodies disclosed herein comprise a CD3 binding moiety (anti-CD3 moiety) and a TAA binding moiety (anti-TAA moiety).

(i) CD3 Binding Moieties

The anti-CD3 moiety in any of the bispecific antibodies disclosed herein comprises an antigen-binding fragment specific to a CD3 molecule, for example, human CD3. In some embodiments, the anti-CD3 moiety comprises a heavy chain variable region (V_H) and a light chain variable region (V_L). In some instances, the anti-CD3 moiety may be derived from a reference anti-CD3 antibody. Exemplary reference anti-CD3 antibodies include CTA.02, CTA.03, CTA.04, or CTA.05. The structural information of these reference anti-CD3 antibodies are provided in **Table 1** below (heavy chain and light chain complementary determining regions (CDRs) based on the Kabat scheme are in boldface and underlined).

Table 1. Reference anti-CD3 Antibodies

Reference Antibody		Amino Acid Sequences	SEQ ID NO
CTA.02	VH CDR1	RYTMH	1
	VH CDR2	<u>YINPSRGYTNYNQK</u> FED	2
	VH CDR3	YYDDHYCLDY	3
	VH	QVQLQQSGAELARPGASVKMSCKASGYTFT <u>RYTMH</u> WVKQRPQG GLEWIG <u>YINPSRGYTNYNQK</u> FDKATLTDDKSSSTAYMQLSSL TSEDSAVYYCARY <u>YYDDHYCLDY</u> WGQGTTLTVSS	4

	VL CDR1	SASSSVSYMN	5
	VL CDR2	DTSKLAS	6
	VL CDR3	QQWSSNPFT	7
	VL	QIVLTQSPAIMASASPGEKVTMTCS SASSSVSYMN WYQQKSGTSP KRWIY DTSKLAS GVPAPHFRGSGSGTSYSLTISGMEADAATYY CQQWSSNPFT FGSGTKLEINR	8
	scFv	GGSGGG QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHW VKQRPQGGLIEWIGYINPSRGYTNYNQKFKDKATLTDDKSSSTA YMQLSSTLSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS GGG GGSGGGSGGGG SQIVLTQSPAIMASASPGEKVTMTCSASSSVSY MNWYQQKSGTSPKRWIYDTSKLASGVPAPHFRGSGSGTSYSLTI SGMEADAATYYCQQWSSNPFTFGSGTKLEINR	9 250 (no N-terminus peptide linker)
	VH-CH1	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPQG GLEWIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSTL SEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSAKTTAPSVYP LAPVCGGTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDDK I	10
CTA.03	VL-Ck	QIVLTQSPAIMASASPGEKVTMTCSASSSVSYMNWYQQKSGTSP KRWIYDTSKLASGVPAPHFRGSGSGTSYSLTISGMEADAATYY CQQWSSNPFTFGSGTKLEINRADTAPTVSIFPPSSEQLTSGGA SVVCFLNFPKIDINVKKIDGSEKQNGVLNSWTDQDSKDY SMSTLTTLTKDEYERHNSYTCETHKSTSPIVKSFNRNEC	11
	VH CDR1	SFPMA	12
	VH CDR2	TISTSGGRYYRDSVKG	13
	VH CDR3	FRQYSGGFDY	14
	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSS SFPMA WVRQAPGK GLEWVST TISTSGGRYYRDSVKG RFTISRDNKNTLYLQMNLSL RAEDTAVYYCAK FRQYSGGFDY WGQGTTLTVSS	15
	VL CDR1	TLSSGNIENNYVH	16
	VL CDR2	DDDKRPD	17
	VL CDR2-02	DDDKRPE	18
	VL CDR3	HSYVSSFN	19
	VL	DIQLTQPNVSVSTSLGSTVKLSCT TLSSGNIENNYVH WYQLYEGR SPTTMIY DDDKRPD GVPDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCH HSYVSSFN FGGGTKLTVLRQ	20
	VL-01	DIQLTQPNVSVSTSLGSTVKLSCT TLSSGNIENNYVH WYQLYEGR SPTTMIY DDDKRPDA GVPDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCH HSYVSSFN FGGGTKLTVLRQ	21
	VL-02	DIQLTQPNVSVSTSLGSTVKLSCT TLSSGNIENNYVH WYQLYEGR SPTTMIY DDDKRPE GVPDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCH HSYVSSFN FGGGTKLTVLRQ	249
	scFv	GGSGGG EVQLLESGGGLVQPGGSLRLSCAASGFTFSS SFPMA WVRQAPGK GLEWVSTISTSGGRYYRDSVKGRFTISRDNKNTLYLQMNLSLRAEDTAVYYCAK FRQYSGGFDYWGQGTTLTVSS GGG GGSGGGSGGGG DIQLTQPNVSVSTSLGSTVKLSCT TLSSGNIEN NYVHWYQLYEGRSPTTMIYDDDKRPDGVDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCHSYVSSFNFGGGTKLTVLRQ	22 251 (no N-terminus peptide linker)
	VH-CH1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSS SFPMA WVRQAPGK GLEWVSTISTSGGRYYRDSVKGRFTISRDNKNTLYLQMNLSL RAEDTAVYYCAKFRQYSGGFDYWGQGTTLTVSSASTKGPVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHPKPSNTKVDDK	23

		KVEPKSC	
	VL-C _λ	DIQLTQPNVSVSTSLGSTVKLSCTLSGGNIENNYVHWYQLYEGR SPTTMIYDDDKRPDGVDPDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCHSYVSSFNVFGGGKLTVLRLQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQS NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC S	24
	VL-C _λ -01	DIQLTQPNVSVSTSLGSTVKLSCTLSGGNIENNYVHWYQLYEGR SPTTMIYDDDKRPD AV PDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCHSYVSSFNVFGGGKLTVLRLQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQS NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC S	228
	VL-C _λ -02	DIQLTQPNVSVSTSLGSTVKLSCTLSGGNIENNYVHWYQLYEGR SPTTMIYDDDKRP E GVDPDRFSGSIDRSSNSAFLTIHNVAIEDE AIYFCHSYVSSFNVFGGGKLTVLRLQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQS NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC S	25
CTA.04	VH CDR1	RYTME	26
	VH CDR2	YINPSRGYTNYNQKVKD	27
	VH CDR3	YYDDHYCLDY	28
	VH	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRY TMH WVRQAPGK GLEWIG YINPSRGYTNYNQKVKD RFTISRDNKNTAFLQMDSL RPEDTGVYFCARY YYDDHYCLDY WGQGPVTVSS	29
	VL CDR1	SASSSVSYMN	30
	VL CDR2	DTSKLAS	31
	VL CDR3	QQWSSNPFT	32
	VL	DIQMTQSPSSLSASVGDRVITITC SASSSVSYMN WYQTPGKAP KRWIY DTSKLAS GVPSRFSGSGSGTDYFTFTISLQPEDATYY CQQWSSNPFT FGQGTKLQITR	33
	scFv	GGSGGGG QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHW VRQAPGKGLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNTA FLQMDSLRPEDTGVYFCARYDDHYCLDYWGQGPVTVSS GGG GGSGGGGGGGG DIQMTQSPSSLSASVGDRVITITCSASSSVSY MNWYQTPGKAPKRWIYDTSKLASGVPSRFSGSGSGTDYFTFTI SSLQPEDATYYCQQWSSNPFTFGQGTKLQITR	34 252 (no N-terminus peptide linker)
	VH-CH1	QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKVKDRFTISRDNKNTAFLQMDSL RPEDTGVYFCARYDDHYCLDYWGQGPVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSC	35
	VL-C _κ	DIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWYQTPGKAP KRWIYDTSKLASGVPSRFSGSGSGTDYFTFTISLQPEDATYY CQQWSSNPFTFGQGTKLQITRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSITLTSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC	36
CTA.05	VH CDR1	SYTME	37
	VH CDR2	YINPSRGYTHYNQKLED	38
	VH CDR3	SAYDYDGFAY	39
	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFI SYTMH WVRQAPGQ	40

		GLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSL RSED TAVYYCARSAYDYDGFAYWGQGLVTVSS	
	VL CDR1	SASSSVSYMN	41
	VL CDR2	DTSELAS	42
	VL CDR3	QQWSSNPPT	43
	VL	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAP KRLIYDTSKLASGVPSRFSGSGSGTDFTLTISLQPEDFATYY CQQWSSNPPTFGGGTKVEIK	44
	scFv	GGSGGGQVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHW VRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTA YMESSLRSED TAVYYCARSAYDYDGFAYWGQGLVTVSSGG GGSGGGSGGGGSDIQMTQSPSSLSASVGDRTITCSASSSVS YMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLT ISLQPEDFATYYCQQWSSNPPTFGGGTKVEIK	45 253 (no N-terminus peptide linker)
	VH-CH1	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQ GLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSL RSED TAVYYCARSAYDYDGFAYWGQGLVTVSSASTKGPSVF PLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVAIVTVPSSNFGTQTYTCNVDPKPKNTK VDKTV	46
	VL-Ck	DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAP KRLIYDTSKLASGVPSRFSGSGSGTDFTLTISLQPEDFATYY CQQWSSNPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTY SLSTLTLSKADYEKHKLYACEVTHQGLSPVTKSFNRGEC	47

An anti-CD3 binding moiety (and an anti-TAA binding moiety disclosed below) derived from a reference antibody refers to binding moieties having substantially similar structural and functional features as the reference antibody. Structurally, the binding moiety may have the same heavy and/or light chain complementary determining regions or the same V_H and/or V_L chains as the reference antibody. Alternatively, the binding moiety may only have a limited number of amino acid variations in one or more of the framework regions and/or in one or more of the CDRs without significantly affecting its binding affinity and binding specificity relative to the reference antibody.

In some embodiments, the anti-CD3 binding moiety may comprise the same heavy chain CDRs as those in antibody CTA.02, which are provided in **Table 1** above. Alternatively or in addition, the anti-CD3 binding moiety may have the same light chain CDRs as those in antibody CTA.02, which are also provided in **Table 1** above. Such an anti-CD3 binding moiety may comprise the same V_H and/or V_L chains as CTA.02. Alternatively, the anti-CD3 binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTA.02. For example, the anti-CD3 binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTA.02.

In some embodiments, the anti-CD3 moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTA.02. For example, the anti-CD3 moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTA.02.

5 Alternatively or in addition, the anti-CD3 antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTA.02. As used herein, “individually” means that one CDR of an antibody shares the indicated sequence identity relative to the corresponding CDR of a reference antibody (*e.g.*, the anti-CD3 reference antibodies provided in **Table 1** above or any
10 of the anti-TAA reference antibodies disclosed below). “Collectively” means that three V_H or V_L CDRs of an antibody in combination share the indicated sequence identity relative the corresponding three V_H or V_L CDRs of the reference antibody in combination.

In some instances, the anti-CD3 moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain
15 and light chain CDRs collectively relative to those in the CDRs of CTA.02. In some instances, the anti-CD3 moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTA.02 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some embodiments, the anti-CD3 binding moiety may comprise the same heavy
20 chain CDRs as those in antibody CTA.03, which are provided in **Table 1** above. Alternatively or in addition, the anti-CD3 binding moiety may have the same light chain CDRs as those in antibody CTA.03, which are also provided in **Table 1** above. Such an anti-CD3 binding moiety may comprise the same V_H and/or V_L chains as CTA.03. Alternatively, the anti-CD3 binding moiety may comprise amino acid variations in one or more of the framework
25 regions relative to the corresponding framework regions in CTA.03. For example, the anti-CD3 binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTA.03. In one specific example, the anti-CD3 moiety disclosed herein comprises a mutation at position G58 of the VL chain
30 relative to CTA.03, for example, an amino acid residue substitution (*e.g.*, G58A). See, *e.g.*, CTA.03 VL-01 in **Table 1** above.

In some embodiments, the anti-CD3 moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTA.03. For example, the anti-CD3 moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%)

sequence identity, individually or collectively, as compared with the V_H CDRs of CTA.03. Alternatively or in addition, the anti-CD3 antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTA.03.

5 In some instances, the anti-CD3 moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTA.03. In some instances, the anti-CD3 moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTA.03 and comprise one or more amino acid variations in one or more of the other heavy
10 chain and light chain CDRs. In specific examples, the anti-CD3 moiety disclosed herein may comprise a mutation at position D57 of the VL chain relative to that of CTA.03, for example, an amino acid residue substitution such as D57E. See, *e.g.*, CTA.03 VL-02 in **Table 1**.

 In some examples, the anti-CD3 binding moiety may comprise the same heavy chain CDRs as those in antibody CTA.04, which are provided in **Table 1** above. Alternatively or in
15 addition, the anti-CD3 binding moiety may have the same light chain CDRs as those in antibody CTA.04, which are also provided in **Table 1** above. Such an anti-CD3 binding moiety may comprise the same V_H and/or V_L chains as CTA.04. Alternatively, the anti-CD3 binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTA.04. For example, the anti-CD3 binding moiety
20 may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTA.04.

 In some embodiments, the anti-CD3 moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTA.04. For example, the anti-CD3 moiety
25 may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTA.04. Alternatively or in addition, the anti-CD3 antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTA.04.

30 In some instances, the anti-CD3 moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTA.04. In some instances, the anti-CD3 moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTA.04 and comprise one or more amino acid variations in one or more of the other heavy

chain and light chain CDRs.

In some examples, the anti-CD3 binding moiety may comprise the same heavy chain CDRs as those in antibody CTA.05, which are provided in **Table 1** above. Alternatively or in addition, the anti-CD3 binding moiety may have the same light chain CDRs as those in
 5 antibody CTA.05, which are also provided in **Table 1** above. Such an anti-CD3 binding moiety may comprise the same V_H and/or V_L chains as CTA.05. Alternatively, the anti-CD3 binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTA.05. For example, the anti-CD3 binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10,
 10 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTA.05.

In some embodiments, the anti-CD3 moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTA.05. For example, the anti-CD3 moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%)
 15 sequence identity, individually or collectively, as compared with the V_H CDRs of CTA.05. Alternatively or in addition, the anti-CD3 antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTA.05.

In some instances, the anti-CD3 moiety may comprise up to 10 amino acid variations
 20 (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTA.05. In some instances, the anti-CD3 moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTA.05 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

25 (ii) TAA Binding Moiety

In addition to the anti-CD3 binding moiety, any of the bispecific antibodies disclosed herein further comprises a second binding moiety specific to a tumor associated antigen. The term “tumor-associated antigen” (TAA) is well-understood in the art, and refers to a molecule
 30 that is differentially expressed on and/in cancerous cells relative to non-cancerous cells of the same cell type. Non-limiting examples of TAA include CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD30, CD33, CD34, CD37, CD38, CD40, CD43, CD44v6, CD47, CD50, CD52, CD56, CD63, CD72a, CD74, CD78, CD79a, CD79b, CD86, CD134, CD137, CD138, CD248, CD319, α v β 3, α 5 β 1, human epidermal growth factor receptor (EGFR or HER1), HER2, HER3,

HER4, vascular endothelial growth factor receptor 1 (VEGFR-1), VEGFR-2, VEGFR-3, TRAIL-R2, carbohydrate antigen 19-9 (CA 19-9), carbohydrate antigen 125 (CA 125), carcinoembryonic antigen (CEA), mucin 1 (MUC 1), MUC2, MUC3, MUC4, MUC5, MUC7, ganglioside GD2, ganglioside GD3, ganglioside GM2, carbonic anhydrase IX (CAIX), sonic hedgehog (SHH), melanoma chondroitin sulfate proteoglycan (MCSP), chondroitin sulfate proteoglycan 4 (CSPG4), six-transmembrane epithelial antigen of prostate (STEAP), A33 antigen, desmoglein-2 (Dsg2), Dsg3, Dsg4, E-cadherin neoepitope, fetal nicotinic acetylcholine receptor (fnAChR), muellerian inhibitory substance receptor type II (MISIIR), tumor-associated antigen L6 (TAL6), Thomsen-Friedenreich (TF) antigen, EPHA1, EPHA2, EPHA3, EPHA4, EPHA7, EPHA8, EPHA10, EPHB4, cancer testis antigen (CTA), NY-BR1, tumor-associated glycoprotein 72 (TAG-72), alpha-fetoprotein (AFP), brother of the regulator of the imprinted site (BORIS), B-cell activating factor (BAFF), extradomain-B fibronectin (EDB-FN), glycoprotein A33 (GPA33), tenascin-C (TNC), melanoma-associated antigen (MAGE), GAGE, BAGE, prostate stem cell antigen (PSCA), mesothelin, mucine-related Tn, Sialyl Tn, globo H, stage-specific embryonic antigen-4 (SSEA-4), epithelial cell adhesion molecule (EpCAM), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1), programmed cell death 1 ligand 1 (PD-L1), prostate-specific membrane antigen (PSMA), fibroblast activation protein (FAP), vascular cell adhesion protein 1 (VCAM-1), insulin-like growth factor receptor (IGFR), or hepatocyte growth factor receptor (HGFR).

In some embodiments, the anti-TAA binding moiety comprises a heavy chain variable region (V_H) and a light chain variable region (V_L). In some examples, the anti-TAA binding moiety is specific to CD20 (*e.g.*, human CD20). In some examples, the anti-TAA binding moiety is specific to CD19 (*e.g.*, human CD19). In some examples, the anti-TAA binding moiety is specific to EGFR (*e.g.*, human EGFR). In some examples, the anti-TAA binding moiety is specific to HER2 (*e.g.*, human HER2). In some examples, the anti-TAA binding moiety is specific to PSMA (*e.g.*, human PSMA). In some examples, the anti-TAA binding moiety is specific to CEA (*e.g.*, human CEA). In some examples, the anti-TAA binding moiety is specific to EpCAM (*e.g.*, human EpCAM). In some examples, the anti-TAA binding moiety is specific to FAP (*e.g.*, human FAP). In some examples, the anti-TAA binding moiety is specific to PDL1 (*e.g.*, human PDL1). In some examples, the anti-TAA binding moiety is specific to CD38 (*e.g.*, human CD38). In some examples, the anti-TAA binding moiety is specific to CD33 (*e.g.*, human CD33). In some examples, the anti-TAA binding moiety is specific to HGFR (cMET) (*e.g.*, human cMET). In some examples, the anti-TAA binding

moiety is specific to CD47 (*e.g.*, human CD47). In some examples, the anti-TAA binding moiety is specific to TRAIL-R2 (*e.g.*, human TRAIL-R2). In some examples, the anti-TAA binding moiety is specific to mesothelin (*e.g.*, human mesothelin). In some examples, the anti-TAA binding moiety is specific to GD2 (*e.g.*, human GD2).

5 In some instances, the anti-TAA moiety may be derived from a reference anti-TAA antibody. Exemplary reference anti-TAA antibodies include CTAT.01-CTAT.16. The structural information of these reference anti-CD3 antibodies are provided in **Table 2** below (heavy chain and light chain complementary determining regions (CDRs) based on the Kabat scheme are in boldface and underlined).

10

Table 2. Reference Anti-Tumor Associated Antigen Antibodies

Reference Antibody (TAA)		Amino Acid Sequences	SEQ ID NO
CTAT.01 (CD20)	VH CDR1	SYNMH	48
	VH CDR2	AIYPGNGDTSYNQKFKG	49
	VH CDR3	STYYGGDWYFNV	50
	VH	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEW IGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVY YCARSTYYGGDWYFNVWGAGTTTVSA	51
	VL CDR1	RASSSVSYIH	52
	VL CDR2	ATSNLAS	53
	VL CDR3	QQWTSNPPT	54
	VL	QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWQQKPGSSPKPWI YATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK	55
	scFv	GGGGGGQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTTVSAAGGGGGGGGGGGGGSQIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK	56 254 (no N-terminus peptide linker)
	VH-CH1	QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEW IGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVY YCARSTYYGGDWYFNVWGAGTTTVSAASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSC	57
	VL-Ck	QIVLSQSPAILASAPGEKVTMTCRASSSVSYIHWQQKPGSSPKPWI YATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSLTLSKADYEKH KVIACEVTHQGLSSPVTKSFNRGEC	58
CTAT.02 (CD19)	VH CDR1	DYGV	59

	VH CDR2	VIWGSETTYNSALKS	60
	VH CDR3	HYYYGGSYAMDY	61
	VH	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEW LGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIYY CAKHYYGGSYAMDYWGQGTSTVTVSS	62
	VL CDR1	RASQDISKYL N	63
	VL CDR2	WTSRLHS	64
	VL CDR3	QQGNTLPYT	65
	VL	DIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKPDGTVKLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PYTFGGGKLEIT	66
	VL-01	DIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKPDATV KLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PYTFGGGKLEIT	67
	VL-02	DIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKEGTV KLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PYTFGGGKLEIT	68
	scFv	GGGSGGGEVKLQESGPGIVAPSQSLSVTCTVSGVSLPDYGVSWIRQP PRKGLEWLGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIYYCAKHYYGGSYAMDYWGQGTSTVTVSS GGGSGGGSGGGG SDIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKPDGTVKLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT LPYTFGGGKLEIT	69 255 (no N-terminus peptide linker)
	scFv-01	GGGSGGGEVKLQESGPGIVAPSQSLSVTCTVSGVSLPDYGVSWIRQP PRKGLEWLGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIYYCAKHYYGGSYAMDYWGQGTSTVTVSS GGGSGGGSGGGG SDIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKPDATV KLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT LPYTFGGGKLEIT	70 256 (no N-terminus peptide linker)
	scFv-02	GGGSGGGEVKLQESGPGIVAPSQSLSVTCTVSGVSLPDYGVSWIRQP PRKGLEWLGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIYYCAKHYYGGSYAMDYWGQGTSTVTVSS GGGSGGGSGGGG SDIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKEGTV KLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNT LPYTFGGGKLEIT	71 257 (no N-terminus peptide linker)
	VH-CH1	EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPPRKGLEW LGVIWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTD DTAIYY CAKHYYGGSYAMDYWGQGTSTVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDRVEPKSCDK	72
	VL-Ck	DIQMTQTTSSLSASLGDRVTISCRASQDISKYL N WYQQKPDGTVKLL IYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTL PYTFGGGKLEITRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKADY EK HKLYACEVTHQGLSSPVTKSFNRGEC	73
CTAT.03 (EGFR)	VH CDR1	NYGVH	74
	VH CDR2	VIWGGNTDYNTFFTS	75
	VH CDR3	ALTYDYEFAY	76

	VH	QVQLKQSGPGLVQPSSQLSITCTVSGFSLT NYGVH WVRQSPGKGLEW LGVIWSSGGNTDYN TPFTS RSLKINKDNSKQVFFKMNLSQSNDAIYY CARALTYDYEFAYWGQGLVTVSA	77
	VL CDR1	RASQSIGTNIH	78
	VL CDR2	YASESIS	79
	VL CDR3	QQNNNNWFET	80
	VL	DILLTQSPVILSVSPGERVSFSC RASQSIGTNIH HWYQORTNGSPRLL IKY ASESIS GIPSRFSGSGSGTDFTLINSVESEDIADYYC QQNNNNW PTTFGAGTKLELK	81
	scFv	GGGSGGG QVQLKQSGPGLVQPSSQLSITCTVSGFSLT NYGVH WVRQSPGKGLEWLGVIWSSGGNTDYN TPFTS RSLKINKDNSKQVFFKMNLSQSNDAIYYCARALTYDYEFAYWGQGLVTVSA GGGSGGGSGGGG DILLTQSPVILSVSPGERVSFSC RASQSIGTNIH HWYQORTNGSPRLL IKY ASESIS GIPSRFSGSGSGTDFTLINSVESEDIADYYC QQNNNNW PTTFGAGTKLELK	82 258 (no N-terminus peptide linker)
	VH-CH1	QVQLKQSGPGLVQPSSQLSITCTVSGFSLT NYGVH WVRQSPGKGLEW LGVIWSSGGNTDYN TPFTS RSLKINKDNSKQVFFKMNLSQSNDAIYY CARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVPSSSLGTQTYICNVNHKPSNTKVDKRVPEPKSCDK	83
CTAT.04 (HER2)	VL-Ck	DILLTQSPVILSVSPGERVSFSC RASQSIGTNIH HWYQORTNGSPRLL IKY ASESIS GIPSRFSGSGSGTDFTLINSVESEDIADYYC QQNNNNW PTTFGAGTKLELK RRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK HKLYACEVTHQGLSSPVTKSFNRGEC	84
	VH CDR1	DTYIH	85
	VH CDR2	RIYPTNGYTRYADSVKQ	86
	VH CDR3	WGDDGFYAMDY	87
	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIK DTYIH WVRQAPGKGLEW VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCSR WGDDGFYAMDY WGQGLVTVSS	88
	VL CDR1	RASQDVNTAVA	89
	VL CDR2	SASFLYS	90
	VL CDR3	QQHYTTPPT	91
	VL	DIQMTQSPSSLSASVGDRVTITC RASQDVNTAVA WYQQKPGKAPKLL IY SASFLYS GVPSRFSGSRSGTDFLTISLQPEDFATYYC QQHYTT PTTFGQGTKEIK	92
	scFv	GGGSGGG EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCSR WGDDGFYAMDY WGQGLVTVSS GGGSGGGSGGGG SDI QMTQSPSSLSASVGDRVTITC RASQDVNTAVA WYQQKPGKAPKL LIYSASFLYSGVPSRFSGSRSGTDFLTISLQPEDFATYYC QQHYT TPPTFGQGTKEIK	93 259 (no N-terminus peptide linker)
	VH-CH1	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEW VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVY YCSR WGDDGFYAMDY WGQGLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEPKSCDK	94

	VL-Ck	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLL IYSASFVLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHYTT PPTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEK HKLYACEVTHQGLSSPVTKSFNRGEC	95
CTAT.05 (PSMA)	VH CDR1	EYTIH	96
	VH CDR2	NINPNNGGTTYNQKFED	97
	VH CDR3	GWNFY	98
	VH	EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEW IGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDATVY YCAAGWNFDYWGQGTITVTVSS	99
	VL CDR1	KASQDVGTAVD	100
	VL CDR2	WASTRHT	101
	VL CDR3	QQYNSYPLT	102
	VL	DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLL IYWASTRHTGVPDRFTGSGSGTDFTLTISLQPEDFADYFCQQYNSY PLTFGGGKLEIK	103
	scFv	GGGSGGGEVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKA SGKGLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLR SEDATVYYCAAGWNFDYWGQGTITVTVSSGGGSGGGSGGGSDIVM TQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLLIYWA STRHTGVPDRFTGSGSGTDFTLTISLQPEDFADYFCQQYNSYPLTF GGGKLEIK	104 260 (no N-terminus peptide linker)
	VH-CH1	EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQASGKGLEW IGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDATVY YCAAGWNFDYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	105
	VL-Ck	DIVMTQSPSSLSASVGDRVTITCKASQDVGTAVDWYQQKPGKAPKLL IYWASTRHTGVPDRFTGSGSGTDFTLTISLQPEDFADYFCQQYNSY PLTFGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEK HKLYACEVTHQGLSSPVTKSFNRGEC	106
CTAT.06 (CEA)	VH CDR1	EYGMN	107
	VH CDR2	WINTKSGEATYVEEFKG	108
	VH CDR3	WDFYDYVDEAMY	109
	VH	QVQLVQSGSELKKPGASVKVCKASGYTFTEYGMNVWRQAPGQGLEW MGWINTKSGEATYVEEFKGRFVFLDTSVSTAYLQISSLKAEDATVY YCARWDFYDYVDEAMYWGQGTITVTVSS	110
	VL CDR1	KASQTVSANVA	111
	VL CDR2	LASYRYR	112
	VL CDR3	RQYYTYPLFT	113
	VL	DIQMTQSPSSLSASVGDRVTITCKASQTVSANVAWYQQKPGKAPKLL IYLASYRYRGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQYYTY PLFTFGGKLEIK	114

	scFv	GGGSGGG QVQLVQSGSELKKPGASVKVSCASGYTFTEYGMNVWRQA PGQGLEWMGWINTKSGEATYVEEFKGRFVFLDTSVSTAYLQISSLK AEDTAVYYCARWDFYDYVDEAMYWGQGTITVTVSS GGGSGGGSGGG GSD IQMTQSPSSLSASVGDRVTITCKASQTVSANVAWYQQKPGKAPK LLIYLASYRYRGVPSRFSGSGSGTDFLTITSSLPEDFATYYCHQYY TYPLTFGQGTKLEIK	115 261 (no N-terminus peptide linker)
	VH-CH1	QVQLVQSGSELKKPGASVKVSCASGYTFTEYGMNVWRQAPGQGLEW MGWINTKSGEATYVEEFKGRFVFLDTSVSTAYLQISSLKAEADTAVY YCARWDFYDYVDEAMYWGQGTITVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	116
	VL-Ck	DIQMTQSPSSLSASVGDRVTITCKASQTVSANVAWYQQKPGKAPKLL IYLASYRYRGVPSRFSGSGSGTDFLTITSSLPEDFATYYCHQYYTY PLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYE KHKLYACEVTHQGLSSPVTKSFNRGEC	117
CTAT.07 (EpCAM)	VH CDR1	NYGMN	118
	VH CDR2	WINTYTGESTYADSFKG	119
	VH CDR3	FAIKGDY	120
	VH	EVQLVQSGPGLVQPGGSVRISCAASGYTFT NYGMN WVKQAPGKGLEW MGW WINTYTGESTYADSFKGR FTFSLDTSASAAYLQINSLRAEDTAVY YCAR FAIKGDY WGQGTLLTVSS	121
	VL CDR1	RSTKSLHLSNGITYLY	122
	VL CDR2	QMSNLAS	123
	VL CDR3	AQNLEIPRT	124
	VL	DIQMTQSPSSLSASVGDRVTIT CRSTKSLHLSNGITYLY WYQQKPGK APKLLIY QMSNLAS GVPSRFSGSGSGTDFLTITSSLPEDFATYY CA QNLEIPRT FGQGTKVELK	125
	scFv	GGGSGGG EVQLVQSGPGLVQPGGSVRISCAASGYTFTNYGMNWVKQA PGKLEWMGWINTYTGESTYADSFKGRFTFSLDTSASAAYLQINSLR AEDTAVYYCARFAIKGDYWGQGTLLTVSS GGGSGGGSGGGSGGSD IQ MTQSPSSLSASVGDRVTITCRSTKSLHLSNGITYLYWYQQKPGKAPK LLIYQMSNLASGVPSRFSGSGSGTDFLTITSSLPEDFATYYCAQNL EIPRTFGQGTKVELK	126 262 (no N-terminus peptide linker)
	VH-CH1	EVQLVQSGPGLVQPGGSVRISCAASGYTFTNYGMNWVKQAPGKGLEW MGWINTYTGESTYADSFKGRFTFSLDTSASAAYLQINSLRAEDTAVY YCARFAIKGDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	127
	VL-Ck	DIQMTQSPSSLSASVGDRVTITCRSTKSLHLSNGITYLYWYQQKPGK APKLLIYQMSNLASGVPSRFSGSGSGTDFLTITSSLPEDFATYYCA QNLEIPRTFGQGTKVELKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSK ADYEKHKLYACEVTHQGLSSPVTKSFNRGEC	128
CTAT.08 (FAP)	VH CDR1	EYTIH	129
	VH CDR2	GINPNNGIPNYNQKFKG	130
	VH CDR3	RRIAYGYDEGHAMDY	131
	VH	QVQLVQSGAEVKKPGASVKVSCKTSRYTFT EYTIH WVRQAPGQRLEW	132

		IGGINPNNGIPNYNQKFGRVTITVDTASTAYMELSSLRSED TAVY YCARRRIAYGYDEGHAMDYWGQGLTVTVSS	
	VL CDR1	RSSQSLLYSRNQKNYLA	133
	VL CDR2	WASTRES	134
	VL CDR3	QQYFSYPLT	135
	VL	DIVMTQSPDSLAVSLGERATINCKSSQSLLYSRNQKNYLAWYQQKPG QPPKLLIFWASTRESGVPDRFSGSGFGTDFTLTISSLQAEDVAVYYC QQYFSYPLTFGQGTKVEIK	136
	scFv	GGGSGGGQVQLVQSGAEVKKPGASVKVSCKTSRYTFTEYTIHWVRQA PGQRLEWIGGINPNNGIPNYNQKFGRVTITVDTASTAYMELSSLR SED TAVYYCARRRIAYGYDEGHAMDYWGQGLTVTVSS GGGSGGGGS GGGSDIVMTQSPDSLAVSLGERATINCKSSQSLLYSRNQKNYLAWY QQKPGQPPKLLIFWASTRESGVPDRFSGSGFGTDFTLTISSLQAEDV AVYYCQQYFSYPLTFGQGTKVEIK	137 263 (no N-terminus peptide linker)
	VH-CH1	QVQLVQSGAEVKKPGASVKVSCKTSRYTFTEYTIHWVRQAPGQRLEW IGGINPNNGIPNYNQKFGRVTITVDTASTAYMELSSLRSED TAVY YCARRRIAYGYDEGHAMDYWGQGLTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	138
	VL-Ck	DIVMTQSPDSLAVSLGERATINCKSSQSLLYSRNQKNYLAWYQQKPG QPPKLLIFWASTRESGVPDRFSGSGFGTDFTLTISSLQAEDVAVYYC QQYFSYPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLS KADYEEKHKLKACEVTHQGLSSPVTKSFNRGEC	139
CTAT.09 (PDL1)	VH CDR1	DSWIH	140
	VH CDR2	WISPYGGSTYYADSVK	141
	VH CDR3	RHWPGGFDY	142
	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTTFSDSWIHWVRQAPGKGLEW VAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAED TAVY YCARRHWPGGFDYWGQGLTVTVSS	143
	VL CDR1	RASQDVSTAVA	144
	VL CDR2	SASFLYS	145
	VL CDR3	QQYLYHPAT	146
	VL	DIQMTQSPSSLSASVGRVTITCRASQDVSTAVAWYQQKPGKAPKLL IYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYH PATFGQGTKVEIK	147
	scFv	GGGSGGGEVQLVESGGGLVQPGGSLRLSCAASGFTTFSDSWIHWVRQA PGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLR AED TAVYYCARRHWPGGFDYWGQGLTVTVSS GGGSGGGSGGGGS IQMTQSPSSLSASVGRVTITCRASQDVSTAVAWYQQKPGKAPKLLI YSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHP ATFGQGTKVEIK	148 264 (no N-terminus peptide linker)
	VH-CH1	EVQLVESGGGLVQPGGSLRLSCAASGFTTFSDSWIHWVRQAPGKGLEW VAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAED TAVY YCARRHWPGGFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	149
	VL-Ck	DIQMTQSPSSLSASVGRVTITCRASQDVSTAVAWYQQKPGKAPKLL	150

		IYSASFLYSGVPSRFSGSGSGTDFTLTISSLPEDFATYYCQQYLYH PATFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	
CTAT.10 (CD38)	VH CDR1	SFAMS	151
	VH CDR2	AISGSGGGTYADSVKG	152
	VH CDR3	DKILWFGPEVFDY	153
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFTFNS SFAMS WVRQAPGKGLEW VSA ISGSGGGTYADSVKGR FTISRDN SKNTLYLQMN SLRAEDTAVY FCAKD KILWFGPEVFDY WGQGT LVTVSS	154
	VL CDR1	RASQSVSSYLA	155
	VL CDR2	DASNPA	156
	VL CDR3	QQRSNWPPT	157
	VL	EIVLTQSPATLSLSPGERATLSC RASQSVSSYLA WYQQKPGQAPRLL IYD ASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQR SNW PPT FGQGTKEIK	158
	scFv	GGGSGGGE EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQA PGKLEWVSAISGSGGGTYADSVKGRFTISRDN SKNTLYLQMN SLR AEDTAVYFCAKDILWFGPEVFDYWGQGT LVTVSS GGGSGGGGGGGG GGSE IIVLTQSPATLSLSPGERATLSC RASQSVSSYLA WYQQKPGQAP RLLIYDASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQR SNWPPTFGQGTKEIK	159 265 (no N-terminus peptide linker)
	VH-CH1	EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEW VSAISGSGGGTYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVY FCAKDILWFGPEVFDYWGQGT LVTVSSASTKGPSVFPLAPSSKST SGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDK	160
	VL-Ck	EIVLTQSPATLSLSPGERATLSC RASQSVSSYLA WYQQKPGQAPRLL IYD ASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYC QQR SNW PPT FGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC	161
CTAT.11 (CD33)	VH CDR1	DSNIH	162
	VH CDR2	YIYPYNGGTDYNQKFN	163
	VH CDR3	GNPWLAY	164
	VH	EVQLVQSGAEVKKPGSSVKVSKASGYTIT DSNIH WVRQAPGQSLEW IGY IYPYNGGTDYNQKFN RATLTVDNPTNTAYMELSSLRSEDTAFY YCVN GNPWLAY WGQGT LVTVSS	165
	VL CDR1	RASESLDNYGIRFLT	166
	VL CDR2	AASNQGS	167
	VL CDR3	QQTEVPWS	168
	VL	DIQLTQSPSTLSASVGRVTIT C RASESLDNYGIRFLTWFQQKPGKA PKLLMY AASNQGS GVPSRFSGSGSGTEFTLTISLQPDFAVYYC QQ TKEVPWS FGQGTKEVEK	169
	scFv	GGGSGGGE EVQLVQSGAEVKKPGSSVKVSKASGYTITDSNIHWVRQA	170

		PGQSLIEWIGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLR SEDTAFYYCVNGNPWLAYWGQGTTLTVSS GGGSGGGSGGGSG DIQ LTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWQKPGKAPKL LMYAASNQSGGVP SRFSGSGSGTEFTLTISLQPDDEFATYYCQQTKE VPWSFGQGTKVEVK	266 (no N-terminus peptide linker)
	VH-CH1	EVQLVQSGAEVKKPGSSVKVSKASGYTITDSNIHWVRQAPGQSLW IGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDTAFY YCVNGNPWLAYWGQGTTLTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSVVTVP SSSLGKTITCNVDHKPSNTKVDKRV	171
	VL-Ck	DIQLTQSPSTLSASVGDRVTITCRASESLDNYGIRFLTWQKPGKA PKLLMYAASNQSGGVP SRFSGSGSGTEFTLTISLQPDDEFATYYCQ TKEVPWSFGQGTKVEVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYFPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC	172
CTAT.12 (HGFR (cMET))	VH CDR1	SYWLH	173
	VH CDR2	MIDPSNSDTRFNENFKD	174
	VH CDR3	YRSYVTPLDY	175
	VH	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWLHWVRQAPGKGLEW VGMIDPSNSDTRFNENFKDRFTISADTSKNTAYLQMNSLRAEDTAVY YCATYRSYVTPLDYWGQGTTLTVSS	176
	VL CDR1	KSSQSLLYTSSQKNYLA	177
	VL CDR2	WASTRES	178
	VL CDR3	QYYAYPWT	179
	VL	DIQMTQSPSSLSASVGDRVTITCKSSQSLLYTSSQKNYLAWYQQKPG KAPKLLIYWASTRESGVP SRFSGSGSGTDFLTISLQPEDDEFATYYC QYYAYPWTFGQGTKVEIK	180
	scFv	GGGSGGGE EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWLHWVRQA PGKGLEWVGMIDPSNSDTRFNENFKDRFTISADTSKNTAYLQMNSLR AEDTAVYYCATYRSYVTPLDYWGQGTTLTVSS GGGSGGGSGGGSG DIQMTQSPSSLSASVGDRVTITCKSSQSLLYTSSQKNYLAWYQQKPG KAPKLLIYWASTRESGVP SRFSGSGSGTDFLTISLQPEDDEFATYYC QYYAYPWTFGQGTKVEIK	181 267 (no N-terminus peptide linker)
	VH-CH1	EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWLHWVRQAPGKGLEW VGMIDPSNSDTRFNENFKDRFTISADTSKNTAYLQMNSLRAEDTAVY YCATYRSYVTPLDYWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSVVTVP SSSLGKTQYICNVNHKPSNTKVDKKVEPKSCDK	182
	VL-Ck	DIQMTQSPSSLSASVGDRVTITCKSSQSLLYTSSQKNYLAWYQQKPG KAPKLLIYWASTRESGVP SRFSGSGSGTDFLTISLQPEDDEFATYYC QYYAYPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLS KADYEHKVKYACEVTHQGLSSPVTKSFNRGEC	183
CTAT.13 (CD47)	VH CDR1	NYNMH	184
	VH CDR2	TIYPGNDDTSYNQKFKD	185
	VH CDR3	GGYRAMDY	186
	VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTNYNMHWVRQAPGQRLEW MGTIYPGNDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVY	187

		YCARGGYRAMDYWGQGLTVTVSS	
	VL CDR1	RSSQSIVYSNGNTYLG	188
	VL CDR2	KVSNRFS	189
	VL CDR3	FQGSHPVYT	190
	VL	DIVMTQSPLSLPVTGPGEPAISCRSSQSIVYSNGNTYLGWYLQKPGQ SPQLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCF QGSHVPYTFGQGTKLEIK	191
	scFv	GGGSGGGQVQLVQSGAEVKKPGASVKVSKASGYTFTNINMHVVRQA PGQRLEWMGTIYPGNDTSYNQKFKDRVTITADTSASTAYMELSSLR SEDTAVYYCARGGYRAMDYWGQGLTVTVSSGGGSGGGSGGGSDI VMTQSPLSLPVTGPGEPAISCRSSQSIVYSNGNTYLGWYLQKPGQSP QLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQ SHVPYTFGQGTKLEIK	192 268 (no N-terminus peptide linker)
	VH-CH1	QVQLVQSGAEVKKPGASVKVSKASGYTFTNINMHVVRQAPGQRLEW MGTIYPGNDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVY YCARGGYRAMDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK	193
	VL-Ck	DIVMTQSPLSLPVTGPGEPAISCRSSQSIVYSNGNTYLGWYLQKPGQ SPQLLIYKVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCF QGSHVPYTFGQGTKLEIKRTVAAPSVFIAPPDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSK ADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC	194
CTAT.14 (TRAIL-R 2)	VH CDR1	SGDYFWS	195
	VH CDR2	HIHNSGTTYYNPSLKS	196
	VH CDR3	DRGGDYYYGMDV	197
	VH	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYFWSWIRQLPGKGL EWIGHIHNSGTTYYNPSLKSRTISVDTSKKQFSLRLSSVTAADTAV YYCARDRGGDYYYGMDVWGQGLTVTVSS	198
	VL CDR1	RASQGISRSYLA	199
	VL CDR2	GASSRAT	200
	VL CDR3	QQFGSSPWT	201
	VL	EIVLTQSPGTLSPGERATLSCRASQGISRSYLAWYQKPGQAPSL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQFGS SPWTFGQGTKVEIK	202
	scFv	GGGSGGGQVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYFWSWIR QLPGKGLEWIGHIHNSGTTYYNPSLKSRTISVDTSKKQFSLRLSSV TAADTAVYYCARDRGGDYYYGMDVWGQGLTVTVSSGGGSGGGSGG GGSEIVLTQSPGTLSPGERATLSCRASQGISRSYLAWYQKPGQA PSLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ FGSSPWTFGQGTKVEIK	203 269 (no N-terminus peptide linker)
	VH-CH1	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYFWSWIRQLPGKGL EWIGHIHNSGTTYYNPSLKSRTISVDTSKKQFSLRLSSVTAADTAV YYCARDRGGDYYYGMDVWGQGLTVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSL SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK	204
	VL-Ck	EIVLTQSPGTLSPGERATLSCRASQGISRSYLAWYQKPGQAPSL LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQFGS	205

		SPWTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC	
CTAT.15 (mesotheli n)	VH CDR1	SGSYYS	206
	VH CDR2	YIYSGSTNYPNPSLKS	207
	VH CDR3	EGKNGAFDI	208
	VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSVSS SGSYYS SWIRQPPGKGL EWIGY YIYSGSTNYPNPSLKS SRVTISVDTSKNQFSLKLSSVTAADTAV YYCARE EGKNGAFDI WGQGMVTVSS	209
	VL CDR1	RASQSISSYLN	210
	VL CDR2	AASSLQS	211
	VL CDR3	QQSYSTPLT	212
	VL	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPGKAPKLL IYA AASSLQS GVPSGFSGSGSGTDFLTITISLQPEDFATYYC QQSYST PLTFGGGTKEIK	213
	scFv	GGSGGG QVQLQESGPGLVKPSSETLSLTCTVSGGSVSSGSYYWSWIR QPPGKGLEWIGYIYSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSV TAADTAVYYCAREGKNGAFDIWGQGMVTVSS GGSGGGGGGGGG DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSGFSGSGSGTDFLTITISLQPEDFATYYCQQSYST PLTFGGGTKEIK	214 270 (no N-terminus peptide linker)
	VH-CH1	QVQLQESGPGLVKPSSETLSLTCTVSGGSVSSGSYYWSWIRQPPGKGL EWIGYIYSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVTAADTAV YYCAREGKNGAFDIWGQGMVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK	215
	VL-Ck	DIQMTQSPSSLSASVGDRVITITCRASQSISSYLNWYQQKPGKAPKLL IYAASSLQSGVPSGFSGSGSGTDFLTITISLQPEDFATYYCQQSYST PLTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY REAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC	216
CTAT.16 (GD2)	VH CDR1	GYNMN	217
	VH CDR2	AIDPYYGGTSYNQKFKG	218
	VH CDR3	GMEY	219
	VH	EVQLQSGPELEKPGASVMISCKASGSSFT GYNMN WVRQNIQKSLEW IG AIDPYYGGTSYNQKFKG GRATLTVDKSSSTAYMHLKSLTSEDSAVY YCVS GMEY WGQTSVTVSS	220
	VL CDR1	RSSQSLVHRNGNTYLH	221
	VL CDR2	KVSNRFS	222
	VL CDR3	SQSTHVPPLT	223
	VL	EIVMTQSPATLSVSPGERATLSC RSSQSLVHRNGNTYLH HWYLQKPGQ SPKLLIH KVSNRFS GVDPDRFSGSGSGTDFLTISKISRVEAEDLGVYFCS QSTHVPPLT FGAGTKLELK	224
	scFv	GGSGGG EVQLQSGPELEKPGASVMISCKASGSSFTGYNMN WVRQNI IGKSLEWIG AIDPYYGGTSYNQKFKG GRATLTVDKSSSTAYMHLKSLT	225

		SEDSAVYYCVSGMEYWGQGTSTVTSS GGGGSGGGSGGGGS EIVMTQ SPATLSVSPGERATLSCRSSQSLVHRNGNTYLHWYLQKPGQSPKLLI HKVSNRFGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVP PLTFGAGTKLELK	271 (no N-terminus peptide linker)
	VH-CH1	EVQLQSGPELEKPGASVMISCKASGSSFTGYNMNWRQNIGKSLEW IGAIDPYYGGSYNQKFKGRATLTVDKSSSTAYMHLKSLTSEDSAVY YCVSGMEYWGQGTSTVTSSASTKGPSVFPLAPSSKSTSGGTAAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDK	226
	VL-Ck	EIVMTQSPATLSVSPGERATLSCRSSQSLVHRNGNTYLHWYLQKPGQ SPKLLIHKVSNRFGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYFCS QSTHVPPLTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSPVTKSFNRGEC	227

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.01, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.01, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.01. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.01. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.01.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.01. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.01. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.01.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.01. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.01 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain

CDRs as those in antibody CTAT.02, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.02, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.02. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.02. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.02.

In one specific example, the anti-TAA moiety disclosed herein comprises a mutation at position G42 of the VL chain relative to CTAT.02, for example, an amino acid residue substitution (*e.g.*, G42A). See, *e.g.*, CTAT.02 VL-01 in **Table 2** above. In another specific example, the anti-TAA moiety disclosed herein comprises a mutation at position D41 of the VL chain relative to CTAT.02, for example, an amino acid residue substitution (*e.g.*, D41E). See, *e.g.*, CTAT.02 VL-02 in **Table 2** above.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.02. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.02.

Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.02.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.02. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.02 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.03, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.03, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.03. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions

relative to the corresponding framework regions in CTAT.03. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.03.

5 In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.03. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.03. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at
10 least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.03.

 In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.03. In some
15 instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.03 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

 In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.04, which are provided in **Table 2** above. Alternatively or in
20 addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.04, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.04. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.04. For example, the anti-TAA
25 binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.04.

 In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.04. For example, the anti-TAA moiety
30 may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.04. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.04.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.04. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.04 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.05, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.05, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.05. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.05. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.05.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.05. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.05. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.05.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.05. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.05 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.06, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.06, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.06. Alternatively, the anti-TAA

binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.06. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.06.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.06. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.06. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.06.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.06. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.06 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.07, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.07, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.07. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.07. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.07.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.07. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.07. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as

compared with the V_L CDRs as CTAT.07.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.07. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.07 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.08, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.08, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.08. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.08. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.08.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.08. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.08. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.08.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.08. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.08 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.09, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.09, which are also provided in **Table 2** above. Such an anti-TAA binding

moiety may comprise the same V_H and/or V_L chains as CTAT.09. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.09. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.09.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.09. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.09. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.09.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.09. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.09 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.10, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.10, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.10. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.10. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.10.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.10. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.10. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at

least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.10.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.10. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.10 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.11, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.11, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.11. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.11. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.11.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.11. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.11. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.10.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.11. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.11 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.12, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in

antibody CTAT.12, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.12. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.12. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.12.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.12. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.12. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.12.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.12. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.12 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.13, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.13, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.13. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.13. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.13.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.13. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.13.

Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.13.

5 In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.13. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.13 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

10 In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.14, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.14, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.14. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.14. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.14.

20 In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.14. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.14. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.14.

30 In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.14. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.14 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.15, which are provided in **Table 2** above. Alternatively or in

addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.15, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.15. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.15. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.15.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.15. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.15. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.15.

In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.15. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.15 and comprise one or more amino acid variations in one or more of the other heavy chain and light chain CDRs.

In some examples, the anti-TAA binding moiety may comprise the same heavy chain CDRs as those in antibody CTAT.16, which are provided in **Table 2** above. Alternatively or in addition, the anti-TAA binding moiety may have the same light chain CDRs as those in antibody CTAT.16, which are also provided in **Table 2** above. Such an anti-TAA binding moiety may comprise the same V_H and/or V_L chains as CTAT.16. Alternatively, the anti-TAA binding moiety may comprise amino acid variations in one or more of the framework regions relative to the corresponding framework regions in CTAT.16. For example, the anti-TAA binding moiety may comprise, collectively, up to 15 amino acid variations (*e.g.*, up to 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more framework regions relative to the corresponding framework regions in CTAT.16.

In some embodiments, the anti-TAA moiety may comprise a certain level of variations in one or more of the CDRs relative to those of CTAT.16. For example, the anti-TAA moiety may comprise heavy chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%)

sequence identity, individually or collectively, as compared with the V_H CDRs of CTAT.16. Alternatively or in addition, the anti-TAA antibody may comprise light chain CDRs that are at least 80% (*e.g.*, 85%, 90%, 95%, or 98%) sequence identity, individually or collectively, as compared with the V_L CDRs as CTAT.16.

5 In some instances, the anti-TAA moiety may comprise up to 10 amino acid variations (*e.g.*, up to 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variations) in one or more of the heavy chain and light chain CDRs collectively relative to those in the CDRs of CTAT.16. In some instances, the anti-TAA moiety may comprise the same heavy chain CDR3 as the heavy chain CDR3 of CTAT.16 and comprise one or more amino acid variations in one or more of the
10 other heavy chain and light chain CDRs.

(iii) Anti-CD3/Anti-TAA Bispecific Antibodies

The bispecific antibody disclosed herein may be in any suitable format as those known in the art, for example, those disclosed in *Mol. Immunol.* 67(2):95-106 (2015), the relevant
15 disclosures of which are incorporated by reference for the subject matter and purpose referenced herein. Some examples are provided below. See also FIGs. 1A-1N.

In some embodiments, the bispecific antibody disclosed herein may comprise one antigen binding moiety in Fab format and the other antigen binding moiety in single chain variable fragment (scFv) format. Such a bispecific antibody may comprise two polypeptides,
20 one comprising the heavy or light chain of the Fab fragment linked to the scFv fragment and the other comprising the light or heavy chain of the Fab that is not linked to the scFv fragment.

In some instances, a Fab fragment comprises two polypeptide chains, one comprising a V_H domain linked to a fragment of a heavy chain constant region (*e.g.*, CH1) and the other one comprising a V_L domain linked to a light chain constant region. The heavy chain constant
25 region fragment may be from any Ig subclass, for example, IgG, IgA, IgE, IgD, or IgM. In some examples, the heavy chain constant region fragment is from an IgG molecule (*e.g.*, a human IgG molecule). The light chain constant region may be a kappa chain or a lambda chain (*e.g.*, a human kappa or lambda chain). An scFv fragment comprises a V_H domain and a V_L domain linked by a peptide linker. See *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and
30 Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883. In some instances, the scFv fragment has, from N-terminus to C-terminus, the V_H-linker-V_L orientation. Alternatively, the scFv fragment has, from N-terminus to C-terminus, the V_L-linker-V_H orientation. In the bispecific antibody, the scFv fragment may be linked to the heavy chain of the Fab fragment. Alternatively, the scFv may be linked to the light chain of the Fab fragment. See FIGs. 1A-1H.

In some examples, the bispecific antibody disclosed herein may comprise the anti-CD3 binding moiety in Fab format and the anti-TAA binding moiety in scFv format. Exemplary illustrations are provided in FIGs. 1A-1D. The anti-CD3 Fab comprises a heavy chain VH-CH1 domain and a light chain VL-C κ or VL-C λ domain. The anti-TAA scFv comprises a VH domain and a VL domain. FIGs. 1A-1D. In some instances, the anti-CD3 Fab may be linked to the anti-TAA scFv via a peptide linker disposed between the CH1 domain of the anti-CD3 Fab heavy chain and the VH domain of the anti-tumor scFv. An exemplary illustration is provided in FIG. 1A. In other instances, the CH1 domain of the anti-CD3 Fab heavy chain can be linked to the VL domain of the anti-tumor scFv as illustrated in FIG. 1B. In other instances, the anti-TAA scFv can be linked to the C κ or C λ domain of the anti-CD3 Fab light chain via the VL domain of the scFv (FIG. 1C), or via the VH domain of the anti-tumor scFv (FIG. 1D). Examples of anti-CD3 Fab heavy chain (VH-CH1) and light chains (VL-C κ) and examples of anti-TAA scFv fragments are provided in **Tables 1** and **2**, respectively. Any combination of such is within the scope of the present disclosure.

In some examples, the bispecific antibody disclosed herein may comprise the anti-TAA binding moiety in Fab format and the anti-CD3 binding moiety in scFv format. Exemplary illustrations are provided in FIGs. 1E-1H. The anti-TAA Fab comprises a heavy chain VH-CH1 domain and a light chain VL-C κ or VL-C λ domain. The anti-CD3 scFv comprises a VH domain and a VL domain. FIGs. 1E-1H. In some instances, the anti-TAA Fab may be linked to the anti-CD3 scFv via a peptide linker disposed between the CH1 domain of the anti-TAA Fab heavy chain and the VH domain of the anti-CD3 scFv. An exemplary illustration is provided in FIG. 1E. In other instances, the CH1 domain of the anti-TAA Fab heavy chain can be linked to the VL domain of the anti-CD3 scFv as illustrated in FIG. 1F. In other instances, the anti-CD3 scFv can be linked to the C κ or C λ domain of the anti-TAA Fab light chain via the VL domain of the scFv (FIG. 1G), or via the VH domain of the anti-CD3 scFv (FIG. 1H). Examples of anti-TAA Fab heavy chain (VH-CH1) and light chains (VL-C κ) and examples of anti-CD3 scFv fragments are provided in **Tables 2** and **1**, respectively. Any combination of such is within the scope of the present disclosure.

In some embodiments, the bispecific antibody disclosed herein may comprise both antigen binding moieties in scFv format. Exemplary illustrations are provided in FIGs. 1I to 1L.

In some examples, the VH domain of anti-CD3 scFv may be linked to the VH domain of the anti-TAA scFv via a peptide linker (FIG. 1I). In some examples, the VH domain of anti-CD3 scFv may be linked to the VL domain of the anti-TAA scFv via a peptide linker (FIG.

1J). In some examples, the VL domain of anti-CD3 scFv may be linked to the VH domain of the anti-TAA scFv via a peptide linker (FIG. 1K). In other examples, the VL domain of anti-CD3 scFv may be linked to the VH domain of the anti-TAA scFv via a peptide linker (FIG. 1L). Exemplary anti-CD3 scFv fragments and exemplary anti-TAA scFv fragments are
5 provided in **Tables 1** and **2**, respectively. Any combination thereof for constructing a bispecific antibody is within the scope of the present disclosure.

In yet other embodiments, the bispecific antibodies disclosed herein may comprise one or more Fc regions, which may optionally a "knob into hole" structure, in which a knob in the CH2 domain, the CH3 domain, or both of the first heavy chain is created by replacing several
10 amino acid side chains with alternative ones, and a hole in the juxtaposed position at the CH3 domain of the second heavy chain is created by replacing appropriate amino acid side chains with alternative ones. Exemplary illustrations are provided in FIGs. 1M and 1N.

Typically, the terms "a knob and a hole" or "knobs-into-holes" are used interchangeably herein. Knobs-into-holes amino acid changes is a rational design strategy
15 known in the art for heterodimerization of the heavy (H) chains in the production of bispecific IgG antibodies. Carter, J. Immunol. Methods, 248(1-2):7-15 (2001), the relevant disclosures of which are incorporated by reference herein for the purpose and subject matter referenced herein.

In one example, the "knobs-into-holes" provides an approach as described in, e.g.,
20 Ridgway JBB *et al.*, (1996) Protein Engineering, 9(7): 617-21 and US 5,731,168, the relevant disclosures of each of which are incorporated by reference herein for the purpose and subject matter referenced herein. This approach has been shown to promote the formation of heterodimers of the first polypeptide and the second polypeptide chain, and hinder the assembly of corresponding homodimers. In one aspect, a knob is created by replacing small
25 amino side chains at the interface between CH3 domains with larger ones, whereas a hole is constructed by replacing large side chains with smaller ones. In a specific example, the "knob" mutation comprises T366W and the "hole" mutations comprise T366S, L368A and Y407V (Atwell S *et al.*, (1997) J. Mol. Biol. 270: 26-35).

In some instances, the bispecific antibody may comprise an anti-CD3 binding moiety
30 comprising a first VH-CH1-CH2-CH3 domain and a first VL-C κ or VL-C λ domain, and an anti-TAA binding moiety comprising a second VH-CH1-CH2-CH3 domain and second a VL-C κ or VL-C λ domain. FIG. 1M. The CH2 and/or CH3 in the heavy chain of the anti-CD3 binding moiety that those in the heavy chain of the anti-TAA binding moiety may comprise the knob/hole modifications, allowing for the binding between the two heavy chains. In other

instances, the bispecific antibody may comprise an anti-CD3 binding moiety comprising a first VH-CH1-CH2-CH3 domain and a first VL-C κ or VL-C λ domain, and an anti-TAA scFv linked to a second CH2-CH3 domain. The CH2 and/or CH3 in the heavy chain of the anti-CD3 binding moiety that those in the anti-TAA binding moiety may comprise the knob/hole
 5 modifications, allowing for the binding between the two heavy chains. FIGs. 1N. In this setting, the format of the anti-CD3 binding moiety and the format of the anti-TAA binding moiety may be switched.

The term “peptide linker” refers to a peptide having natural or synthetic amino acid residues for connecting two polypeptides. For example, the peptide linker may be used to
 10 connect one VH domain and one VL domain to form a single chain variable fragment (*e.g.*, scFv); to connect one scFv and one Fab to form a scFv/Fab recombinant antibody; to connect two scFvs to form a scFv/scFv recombinant antibody; or to connect two monovalent antibodies (*e.g.*, two monovalent IgGs), two monovalent antibody fragments (*e.g.*, two monovalent scFv-Fc fusion proteins), or one monovalent antibody and one monovalent antibody fragment
 15 (*e.g.*, one monovalent IgG and one monovalent scFv-Fc fusion protein) thereby forming a divalent antibody. Preferably, the peptide linker is a peptide having at least 5 amino acid residues in length, such as 5 to 100 amino acid residues in length; more preferably, 10 to 30 amino acid residues in length. The peptide linker within scFv is a peptide of at least 5 amino acid residues in length, preferably 15 to 20 amino acid residues in length. Preferably, the
 20 peptide linker comprises a sequence of (G_nS)_m, with G = glycine, S = serine, and n and m are independently a number between 1 to 4. In one example, the linker comprises a sequence of (G₂S)₄. In another example, the linker comprises a sequence or (G₄S)₃.

The peptide linker for linking the first antibody fragment (*i.e.*, anti-CD3 antibody fragment) and the second antibody fragment (*i.e.*, anti-TAA antibody fragment) may be any
 25 peptide suitable for connecting two polypeptides. According to certain embodiments of the present disclosure, the peptide linker is a peptide having at least 5 amino acid residues in length, for example, having 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73,
 30 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more amino acid residues in length. Preferably, the peptide linker of the present recombinant antibody consists of 10 to 30 glycine (G) and/or serine (S) residues.

In some embodiments, the bispecific antibodies described herein specifically bind to one or both of the corresponding target antigen (CD3 and a TAA) or an epitope thereof. An

antibody that “specifically binds” to an antigen or an epitope is a term well understood in the art. A molecule is said to exhibit “specific binding” if it reacts more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen than it does with alternative targets. An antibody “specifically binds” to a target antigen or epitope if it
5 binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically (or preferentially) binds to an antigen (CD3 and/or a TAA) or an antigenic epitope therein is an antibody that binds this target antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens or other epitopes in the same antigen. It is also understood with this definition
10 that, for example, an antibody that specifically binds to a first target antigen may or may not specifically or preferentially bind to a second target antigen. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. In some examples, an antibody that “specifically binds” to a target antigen or an epitope thereof may not bind to other antigens or other epitopes in the same antigen (*i.e.*, only baseline
15 binding activity can be detected in a conventional method).

In some embodiments, a bispecific antibody as described herein has a suitable binding affinity for one or both of the target antigens (*e.g.*, CD3 and a TAA) or antigenic epitopes thereof. As used herein, “binding affinity” refers to the apparent association constant or K_A . The K_A is the reciprocal of the dissociation constant (K_D). The bispecific antibody described
20 herein may have a binding affinity (K_D) of at least 100 nM, 10nM, 1nM, 0.1 nM, or lower for CD3 (*e.g.*, lower than 1nM or 0.1nM). Alternatively, the bispecific antibody described herein may have a binding affinity (K_D) of at least 100 nM, 10nM, 1nM, 0.1 nM, or lower for the TAA.

An increased binding affinity corresponds to a decreased K_D . Higher affinity binding of
25 an antibody for a first antigen relative to a second antigen can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first antigen than the K_A (or numerical value K_D) for binding the second antigen. In such cases, the antibody has specificity for the first antigen (*e.g.*, a first protein in a first conformation or mimic thereof) relative to the second antigen (*e.g.*, the same first protein in a second conformation or mimic thereof; or a second protein).
30 Differences in binding affinity (*e.g.*, for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 90, 100, 500, 1000, 10,000 or 10^5 fold. In some embodiments, any of the anti-CD3 and/or anti-TAA antibodies for making the bispecific antibodies may be further affinity matured to increase the binding affinity of the antibody to the target antigen or antigenic epitope thereof.

Binding affinity (or binding specificity) can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (*e.g.*, using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in HBS-P buffer (10 mM HEPES pH7.4, 150 mM NaCl, 0.005% (v/v) Surfactant P20). These techniques can be used to measure the concentration of bound binding protein as a function of target protein concentration. The concentration of bound binding protein ([Bound]) is generally related to the concentration of free target protein ([Free]) by the following equation:

$$[\text{Bound}] = [\text{Free}] / (K_d + [\text{Free}])$$

It is not always necessary to make an exact determination of K_A , though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, *e.g.*, determined using a method such as ELISA or FACS analysis, is proportional to K_A , and thus can be used for comparisons, such as determining whether a higher affinity is, *e.g.*, 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, *e.g.*, by activity in a functional assay, *e.g.*, an *in vitro* or *in vivo* assay.

Exemplary bispecific antibodies as disclosed herein are provided in **Table 3** below (using anti-CD3 binding moieties from CTA.03 as examples). Anti-CD3 binding moieties from other anti-CD3 reference antibodies (*e.g.*, CTA.02, CTA.04, and CTA.05) are also within the scope of the present disclosure.

Table 3. Exemplary Bispecific Antibodies

Bi-Specific Antibodies		Amino Acid Sequences
CTA03Fab/ CTAT01scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCGGGSGGGQVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTP GRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLSSLTSEDSAVYY CARSTYYGGDWYFNVWGAGTTTVSA GGGGSGGGGSGGGGSQIVLSQSPAILSA SPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSG TSYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIK (SEQ ID NO: 229)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT02scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCGGGSGGGEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP RKGLEWLGVIWGSETTYNSALKSRLTIKDN SKSQVFLKMNSLQTDITAIYYC AKHYYYGGSYAMDYWGQTSVTVSS GGGGSGGGGSGGGGSDIQMTQTSSLSAS LGDRVTISCRASQDISKYNWYQQKPDGTVKLLIYHTSRSLHSGVPSRFSGSGSG TDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT (SEQ ID NO: 230)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)

CTA03Fab/ CTAT03scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSP GKGLEWLGVIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYC ARALTYDYEFAYWGQGTLLVTVSA GGGSGGGSGGGSGGGSD ILLTQSPVILSVSP GERVSFSCRASQSIGTNIHWYQQRTNGSPRLIKYASESISGIPSRFSGSGSGT DFTLSINSVESEDIADYYCQNNNWPPTFGAGTKLELK (SEQ ID NO: 231)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT04scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVQLVESGGGLVQPGGSLRLSCAASGFTNKTDTYIHWVRQAP GKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDYWGQGTLLVTVSS GGGSGGGSGGGSGGGSD IQMTQSPSSLSAS VGDRVITICRASQDVNTAVAWYQQKPGKAPKLLIYSASFYSGVPSRFSGSRSG TDFTLTISLQPEDFATYYCQHYTTPPTFGQGTKVEIK (SEQ ID NO: 232)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT05scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVQLVQSGAEVKKPGASVKISCKTSGYTFTEYTIHWVKQAS GKLEWIGNINPNNGGTTYNQKFEDRATLTVDKSTSTAYMELSSLRSEDATVYY CAAGWNFDYWGQGTITVTVSS GGGSGGGSGGGSGGGSD IVMTQSPSSLSASVGDV TITCKASQDVGTAVDWYQQKPGKAPKLLIYWASTRHTGVPDRFTGSGSGTDFTL TISLQPEDFADYFCQYNSYPLTFGGGTKLEIK (SEQ ID NO: 233)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT06scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG QVQLVQSGSELKKPGASVKVSKASGYTFTEYGMNVWRQAP GQGLEWMGWINTKSGEATYVEEFKGRFVSLDTSVSTAYLQISLKAEDTAVYY CARWDFDYVDEAMYWGQGTITVTVSS GGGSGGGSGGGSGGGSD IQMTQSPSSLSA SVGDRVITITCKASQTVSANVAWYQQKPGKAPKLLIYLASYRYRGVPSRFSGSGS GTDFTLTISLQPEDFATYYCHQYTYPLTFFGQGTKLEIK (SEQ ID NO: 234)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT07scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVQLVQSGPGLVQPGGSVRISCAASGYTFNTYGMNVWKQAP GKLEWMGWINTYTGESTYADSFKGRFTFSLDTSASAAALQINSLRAEDTAVYY CARFAIKGDYWGQGTLLTVSS GGGSGGGSGGGSGGGSD IQMTQSPSSLSASVGDV VTITCRSTKSLHNSGITLYWYQQKPGKAPKLLIYQMSNLASGVPSRFSSSGS GTDFTLTISLQPEDFATYYCAQNLEIPRTFGQGTKVELK (SEQ ID NO: 235)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT08scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG QVQLVQSGAEVKKPGASVKVSKTSRYTFTEYTIHWVRQAP GQRLIEWIGGINPNNGIPNYNQKFGRVITITVDTASTAYMELSSLRSEDATVYY CARRIAYGYDEGHAMDYWGQGTLLVTVSS GGGSGGGSGGGSGGGSD IVMTQSPDS LAVSLGERATINCKSSQLLYSRNQKNYLAWYQQKPGQPPKLLIFWASTRESGV

		PDRFSGSGFGTDFTLTISSLQAEDVAVYYCQQYFSYPLTFGQGTKVEIK (SEQ ID NO: 236)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT09scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGGE EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWRQAP GKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CARRHWPGGFDYWGQGTLLVTVSS GGGSGGGSGGGSGG DIQMTQSPSSLSASVG DRVITITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFYSGVPSRFSGSGSGTD FTLTISLQPEDFATYYCQQYLYHPATFGQGTKVEIK (SEQ ID NO: 237)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT10scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGGE EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAP GKGLEWVSAISGSGGTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYF CAKDKILWFGPEPFDYWGQGTLLVTVSS GGGSGGGSGGGSGG SEIVLTQSPATLS LSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSG SGTDFTLTISLQPEDFAVYYCQQRNWPPTFGQGTKVEIK (SEQ ID NO: 238)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT11scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGGE EVQLVQSGAEVKKPGSSVKVSKASGYTITDSNIHWVRQAP GQSLEWIGYIYPYNGGTDYNQKFKNRATLTVDNPTNTAYMELSSLRSEDFAFY CVNGNPWLAWYWGQGTLLVTVSS GGGSGGGSGGGSGG DIQLTQSPSTLSASVGD VTITCRASESLDNYGIRFLTWFQQKPGKAPKLLMYAASNQSGVPSRFSGSGSG TEFTLTISLQPDFAVYYCQQTKVEPWSFGQGTKVEIK (SEQ ID NO: 239)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT12scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGGE EVQLVESGGGLVQPGGSLRLSCAASGYTFTSYWLHWVRQAP GKGLEWVGMIDPSNSDTRFNPFKDRFTISADTSKNTAYLQMNSLRAEDTAVYY CATYRSYVTPLDYWGQGTLLVTVSS GGGSGGGSGGGSGG DIQMTQSPSSLSASV GDRVTITCKSSQSLLYTSSQKNYLAWYQQKPGKAPKLLIYWASTRESGVPSRFS GSGSGTDFTLTISLQPEDFAVYYCQQYYAYPWTFGQGTKVEIK (SEQ ID NO: 240)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT13scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGGE EVQLVQSGAEVKKPGASVKVSKASGYTFTNYNMHWVRQAP GQRLEWMTIYPGNDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDFAVYY CARGGYRAMDYWGQGTLLVTVSS GGGSGGGSGGGSGG DIQMTQSPSSLSASV PASISCRSSQSIYVSYNGNTYLGWYLQKPGQSPQLLIYKVSNRFSGVDPDRFSGSG SGTDFTLKISRVEAEDVGVYYCFQGSHPVPTFGQGTKLEIK (SEQ ID NO: 241)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT14scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK

		VEPKSC GGGSGGG QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYFWSWIRQ LPGKGLEWIGHIHNSGTTYNPSLKSRTVISVDTSKKQFSLRLSSVTAADTAVY YCARDRGDDYYGMDVWGQGTITVTVSS GGGSGGGSGGGSGGG SEIVLTQSPGTLT LSPGERATLSCRASQGISRSYLAWYQQKPGQAPSLIYGASSRATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQFGSSPWTFGGGTKVEIK (SEQ ID NO: 242)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT015scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG QVQLQESGPGLVKPSQTLSTCTVSGGSVSSGSYYWSWIRQ PPGKGLEWIGIYYSGSTNYNPSLKSRTVISVDTSKNQFSLKSSVTAADTAVY YCAREGKNGAFDIWGQGTMTVTVSS GGGSGGGSGGGSGGG DIQMTQSPSSLSASV GDRVITTCRASQSISSYLWYQQKPGKAPKLLIYAASSLQSGVPSGFSGSGSGT DFTLTISLQPEDFATYYCQSYSTPLTFGGGTKVEIK (SEQ ID NO: 243)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03Fab/ CTAT16scFv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVQLQESGPELEKPGASVMISCKASGSSTGYNNMNVVRQNI GKSLEWIGAIIDPYGGTSYNQKFKGRATLTVDKSSSTAYMHLKSLTSEDSAVYY CVSGMEYWQGTSTVTVSS GGGSGGGSGGGSGGG SEIVMTQSPATLSVSPGERATL SCRSSQSLVHRNGNTYLHWYQLQKPGQSPKLLIHKVSNRFSVGPDRFSGSGSGT FTLKISRVEAEDLGVYFCSQSTHVPPLTFGAGTKLELK (SEQ ID NO: 244)
	2 nd chain	CTA.03 VL-Ck (SEQ ID NO:24)
CTA03-01Fab/ CTAT02-01sc Fv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP RKGLEWLGVIWGSETTYNSALKSRLTIIDKNSKSKVFLKMNSLQTDITAIYYC AKHYYYGGSYANDYWQGTSTVTVSS GGGSGGGSGGGSGGG DIQMTQTSSLSAS LGDRVITISCRASQDISKYLWYQQKPDATVKLLIYHTSRLHSGVPSRFSGSGSG TDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT (SEQ ID NO: 245)
	2 nd chain	CTA.03 VL-Ck-01 (SEQ ID NO: 228)
CTA03-01Fab/ CTAT02-02sc Fv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP RKGLEWLGVIWGSETTYNSALKSRLTIIDKNSKSKVFLKMNSLQTDITAIYYC AKHYYYGGSYANDYWQGTSTVTVSS GGGSGGGSGGGSGGG DIQMTQTSSLSAS LGDRVITISCRASQDISKYLWYQQKPEGTVKLLIYHTSRLHSGVPSRFSGSGSG TDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT (SEQ ID NO: 246)
	2 nd chain	CTA.03 VL-Ck-01 (SEQ ID NO: 228)
CTA03-02Fab/ CTAT02-02sc Fv	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP RKGLEWLGVIWGSETTYNSALKSRLTIIDKNSKSKVFLKMNSLQTDITAIYYC AKHYYYGGSYANDYWQGTSTVTVSS GGGSGGGSGGGSGGG DIQMTQTSSLSAS LGDRVITISCRASQDISKYLWYQQKPEGTVKLLIYHTSRLHSGVPSRFSGSGSG TDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT (SEQ ID NO: 247)
	2 nd chain	CTA.03 VL-Ck-02 (SEQ ID NO: 25)
CTA03-02Fab/ CTAT02-01sc	1 st chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTISTS GGRTYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYSGGFDY WGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS

Fv		GALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSC GGGSGGG EVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYGVSWIRQPP RKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQTDITAIYYC AKHYYYGGSYAMDYWGQTSVTVSS GGGSGGGSGGGSGGG DIQMTQTSSLSAS LGDRVTISCRASQDISKYLNWYQQKPDATVKLLIYHTSRLHSGVPSRFSGSGSG TDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEIT (SEQ ID NO: 248)
	2 nd chain	CTA.03 VL-Ck-01 (SEQ ID NO: 25)

Also provided herein are pharmaceutical compositions comprising any of the bispecific antibodies disclosed herein (or the armed immune cells also disclosed herein), which further comprises a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient may be any inert substance that is combined with an active molecule (such as the bispecific antibody or the armed immune cells) for preparing an agreeable or convenient dosage form. In general, the pharmaceutically acceptable excipient is non-toxic to recipients at the dosages and concentrations employed, and is compatible with other ingredients of the formulation comprising the recombinant antibody. Examples of the pharmaceutically acceptable excipient suitable to be employed in the present pharmaceutical composition include, but are not limited to, water, phosphate buffer, acetate buffer, succinate buffer, citrate buffer, tris(hydroxymethyl)aminomethane (Tris) buffer, phosphate-buffered saline (PBS), Ringer's solution, lactated Ringer's solution, and a combination thereof. Optionally, the pharmaceutical composition may further comprise an agent for storing and/or stabilizing the recombinant antibody, *e.g.*, amino acid residue (such as, histidine (H) or serine (S) residue), glucose, galactose, xylitol, sorbitol, mannitol, sucrose, trehalose, or antioxidant. Other agents may also be added, such as antimicrobial agents, to prevent spoilage upon storage, *i.e.*, to inhibit growth of microbes such as yeasts and molds.

B. Methods for Producing Bispecific Antibodies

Any of the bispecific antibodies described herein can be made by any method known in the art. See, for example, Harlow and Lane, (1998) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York. In some embodiments, the anti-CD3 antibody and/or the anti-TAA antibody for use in making the bispecific antibodies may be produced by the conventional hybridoma technology. Alternatively, the anti-CD3 and/or anti-TAA antibody may be identified from a suitable library (*e.g.*, a human antibody library). In some instances, high affinity fully human CD3 and/or TAA binders may be obtained from a human antibody library, for example, affinity maturation libraries (*e.g.*, having variations in one or more of the CDR regions). There are a number of routine methods known in the art to identify and isolate antibodies capable of binding to the target antigens described herein, including phage display,

yeast display, ribosomal display, or mammalian display technology.

In some embodiments, the bispecific antibodies disclosed herein may be produced by the conventional recombinant technology. In one example, DNA encoding a monoclonal antibodies specific to a target antigen can be readily isolated and sequenced using conventional
5 procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). Once isolated, the DNA may be placed into one or more expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of
10 monoclonal antibodies in the recombinant host cells. See, *e.g.*, PCT Publication No. WO 87/04462. The DNA can then be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., (1984) *Proc. Nat. Acad. Sci.* 81:6851, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a
15 non-immunoglobulin polypeptide.

In some instances, nucleic acids encoding the one or both chains of a bispecific antibody as described herein can be cloned into one expression vector, each nucleotide sequence being in operable linkage to a suitable promoter. In one example, each of the nucleotide sequences encoding the heavy chain and light chain is in operable linkage to a
20 distinct promoter. Alternatively, the nucleotide sequences encoding the heavy chain and the light chain can be in operable linkage with a single promoter, such that both heavy and light chains are expressed from the same promoter. When necessary, an internal ribosomal entry site (IRES) can be inserted between the heavy chain and light chain encoding sequences.

In some examples, the nucleotide sequences encoding the two chains of the antibody
25 are cloned into two vectors, which can be introduced into the same or different cells. When the two chains are expressed in different cells, each of them can be isolated from the host cells expressing such and the isolated heavy chains and light chains can be mixed and incubated under suitable conditions allowing for the formation of the antibody.

Generally, a nucleic acid sequence encoding one or all chains of an antibody can be
30 cloned into a suitable expression vector in operable linkage with a suitable promoter using methods known in the art. For example, the nucleotide sequence and vector can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of a gene. These synthetic linkers

contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/promoter would depend on the type of host cells for use in producing the antibodies.

A variety of promoters can be used for expression of the antibodies described herein, including, but not limited to, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the *Rous sarcoma* virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, *E. coli* lac UV5 promoter, and the herpes simplex tk virus promoter.

Regulatable promoters can also be used. Such regulatable promoters include those using the lac repressor from *E. coli* as a transcription modulator to regulate transcription from lac operator-bearing mammalian cell promoters [Brown, M. et al., *Cell*, 49:603-612 (1987)], those using the tetracycline repressor (tetR) [Gossen, M., and Bujard, H., *Proc. Natl. Acad. Sci. USA* 89:5547-5551 (1992); Yao, F. et al., *Human Gene Therapy*, 9:1939-1950 (1998); Shockelt, P., et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)]. Other systems include FK506 dimer, VP16 or p65 using estradiol, RU486, diphenol murislerone, or rapamycin. Inducible systems are available from Invitrogen, Clontech and Ariad.

Regulatable promoters that include a repressor with the operon can be used. In one embodiment, the lac repressor from *E. coli* can function as a transcriptional modulator to regulate transcription from lac operator-bearing mammalian cell promoters [M. Brown et al., *Cell*, 49:603-612 (1987); Gossen and Bujard (1992); M. Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551 (1992)] combined the tetracycline repressor (tetR) with the transcription activator (VP 16) to create a tetR-mammalian cell transcription activator fusion protein, tTa (tetR-VP 16), with the tetO-bearing minimal promoter derived from the human cytomegalovirus (hCMV) major immediate-early promoter to create a tetR-tet operator system to control gene expression in mammalian cells. In one embodiment, a tetracycline inducible switch is used. The tetracycline repressor (tetR) alone, rather than the tetR-mammalian cell transcription factor fusion derivatives can function as potent trans-modulator to regulate gene expression in mammalian cells when the tetracycline operator is properly positioned downstream for the TATA element of the CMVIE promoter (Yao et al., *Human Gene Therapy*, 10(16):1392-1399 (2003)). One particular advantage of this tetracycline inducible switch is that it does not require the use of a tetracycline repressor-mammalian cells transactivator or repressor fusion protein, which in some instances can be toxic to cells (Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551 (1992); Shockett et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)), to achieve its regulatable effects.

Additionally, the vector can contain, for example, some or all of the following: a

selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; and T7 and SP6 RNA promoters for *in vitro* transcription of sense and antisense RNA. Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art.

Examples of polyadenylation signals useful to practice the methods described herein include, but are not limited to, human collagen I polyadenylation signal, human collagen II polyadenylation signal, and SV40 polyadenylation signal.

Exemplary constructs for producing the bispecific antibodies in various configuration as disclosed herein are provided in FIGs. 2A-2E.

One or more vectors (*e.g.*, expression vectors) comprising nucleic acids encoding any of the antibodies may be introduced into suitable host cells for producing the antibodies. The host cells can be cultured under suitable conditions for expression of the antibody or any polypeptide chain thereof. Such antibodies or polypeptide chains thereof can be recovered by the cultured cells (*e.g.*, from the cells or the culture supernatant) via a conventional method, *e.g.*, affinity purification. If necessary, polypeptide chains of the antibody can be incubated under suitable conditions for a suitable period of time allowing for production of the antibody.

In some embodiments, methods for preparing an antibody described herein involve a recombinant expression vector that encodes both chains of a bispecific antibody as described herein. The recombinant expression vector can be introduced into a suitable host cell (*e.g.*, a dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Positive transformant host cells can be selected and cultured under suitable conditions allowing for the expression of the two polypeptide chains that form the antibody, which can be recovered from the cells or from the culture medium. When necessary, the two chains recovered from the host cells can be incubated under suitable conditions allowing for the formation of the antibody.

In one example, two recombinant expression vectors are provided, each encoding one chain of a bispecific antibody disclosed herein. Both of the two recombinant expression vectors can be introduced into a suitable host cell (*e.g.*, dhfr- CHO cell) by a conventional method, *e.g.*, calcium phosphate-mediated transfection. Alternatively, each of the expression vectors can be introduced into a suitable host cells. Positive transformants can be selected and

cultured under suitable conditions allowing for the expression of the polypeptide chains of the antibody. When the two expression vectors are introduced into the same host cells, the antibody produced therein can be recovered from the host cells or from the culture medium. If necessary, the polypeptide chains can be recovered from the host cells or from the culture medium and then incubated under suitable conditions allowing for formation of the antibody. When the two expression vectors are introduced into different host cells, each of them can be recovered from the corresponding host cells or from the corresponding culture media. The two polypeptide chains can then be incubated under suitable conditions for formation of the antibody.

Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recovery of the antibodies from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

Any of the nucleic acids encoding the bispecific antibody as described herein, vectors (*e.g.*, expression vectors) containing such; and host cells comprising the vectors are within the scope of the present disclosure. Methods for producing such bispecific antibodies (*e.g.*, using host cells via the recombinant technology) are also within the scope of the present disclosure.

II. Armed Immune Cells

In another aspects, provided herein are immune cells armed with any of the bispecific antibodies disclosed herein (*e.g.*, those comprising Fab fragment and/or scFv chains provided in **Tables 1** and **2**, or the exemplary bispecific antibodies provided in **Table 3** above). The bispecific antibody can be displayed on the surface of CD3+ immune cells via binding to the cell surface CD3 molecule.

The immune cells may be any type of immune cells (*e.g.*, human immune cells) expressing surface CD3 or a mixture thereof. Examples include, but are not limited to, T cell, a B cell, a monocyte, and/or macrophage. In some instances, the T cell is a traditional CD4+ and/or CD8+ T cells. In some instances, the T cell is a regulatory T cell (Treg). In other instances, the T cell is a natural killer T cell (NKT). The immune cells may be obtained from a donor such as a human donor (*e.g.*, a healthy donor). Alternatively, the immune cells may be obtained from a cell line or differentiated from stem cells, for example, hematopoietic stem cells, bone marrow cells, umbilical cord blood cells, or induced pluripotent stem cells.

Any of the armed immune cells may be produced by incubating suitable immune cells with any of the bispecific antibodies disclosed herein (*e.g.*, those comprising Fab fragment and/or scFv chains provided in **Tables 1** and **2**, or the exemplary bispecific antibodies

provided in **Table 3** above) under suitable conditions for a suitable period of time. Unlike anti-CD3 antibody alone (*e.g.*, OKT3), incubation of the bispecific antibodies disclosed herein with immune cells result in production of armed immune cells having the bispecific antibody displayed on the cell surface. The bispecific antibody may induce proliferation and/or differentiation of immune cells, such as induce differentiation of naïve T cells into effector cells via binding to the CD3 molecule on T cells via its anti-CD3 binding moiety. The armed immune cells thus produced is capable of targeting cancer cells via recognizing the TAA molecule expressed on the cancer cells by the anti-TAA binding moiety of the bispecific antibody, which is displayed on the surface of the armed immune cells.

10 In some examples, the armed immune cells disclosed herein can be produced using peripheral blood mononuclear cells (PBMCs). For example, PBMCs can be isolated from a donor (*e.g.*, a human donor) using a conventional method. The methods suitable for isolating PBMCs from a donor include, but are not limited to, density centrifugation (*e.g.*, FICOLL® Paque), cell preparation tube (CPT), and SEPMATE™ tube. In some examples, the PBMCs
15 can be isolated from a whole blood sample obtained from a donor via density centrifugation according to the manufacturer's directions. The isolated PBMCs can then be cultivated with the bispecific antibody in a suitable cell culture medium for least 7 days, such as 7, 8, 9, 10, 11, 12, 13 14, or more days; preferably, for at least 14 days. In some embodiments, the number of CD3⁺ immune cells such as T cells (*e.g.*, CD4/CD8 T cells and/or NKT cells) multiplies after
20 cultivation for 7 days. In other embodiments, cultivation is continued for 14 days, and the number of CD3⁺ T cells increases for 3 folds.

In other examples, immune cells from cell culture may be used for making the armed immune cells disclosed herein. The *in vitro* cultured immune cells may be from an established cell line. Alternatively, the immune cells may be differentiated from suitable stem cells, for
25 example, hematopoietic stem cells, bone marrow cells, umbilical cord blood cells, or induced pluripotent stem cells, following conventional methods.

A suitable amount of immune cells (*e.g.*, 3×10^5 cells) may be cultured in a suitable cell culture medium in the presence of about 500 ng to about 3,000 ng (*e.g.*, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,100,
30 2,200, 2,300, 2,400, 2,500, 2,600, 2,700, 2,800, 2,900, or 3,000 ng) of a bispecific antibody for a suitable period of time under suitable conditions to produce the armed immune cells. The cell culture medium may comprise one or more cytokines for sustaining the growth of immune cells such as T cells and/or stimulating the activation of the immune cells. Examples include, but are not limited to, IL-1 β , IL-2, IL-4, IL-6, IL-7, IL-12, IL-18, IL-21, IL-23, IL-25,

IL-27, IL-31, interferon-gamma (IFN- γ), TGF- β , or a combination thereof. Additionally or alternatively, the medium may comprise an antibody or a carbohydrate for the activation purpose, such as an anti-CD28 antibody or a mannose.

In some examples, IL-2 may be used in the culture medium to cultivate PBMCs to produce, e.g., armed CD8⁺ T cells. In other examples, IL-2 and IL-7 may be used in the culture medium to cultivating PBMCs for producing, e.g., armed CD4⁺ T cells. For producing armed Treg cells, IL-2, an anti-CD28 antibody, and mannose may be used in the cell culture medium.

The armed immune cells produced by any of the methods disclosed herein are also within the scope of the present disclosure.

III. Cancer Treatment With Armed Immune Cells

In another aspect, the present disclosure provides a method for treating cancer using the armed immune cells disclosed herein. To practice the method disclosed herein, an effective amount of the armed immune cells or a pharmaceutical composition comprising such can be administered to a subject (e.g., a human) in need of the treatment *via* a suitable route, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time. In some instances, the armed immune cells are autologous to the subject. In other instances, the armed immune cells are allogenic to the subject.

The subject to be treated by the methods described herein can be a mammal, more preferably a human or a non-human primate. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats. A human subject who needs the treatment may be a human patient having, at risk for, or suspected of having a target disease/disorder characterized by carrying tumor cells expressing the target TAA, to which a bispecific antibody binds. Exemplary cancers include, but are not limited to, melanoma, esophageal carcinoma, gastric carcinoma, brain tumor, small cell lung cancer, non-small cell lung cancer, bladder cancer, breast cancer, pancreatic cancer, colon cancer, rectal cancer, colorectal cancer, renal cancer, hepatocellular carcinoma, ovary cancer, prostate cancer, thyroid cancer, testis cancer, head and neck squamous cell carcinoma, leukemia, lymphoma, and myeloma.

Presence of specific tumor associated antigens by specific types of cancer cells are known in the art. For example, B-cell malignancies often involve CD19⁺ (e.g., B-cell acute lymphoblastic leukemia) and/or CD20⁺ cancer cells (e.g., B-cell Non-Hodgkin's lymphoma). EGFR is expressed on various types of cancer, such as lung cancer and colon cancer. HER2 is

associated with, for example, breast cancer. PSMA is associated, for example, prostate cancer. CEA is associated with various types of cancer, including colon, rectum, and pancreatic cancer. EpCAM, FAP, CD47, and TRAIL-R2 are associated with solid tumors. PDL1 is associated with various cancers, such as bladder cancer, non-small cell lung cancer, breast cancer, small
5 cell lung cancer, etc. CD38 is associated with, for example, multiple myeloma. CD33 is associated with, for example, AML. cMET (HGFR) is associated, for example, non-small cell lung cancer. Mesothelin is associated with mesothelioma. GD2 is associated with neuroblastoma. Accordingly, choosing a bispecific antibody disclosed herein that has a suitable anti-TAA binding moiety to treat a particular type of cancer is within the knowledge of a
10 medical practitioner.

A subject having a target cancer can be identified by routine medical examination, e.g., laboratory tests, organ functional tests, CT scans, or ultrasounds. In some embodiments, the subject to be treated by the method described herein may be a human cancer patient who has undergone or is subjecting to an anti-cancer therapy, for example, chemotherapy, radiotherapy,
15 immunotherapy, or surgery. A subject suspected of having any of such target disease/disorder might show one or more symptoms of the disease/disorder. A subject at risk for the disease/disorder can be a subject having one or more of the risk factors for that disease/disorder.

As used herein, "an effective amount" refers to the amount of each active agent
20 required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. Determination of whether an amount of the antibody achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition,
25 size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the individual components or combinations thereof be used, that is, the highest safe
30 dose according to sound medical judgment.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's

immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained continuous release formulations of an antibody may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one example, dosages for an antibody as described herein may be determined empirically in individuals who have been given one or more administration(s) of the antibody. Individuals are given incremental dosages of the agonist. To assess efficacy of the agonist, an indicator of the disease/disorder can be followed.

The particular dosage regimen, *i.e.*, dose, timing and repetition, will depend on the particular individual and that individual's medical history, as well as the properties of the individual agents (such as the half-life of the agent, and other considerations well known in the art).

For the purpose of the present disclosure, the appropriate dosage of armed immune cells as described herein will depend on the specific bispecific antibody on the immune cells, the type of immune cells (or compositions thereof) employed, the type and severity of the disease/disorder, the patient's clinical history and response to the agonist, and the discretion of the attending physician. Typically the clinician will administer armed immune cells, until a dosage is reached that achieves the desired result. Methods of determining whether a dosage resulted in the desired result would be evident to one of skill in the art. Administration of one or more doses of armed immune cells can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the armed immune cells may be essentially continuous over a preselected period of time or may be in a series of spaced dose, *e.g.*, either before, during, or after developing a target disease or disorder.

In some embodiments, the amount of the armed immune cells such as armed T cells administered to the subject can be about 1×10^4 to 1×10^7 cells/Kg body weight of the subject. In certain embodiments, the amount of armed immune cells such as armed T cells can be administered to the subject from about 1×10^5 to 1×10^6 cells/Kg body weight of the subject. The dose can be administered in a single dose, or alternatively in more than one dose.

As used herein, the term "treating" refers to the application or administration of a composition including one or more active agents to a subject, who has a target disease or disorder, a symptom of the disease/disorder, or a predisposition toward the disease/disorder,

with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disorder, the symptom of the disease, or the predisposition toward the disease or disorder.

Alleviating a target disease/disorder includes delaying the development or progression of the disease, or reducing disease severity or prolonging survival. Alleviating the disease or
5 prolonging survival does not necessarily require curative results. As used therein, "delaying" the development of a target disease or disorder means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that
10 "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

"Development" or "progression" of a disease means initial manifestations and/or
15 ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of a target disease or
20 disorder includes initial onset and/or recurrence.

Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the armed immune cells or a pharmaceutical composition comprising such to a subject, depending upon the type of cancer to be treated or the site of the cancer. In some instances, the armed immune cells can be administered via intravenous infusion.

25 In some embodiments, the armed immune cells disclosed herein may be co-used with another anti-cancer agent, for example, a chemotherapeutic agent, an immunotherapeutic agent, or a combination thereof. For example, the armed immune cells disclosed herein may be used in combination with an immune checkpoint inhibitor, such as an anti-PD-1 antibody or an anti-PDL1 antibody. As used herein, the term "combination," "combined," and related
30 terms refers to the simultaneous or sequential administration of multiple therapeutic agents in accordance with this disclosure. For example, the armed immune cells as disclosed herein may be administered with another therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form.

In one example, a method for treating a subject (e.g., a human cancer patient) having

cancer cells expressing a TAA using armed immune cells disclosed herein may comprising the following steps: (a) isolating PBMCs from the patient; (b) cultivating the PBMCs of step (a) with a bispecific antibodies disclosed herein, which comprises a binding moiety specific to the TAA so as to produce armed immune cells such as armed T cells; and (c) administering to the
5 subject an effective amount of the armed immune cells.

In the step (a), the PBMCs can be isolated from the subject. The subject may be any mammal, for example, a human, mouse, rat, chimpanzee, rabbit, monkey, sheep, goat, cat, dog, horse, or pig. Preferably, the subject is a human. The methods suitable for isolating PBMCs from the subject include, but are not limited to, density centrifugation (*e.g.*, FICOLL[®] Paque),
10 cell preparation tube (CPT), and SEPMATE[™] tube.

In the step (b), the isolated PBMCs are cultured in a medium containing the present recombinant antibody for a sufficient period of time (*e.g.*, at least 7 days) so as to produce the TAA-specific T cells. The bispecific antibody is capable of inducing the activation of T cells by its anti-CD3 antibody fragment. The thus-produced armed T cells has the bispecific antibody
15 bound on the surface thereof, and accordingly, may specifically target the cancer cells via the anti-TAA antibody fragment of the bispecific antibody.

In the step (c), the armed immune cells such as armed T cells produced in step (b) can be administered to the subject so as to treat cancer. The amount of T cells administered to the subject is from about 1×10^4 to 1×10^7 cells/Kg body weight of the subject. In certain
20 embodiments, the amount of T cells is administered to the subject from about 1×10^5 to 1×10^6 cells/Kg body weight of the subject. The dose can be administered in a single dose, or alternatively in more than one dose.

Optionally, prior to step (c), the method may further isolating the T cell from the product of step (b) by a method suitable for isolating or purifying immune cells, for example,
25 affinity column, or magnetic beads. Treatment efficacy may be examined via routine practice.

IV. Kits For Cancer Treatment

The present disclosure also provides kits comprising any of the armed immune cells such as armed T cells or any of the bispecific antibodies disclosed herein. Such kits can be
30 used for treating or alleviating a target cancer as disclosed herein. Such kits can include one or more containers comprising the armed immune cells or a bispecific antibody as those described herein.

In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of

administration of the armed immune cells or use of the bispecific antibody to produce the armed immune cells, to treat, delay the onset, or alleviate a target disease as those described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In still other
5 embodiments, the instructions comprise a description of administering an antibody to an individual at risk of the target disease.

 The instructions relating to the use of the armed immune cells such as armed T cells or the bispecific antibody generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages
10 (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the present disclosure 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.

 The label or package insert indicates that the composition is used for treating, delaying
15 the onset and/or alleviating the disease, such as cancer or immune disorders (e.g., an autoimmune disease). Instructions may be provided for practicing any of the methods described herein.

 The kits of this 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
20 the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit 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). The container may also have a sterile access port (for example the container may be an
25 intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is armed immune cells or a bispecific antibody as those described herein.

 Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or
30 associated with the container. In some embodiments, the invention provides articles of manufacture comprising contents of the kits described above.

General techniques

 The practice of the present disclosure will employ, unless otherwise indicated,

conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; *Oligonucleotide Synthesis* (M. J. Gait, ed. 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J. E. Cellis, ed., 1989) Academic Press; *Animal Cell Culture* (R. I. Freshney, ed. 1987); *Introduction to Cell and Tissue Culture* (J. P. Mather and P. E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J. B. Griffiths, and D. G. Newell, eds. 1993-8) J. Wiley and Sons; *Methods in Enzymology* (Academic Press, Inc.); *Handbook of Experimental Immunology* (D. M. Weir and C. C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J. M. Miller and M. P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. eds. 1987); *PCR: The Polymerase Chain Reaction*, (Mullis, et al., eds. 1994); *Current Protocols in Immunology* (J. E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C. A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: a practice approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal antibodies: a practical approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using antibodies: a laboratory manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds. Harwood Academic Publishers, 1995); *DNA Cloning: A practical Approach*, Volumes I and II (D.N. Glover ed. 1985); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds.(1985»; *Transcription and Translation* (B.D. Hames & S.J. Higgins, eds. (1984»; *Animal Cell Culture* (R.I. Freshney, ed. (1986»; *Immobilized Cells and Enzymes* (IRL Press, (1986»; and B. Perbal, *A practical Guide To Molecular Cloning* (1984); F.M. Ausubel *et al.* (eds.).

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

Example 1: Production of Recombinant Bi-Specific Antibodies

In this example, recombinant antibodies respectively having the structures as depicted in FIGs 1A-1L were prepared using the DNA constructs illustrated in FIGs 2A-2E. For anti-CD3 Fab/anti-TAA scFv bi-specific antibodies, the constructs comprise, from

N-terminus to C-terminus, (a) Igk leader sequence (LS), anti-CD3 VL-Ck domain or anti-CD3 VL-C λ domain, internal ribosomal entry site (IRES), LS, anti-CD3 VH-CH1 domain, peptide linker, and anti-TAA scFv (*e.g.*, anti-EGFR scFv) (FIGs. 1A-1D) and FIG. 2A, top two constructs; or (b) LS, anti-CD3 VL-Ck domain or anti-CD3 VL-C λ domain, peptide linker,
 5 anti-TAA scFv (*e.g.*, anti-EGFR scFv), IRES, LS, and anti-CD3 VH-CH1 domain (FIG. 1B and FIG. 2A, bottom two constructs).

For anti-CD3 scFv/anti-TAA Fab, the constructs comprise, from N-terminus to C-terminus, (a) LS, anti-TAA VL-Ck domain (*e.g.*, anti-EGFR VL-Ck domain), IRES, LS, anti-TAA VH-CH1 domain (*e.g.*, anti-EGFR VH-CH1 domain), peptide linker, and anti-CD3
 10 VH-VL domain or anti-CD3 VL-VH domain (FIGs. 1E-1H and FIG. 2B, top two constructs); or (b) LS, anti-TAA VL-Ck domain (*e.g.*, anti-EGFR VL-Ck domain), peptide linker, anti-CD3 VH-VL domain or anti-CD3 VL-VH domain, IRES, LS, and anti-TAA VH-CH1 domain (*e.g.*, anti-EGFR VH-CH1 domain) (FIG. 1F and FIG. 2B, bottom two constructs).

For anti-CD3 scFv/anti-TAA scFv, the constructs comprise, from N-terminus to
 15 C-terminus, LS, anti-TAA scFv (*e.g.*, anti-EGFR scFv), peptide linker, and anti-CD3 VH-VL domain or anti-CD3 VL-VH domain (FIGs. 1I-1L and Fig. 2C).

For anti-CD3 knob/anti-TAA hole antibody, the anti-CD3 knob constructs comprise, from N-terminus to C-terminus, LS, anti-CD3 VL-Ck domain or anti-CD3 VL-C λ domain, IRES, LS, and anti-CD3 VH-CH1-knob Fc, while the anti-tumor hole comprised in sequence,
 20 LS, anti-TAA VL-Ck (*e.g.*, anti-EGFR VL-Ck domain), IRES, LS, and anti-TAA VH-CH1-hole Fc (*e.g.*, anti-EGFR VH-CH1-hole Fc) (FIG. 2D).

For anti-CD3 knob/anti-TAA scFv hole antibody, the anti-CD3 knob construct comprised in sequence, LS, anti-CD3 VL-Ck domain or anti-CD3 VL-C λ domain, IRES, LS, and anti-CD3 VH-CH1-knob Fc, while the anti-tumor hole comprised in sequence, LS,
 25 anti-TAA scFv (*e.g.*, anti-EGFR scFv), peptide linker, and hole Fc (FIG. 2E).

Two recombinant antibodies, respectively designated as CTA02scFv/CTAT03Fab (previously named anti-EGFR Fab/CAT.02 scFv) and CTA01scFv/CTAT03Fab (previously named anti-EGFR Fab/aCD3 scFv, structural information of which is provided in WO2018177371, the relevant disclosures of which is incorporated by reference for the subject
 30 matter and purpose referenced herein), were accordingly prepared. Both the CTA02scFv/CTAT03Fab and CTA01scFv/CTAT03Fab comprised an anti-EGFR Fab and an anti-CD3 scFv, in which the VH-CH1 and VL-Ck domains of the anti-EGFR Fab respectively had the amino acid sequences of SEQ ID NOs: 83 and 84. The anti-CD3 scFv of the CTA02scFv/CTAT03Fab had the amino acid sequence of SEQ ID NO: 9, and the anti-CD3

scFv of the CTA01scFv/CTAT03Fab is provided in WO2018177371 (CTA01 is the same antibody named as OKT3 in WO2018177371).

Example 2: Effect of Recombinant Bi-Specific Antibodies on T cells

5 This example investigates bioactivities of the recombinant bi-specific antibodies disclosed herein.

Binding affinity

The binding affinities of the recombinant antibodies CTA02scFv/CTAT03Fab and CTA01scFv/CTAT03Fab were examined in this example by flow cytometry. Both the
10 CTA02scFv/CTAT03Fab and the CTA01scFv/CTAT03Fab were capable of binding to CD3-positive T cells. The binding affinity of the CTA02scFv/CTAT03Fab was higher than that of the CTA01scFv/CTAT03Fab. FIG. 3. The binding affinity results are provided in Table 4 below.

Table 4 Binding Affinity of Exemplary Bi-Specific Antibodies

Group				Fluorescent signal
T cell				0
T cell	+	1 ug/mL	CTA01scFv/CTAT03Fab	24.44
T cell	+	5 ug/mL	CTA01scFv/CTAT03Fab	50.31
T cell	+	10 ug/mL	CTA01scFv/CTAT03Fab	74.97
T cell	+	1 ug/mL	CTA02scFv/CTAT03Fab	57.95
T cell	+	5 ug/mL	CTA02scFv/CTAT03Fab	104.63
T cell	+	10 ug/mL	CTA02scFv/CTAT03Fab	117.25

Cytotoxic effect

The cytotoxic effect of the recombinant antibody CTA02scFv/CTAT03Fab or the CTA01scFv/CTAT03Fab on cancer cells was evaluated in this example. It was found that about 2.5%, 18.2% and 26.9% of HT-29 cells were killed by CD3⁺/CD8⁺ T cells activated by murine OKT3 at the effect cells to target cells ratio (E/T ratio) of 3:1, 5:1 and 10:1,
20 respectively; about 13.8%, 34.8% and 65.7% of HT-29 cells were killed by CD3⁺/CD8⁺ T cells armed with the CTA01scFv/CTAT03Fab, and about 28.1%, 44.4% and 76.7% of HT-29 cells were killed by T cells armed with the CTA02scFv/CTAT03Fab at the same E/T ratio (FIG. 4 and Table 5).

Table 5 Cytotoxic effect of specified antibodies

E/T ratio	OKT3 activated T cells	CTA01scFv/CTAT03Fab armed T cells	CTA02scFv/CTAT03Fab armed T cells
3:1	2.5±1.8	13.8±0.7	28.1±2.7
5:1	18.2±1.5	34.8±3.3	44.4±3
10:1	26.9±0.6	65.7±3.3	76.7±1.3

The data indicated that both the tested bi-specific antibodies showed higher cytotoxic effects against cancer cells relative to the OKT3 antibody (anti-CD3 antibody) and the

5 CTA02scFv/CTAT03Fab showed better cytotoxicity effect.

Time effects on the amounts of antibodies remained on the surfaces of T cells

The CTA02scFv/CTAT03Fab and the CTA01scFv/CTAT03Fab were respectively incubated with T cells in the presence of 20% FBS for 24 hours. The T cells were then analyzed by flow cytometry to evaluate the residual amounts of the antibodies on the surface

10 of T cells. The results from this assay indicate that the amounts of the antibodies on the surface of the T cells declined over time. FIG. 5 and Table 6 below. Compared to the CTA01scFv/CTAT03Fab, the CTA02scFv/CTAT03Fab was less affected by degradation. About 84.5% of the CTA02scFv/CTAT03Fab still remained on the T cell surface after 24 hours, while the level of the CTA01scFv/CTAT03Fab remained on the T cell surface dropped

15 to about 40% after 24 hours.

Table 6. Percentage of Bi-Specific Antibodies Remaining On Cell Surface Over Time

Time (hour)	CTA02scFv/CTAT03Fab	CTA01scFv/CTAT03Fab
0	100	100
24	84.5±0.6	40±1.4

In sum, the bi-specific antibodies disclosed herein (exemplified by the CTA02scFv/CTAT03Fab antibody) exhibited higher binding affinity to T cells, higher

20 cytotoxicity, and higher level of T cell engagement over time relative to the CTA01scFv/CTAT03Fab control antibody.

Example 2: Construction of Variant Anti-CD3/Anti-Tumor Bispecific Antibodies

A panel of various anti-CD3/anti-tumor-associated antigen (TAA) bispecific antibodies (BsAbs) were constructed by genetic engineering. These BsAbs were derived from 4 anti-CD3 antibodies and 16 anti-TAA antibodies (CD20(CTAT01), CD19(CTAT02), EGFR(CTAT03),
 5 HER2(CTAT04), PSMA(CTAT05), CEA(CTAT06), EpCAM(CTAT07), FAP(CTAT08), PDL1(CTAT09), CD38(CTAT10), CD33(CTAT11), HGFR(CTAT12), CD47(CTAT13), TRAIL-R2(CTAT14), mesothelin (CTAT15) and GD2(CTAT16)). See **Tables 1** and **2** above.

The BsAbs were produced via recombinant technology in mammalian host cells, collected, and examined by SDS-PAGE under reduced conditions and non-reduced conditions.
 10 Briefly, protein electrophoresis with 8% SDS-PAGE in non-reducing conditions and reducing conditions were performed to analyze the structure and molecular weight of various BsAbs comprising an anti-CD3 fragment and an anti-TAA fragment.

FIGs. 6A-6B show the expression and assembly of BsAbs each comprising a Fab of 4 different anti-CD3 antibody (CTA02, CTA03, CTA04, and CTA05; see Table 1 above) and an
 15 anti-CD19 scFv (CTAT02; see **Table 2** above).

FIGs. 7A-7D show the expression and assembly of BsAbs each comprising an anti-CD3 Fab (CTA03; see **Table 1** above) and a scFv of 16 different anti-tumor antibodies (CD20(CTAT01), CD19(CTAT02), EGFR (CTAT03), HER2(CTAT04), PSMA(CTAT05), CEA(CTAT06), EpCAM (CTAT07), FAP(CTAT08), PDL1(CTAT09), CD38(CTAT10),
 20 CD33(CTAT11), HGFR(CTAT12), CD47(CTAT13), TRAIL-R2(CTAT14), mesothelin (CTAT15) and GD2(CTAT16); see **Table 2** above). FIGs. 7E and 7F show expression of BsAbs each comprising a scFv of one of the four anti-CD3 antibodies (CTA02-CTA05) and a Fab fragment of anti-EGFR CTAT03.

25 **Example 3: Anti-CD3/anti-TAA BsAbs Binds T Cells and Targets Tumor Cells Expressing the TAA**

The binding activities of the various anti-CD3/anti-tumor BsAbs to T cells and tumor cells were analyzed using flow cytometry. The BsAbs specific to CD3 and CD19 (CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv, CTA04Fab/CTAT02scFv, and
 30 CTA05Fab/CTAT02scFv) all showed binding activity to CD3⁺ T cells (Jurkat) and CD19⁺ B cell lymphoma (Raji), indicating that T cells armed with such BsAbs could be used to target CD19⁺ disease cells such as CD19⁺ B cell lymphoma. FIG. 8.

The binding activity of BsAbs having a binding fragment to other TAAs were also investigated. As shown in FIGs. 9A-10K:

- CTA03Fab/CTAT02scFv showed binding activity to CD3⁺ T cells (Jurkat) and CD19⁺ B cell lymphoma (Raji) (FIG. 9A);
- CTA03Fab/CTAT03scFv showed binding activity to CD3⁺ T cells (Jurkat) and EGFR⁺ triple negative breast cancer (MDA-MB-231) (FIG. 9B);
- 5 • CTA03Fab/CTAT04scFv showed binding activity to CD3⁺ T cells (Jurkat) and HER2⁺ breast cancer (MCF7/HER2) (FIG. 9C);
- CTA03Fab/CTAT05scFv showed binding activity to CD3⁺ T cells (Jurkat) and PSMA⁺ Prostate cancer (LNCaP) (FIG. 9D);
- CTA03Fab/CTAT07scFv showed binding activity to CD3⁺ T cells (Jurkat) and EpCAM⁺ prostate cancer (LNCaP) (FIG. 9E);
- 10 • CTA03Fab/CTAT08scFv showed binding activity to CD3⁺ T cells (Jurkat) and FAP⁺ mouse fibroblasts cell (3T3/FAP) (FIG. 9F);
- CTA03Fab/CTAT09scFv showed binding activity to CD3⁺ T cells (Jurkat) and PDL1⁺ triple negative breast cancer (MDA-MB-231) (FIG. 9G);
- 15 • CTA03Fab/CTAT10scFv showed binding activity to CD3⁺ T cells (Jurkat) and CD38⁺ B cell lymphoma (Raji) (FIG. 9H);
- CTA03Fab/CTAT11scFv showed binding activity to CD3⁺ T cells (Jurkat) and CD33⁺ human acute myeloid leukemia (HL-60) (FIG. 9I);
- CTA03Fab/CTAT12scFv showed binding activity to CD3⁺ T cells (Jurkat) and HGFR⁺ human lung carcinoma (A549) (FIG. 9J); and
- 20 • CTA03Fab/CTAT13scFv showed binding activity to CD3⁺ T cells (Jurkat) and CD47⁺ breast cancer (MCF7/HER2) (FIG. 9K).

Further, the binding activities of various anti-CD3 scFv/ anti-tumor Fab BsAbs to T
 25 cells and tumor cell were analyzed using flow cytometry. FIG. 9L shows the targeting ability against CD3⁺ T cells (Jurkat) and EGFR⁺ colon cancer (HT-29) of BsAbs consisting of a scFv of 4 different anti-CD3 antibody (CTA02, CTA03, CTA04, CTA05) and an anti-EGFR Fab (CTAT03). The results demonstrated that CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab all
 30 possessed targeting abilities against CD3⁺ T cells (Jurkat) and EGFR⁺ colon cancer (HT-29).

Example 4: Retention Ability of BsAb on T cell Surface

The retention time of BsAb on T cell surface was analyzed using an *in vitro* incubation platform. Briefly, human T cells were incubated with various anti-CD3Fab/anti-CD19scFv

(CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv and CTA05Fab/CTAT02scFv) for 1hr, and then were cultured in medium for 5 min, 24, 48, and 72 hr. After the culture, the residual amount of BsAb on T cell surface was detected using flow cytometry. After 72 hrs, CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv and CTA05Fab/CTAT02scFv were all detected on T cell surface, and CTA03Fab/CTAT02scFv had the highest retention amount on T cell surface. FIG. 10.

Example 5: Preparation of Armed Immune Cells Using BsAbs via One-Step Incubation,

Human peripheral blood mononuclear cells (PBMCs) from a healthy donor were cultured and differentiated into T cells in the presence of the OKT3 antibody, or in the presence of exemplary BsAbs disclosed herein (using CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv and CTA05Fab/CTAT02scFv as examples). All groups were cultured under the same conditions (in an incubator with 5% CO₂ supply and a stable humidity level at 37°C). After 7 days, all groups were analyzed using flow cytometry to measure production of BsAb armed-T cells.

As shown in FIGs. 11A and 11B, the OKT3 anti-CD3 antibody induced differentiation of PBMCs into only normal T cells but not armed-T cells. By contrast, CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv and CTA05Fab/CTAT02scFv BsAbs all successfully produced armed-T cells.

In another experiment, PBMCs were cultured and differentiated into T cells with OKT3 or exemplary anti-CD3 Fab/anti-Tumor scFv BsAbs (CTA03Fab/CTAT03scFv, CTA03Fab/CTAT04scFv, CTA03Fab/CTAT05scFv, CTA03Fab/CTAT07scFv, CTA03Fab/CTAT08scFv, CTA03Fab/CTAT09scFv, CTA03Fab/CTAT10scFv, CTA03Fab/CTAT11scFv, CTA03Fab/CTAT12scFv, and CTA03Fab/CTAT13scFv). All groups were cultured under the same conditions (in an incubator with 5% CO₂ and a stable humidity level at 37°C). After 7 days, all groups were analyzed using flow cytometry to reveal whether BsAb armed-T cells were successfully generated. FIGs 12A and 12B show that the OKT3 antibody led to differentiation of PBMCs into normal T cells, but not armed-T cells were produced. By contrast, CTA03Fab/CTAT03scFv, CTA03Fab/CTAT04scFv, CTA03Fab/CTAT05scFv, CTA03Fab/CTAT07scFv, CTA03Fab/CTAT08scFv, CTA03Fab/CTAT09scFv, CTA03Fab/CTAT10scFv, CTA03Fab/CTAT11scFv, CTA03Fab/CTAT12scFv, and CTA03Fab/CTAT13scFv BsAbs all led to production of armed-T cells.

Further, PBMCs were cultured and differentiated into T cells with OKT3 or various

anti-CD3 scFv/anti-Tumor Fab BsAbs (CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab). All groups were cultured under the same conditions (in an incubator with 5% CO₂ and a stable humidity level at 37°C). After 7 days, all groups were analyzed using flow cytometry to reveal whether BsAb armed-T cells were successfully generated. FIGs. 12C and 12D shows that the traditional OKT3 method caused PBMCs to differentiate only into normal T cells, but not armed-T cells. However, CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab BsAbs all successfully generated armed-T cells.

Example 6: *In Vitro* Toxicity of BsAb-Armed T cells Against Tumor Cells

The cytotoxicity activity of T cells armed with anti-CD3/anti-CD19 BsAbs against CD19⁺ B cell lymphoma (Raji) were investigated in this example. The exemplary anti-CD3/anti-CD19 BsAbs disclosed herein, including CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv and CTA05Fab/CTAT02scFv. T cells cultured with the OKT3 antibody was analyzed using an *in vitro* cytotoxicity assay. No significant cytotoxicity of T cells cultured with OKT3 against CD19⁺ B cell lymphoma (Raji) was observed. Differently, T cells cultured with CTA01Fab/CTAT02scFv, CTA02Fab/CTAT02scFv, CTA03Fab/CTAT02scFv, or CTA05Fab/CTAT02scFv efficiently killed CD19⁺ B cell lymphoma (Raji). Among the tested BsAbs, CTA03Fab/CTAT02scFv armed T cells had the best cytotoxic activity. FIG. 13A.

Further, the EGFR⁺ colon cancer (HT-29) killing activity of T cells armed with anti-CD3scFv/anti-EGFRFab BsAb consisting of 5 different anti-CD3 scFv (CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab) and T cells cultured with traditional OKT3 antibody was analyzed using an *in vitro* cytotoxicity assay. FIG. 13B shows that the T cells incubated with OKT3 did not efficiently kill EGFR⁺ colon cancer (HT-29), but the armed T cells cultured with CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab efficiently killed EGFR⁺ colon cancer (HT-29). FIG. 13B.

In another experiment, the tumor cell killing activity of armed T cells generated with various anti-CD3 Fab/anti-Tumor scFv BsAb (including CTA03Fab/CTAT03scFv, CTA03Fab/CTAT04scFv, CTA03Fab/CTAT05scFv, CTA03Fab/CTAT07scFv, CTA03Fab/CTAT08scFv, CTA03Fab/CTAT9scFv, CTA03Fab/CTAT10scFv,

CTA03Fab/CTAT11scFv, CTA03Fab/CTAT12scFv and CTA03Fab/CTAT13scFv) were further analyzed. As shown in FIGs. 14A and 14B, CTA03Fab/CTAT03scFv armed-T cells efficiently killed EGFR⁺ colon cancer cells HT29 and HCT-116. FIG. 14C shows that CTA03Fab/CTAT04scFv armed-T cells efficiently killed HER2⁺ Breast cancer (MCF7/HER2). FIG. 14D shows that CTA03Fab/CTAT05scFv armed-T cells efficiently killed PSMA⁺ Prostate cancer (LNCaP). FIG. 14E shows that CTA03Fab/CTAT07scFv armed-T cells efficiently killed EpCAM⁺ Prostate cancer (LNCaP). Further, FIGs. 14F-14G show that CTA03Fab/CTAT08scFv armed-T cells efficiently killed FAP⁺ mouse fibroblasts cell (3T3/FAP).

In addition, FIG. 15A shows that CTA03Fab/CTAT09scFv armed-T cells efficiently killed PDL1⁺ triple negative breast cancer (MDA-MB-231). FIG. 15B shows that CTA03Fab/CTAT10scFv armed-T cells efficiently killed CD38⁺B cell lymphoma (Raji). FIG. 15C shows that CTA03Fab/CTAT11scFv armed-T cells efficiently killed CD33⁺ human acute myeloid leukemia (HL-60). FIG. 15D shows that CTA03Fab/CTAT12scFv armed-T cells efficiently killed HGFR⁺ human lung carcinoma (A549). FIG. 15E shows that CTA03Fab/CTAT13scFv armed-T cells efficiently killed CD47⁺ Breast cancer (MCF7/HER2).

Example 7: *In Vivo* Anti-Cancer Activity of CTA03Fab/CTAT02scFv Armed-T cells

In vivo cancer inhibition of anti-CD3Fab/anti-CD19scFv (CTA01Fab/CTAT02scFv and CTA03Fab/CTAT02scFv) armed-T cells was evaluated. CTA01Fab/CTAT02scFv armed-T cells and CTA03Fab/CTAT02scFv armed-T cells were i.v. injected into SCID mice bearing with B cell lymphoma (Raji). Body weight, survival rate and incidence of hindlimb paralysis were recorded. FIGs. 16A-16C show that CTA03Fab/CTAT02scFv armed-T cells had the best therapeutic efficacy to effectively inhibit cancer.

Example 8: *In Vivo* Anti-Tumor Activity of CTA03Fab/CTAT03scFv Armed-T cells and CTA03Fab/CTAT04scFv Armed-T cells

In vivo tumor inhibition of anti-CD3Fab/anti-EGFRscFv (CTA03Fab/CTAT03scFv) armed-T cells and anti-CD3Fab/anti-HER2scFv (CTA03Fab/CTAT04scFv) armed-T cells were evaluated. CTA03Fab/CTAT03scFv armed-T cells and CTA03Fab/CTAT04scFv armed-T cells were i.v. injected into patient-derived xenograft (PDX) mice models bearing with human triple-negative breast cancer. Body weight and tumor size were recorded. FIGs. 17A-17B show that CTA03Fab/CTAT03scFv armed-T cells and CTA03Fab/CTAT04scFv armed-T cells both efficiently inhibited the tumor growth of human triple-negative breast cancer.

Example 9: One-Step Incubation for Producing Armed NKT Cells from PBMCs Using BsAbs

Human peripheral blood mononuclear cells (PBMCs) from a healthy donor were cultured and differentiated into NKT cells (CD8⁺ CD56⁺) with the OKT3 traditional method, or with CTA03Fab/CTAT03scFv, CTA03Fab/CTAT04scFv and CTA03Fab/CTAT05scFv BsAbs. All groups were cultured in the same environment (an incubator with 5% CO₂ and a stable humidity level at 37°C). After 7 days, all groups were analyzed using flow cytometry to reveal whether BsAb armed-NKT cells were successfully generated. FIGs. 18A and 18B show that OKT3 method induced PBMCs to differentiate into only normal NKT cells, but not formation of armed-T cells. Differently, CTA03Fab/CTAT03scFv, CTA03Fab/CTAT04scFv and CTA03Fab/CTAT05scFv BsAbs all successfully generated armed-NKT cells.

In a similar assay, human peripheral blood mononuclear cells (PBMCs) from a healthy donor were cultured and differentiated into NKT cells (CD8⁺ CD56⁺) in the presence of the OKT antibody, or with CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab BsAbs. All groups were cultured in the same environment (an incubator with 5% CO₂ and a stable humidity level at 37°C). After 7 days, all groups were analyzed using flow cytometry to reveal whether BsAb armed-NKT cells were successfully generated. FIG. 34 shows that the traditional OKT3 method caused PBMCs to differentiate into only normal NKT cells, but not armed-T cells. However, CTA01scFv/CTAT03Fab, CTA02scFv/CTAT03Fab, CTA03scFv/CTAT03Fab, CTA04scFv/CTAT03Fab, and CTA05scFv/CTAT03Fab BsAbs all successfully generated armed-NKT cells.

Example 10: Construction of CTA03Fab/CTAT02scFv BsAb With Point Mutations and Characterization Thereof

Point mutations were introduced into CTA03Fab/CTAT02scFv BsAb by genetic engineering, resulting in BsAbs CTA03-01Fab/CTAT02-01scFv (CTA03Fab(VLG58A) / CTAT02scFv(VLG42A)), CTA03-01Fab/CTAT02-02scFv (CTA03Fab(VLG58A) / CTAT02scFv(VLD41E)), CTA03-02Fab/CTAT02-02scFv (CTA03Fab(VLD57E) / CTAT02scFv(VLD41E)), and CTA03-02Fab/CTAT02-01scFv (CTA03Fab(VLD57E) / CTAT02scFv(VLG42A)). More specifically, point mutations G58A and D57E were introduced into the VL of CTA03 and G42A and D41E were introduced into the VL of CTAT02. See Table 2 above.

Binding abilities of the BsAbs against CD3⁺ T cells (Jurkat) and CD19⁺ B cells lymphoma (Raji) were analyzed using flow cytometry. FIGs. 19A-19B show that

CTA03-01Fab/CTAT02-01scFv, CTA03-01Fab/CTAT02-02scFv, CTA03-02Fab/CTAT02-02scFv and CTA03-02Fab/CTAT02-01scFv BsAbs all possessed targeting abilities against CD3⁺ T cells (Jurkat) and CD19⁺ B cells lymphoma (Raji). Additionally, binding of the BsAbs to the target cells is in a dose-dependent manner.

5 The killing activity against CD19⁺ B cell lymphoma by the armed T cells generated with the point-mutant BsAbs (CTA03-01Fab/CTAT02-01scFv, CTA03-01Fab/CTAT02-02scFv, CTA03-02Fab/CTAT02-02scFv and CTA03-02Fab/CTAT02-01scFv) were compared with that of the original CTA03Fab/CTAT02scFv BsAb armed-T cells. FIG. 19C shows that T cells incubated with
10 OKT3 did not show cytotoxicity against CD19⁺ B cell lymphoma (Raji). On the other hand, the armed-T cells cultured with CTA03-01Fab/CTAT02-01scFv, CTA03-01Fab/CTAT02-02scFv, CTA03-02Fab/CTAT02-02scFv or CTA03-02Fab/CTAT02-01scFv efficiently killed CD19⁺ B cell lymphoma (Raji), and the cytotoxicity were all better than the parent CTA03Fab/CTAT02scFv BsAb.

15

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature
20 disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

25

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages
30 described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive

5 teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and
10 equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually
15 inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

15 All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

20 The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements
25 specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

30 As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly

one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.”

5 “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For

10 example, “about” can mean within an acceptable standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to $\pm 20\%$, preferably up to $\pm 10\%$, more preferably up to $\pm 5\%$, and more preferably still up to $\pm 1\%$ of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 2-fold, of a value. Where particular values are described in the

15 application and claims, unless otherwise stated, the term “about” is implicit and in this context means within an acceptable error range for the particular value. In some embodiments, the hinge domain is a hinge domain of a naturally occurring protein.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element

20 selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or

25 unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally

30 including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of

the method is not necessarily limited to the order in which the steps or acts of the method are recited.