

Mallone, R. *et al.* (2005) “*Targeting T Lymphocytes For Immune Monitoring And Intervention In Autoimmune Diabetes*,” *Am. J. Ther.* 12(6):534–550). CD3 is the receptor that binds to the TCR (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*,” *Immunology* 129(2):170-177; Guy, C.S. *et al.* (2009) “*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex*,” *Immunol. Rev.* 232(1):7-21; St. Clair, E.W. (Epub 2009 Oct 12) “*Novel Targeted Therapies For Autoimmunity*,” *Curr. Opin. Immunol.* 21(6):648-657; Baeuerle, P.A. *et al.* (Epub 2009 Jun 9) “*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*,” *Cancer Res.* 69(12):4941-4944; Smith-Garvin, J.E. *et al.* (2009) “*T Cell Activation*,” *Annu. Rev. Immunol.* 27:591-619; Renders, L. *et al.* (2003) “*Engineered CD3 Antibodies For Immunosuppression*,” *Clin. Exp. Immunol.* 133(3):307-309).

**[0017]** The TCR and CD3 complex, along with the CD3  $\zeta$  chain zeta chain (also known as T-Cell receptor T3 zeta chain or CD247) comprise the “**TCR complex**” (van der Merwe, P.A. *etc.* (epub Dec. 3, 2010) “*Mechanisms For T Cell Receptor Triggering*,” *Nat. Rev. Immunol.* 11:47-55; Wucherpfennig, K.W. *et al.* (2010) “*Structural Biology of the T Cell Receptor: Insights into Receptor Assembly, Ligand Recognition, and Initiation of Signaling*,” *Cold Spring Harb. Perspect. Biol.* 2:a005140). The complex is particularly significant since it contains a large number (ten) of immunoreceptor tyrosine-based activation motifs (ITAMs).

**[0018]** Multispecific molecules comprising a CD3 Binding Domain and a binding domain specific for a Disease Antigen (“**DA**”) expressed on a target cell are capable of mediating redirected T-cell killing of such target cells. However, due to the affinity of such molecules for CD3, such molecules may be too potent, so as to exhibit undesirable cytokine release from the stimulated T-cells. Thus, despite prior advances in identifying the molecules involved in mammalian immune responses, a need remains for improved therapies for treating cancers and infectious diseases. The present invention provides a panel of variant CD3-Binding Domains having a range of binding kinetics, which may be used to modulate the cell killing and/or cytokine release activities of such multispecific molecules to enhance the therapeutic window. The present invention is directed to this and other goals.

## SUMMARY

**[0019]** The present disclosure is directed to multispecific Binding Molecules (*e.g.*, a bispecific antibody, a diabody, a bispecific scFv, a trivalent molecule, a TandAb®, a BiTE®

*etc.*) comprising a CD3-Binding Domain capable of binding an epitope of CD3 and also a Disease Antigen-Binding Domain capable of binding an epitope of a Disease Antigen (“**DA**”) (*e.g.*, a “**DA x CD3 Binding Molecule**”). The disclosure particularly concerns such **DA x CD3 Binding Molecules** comprising a variant CD3-Binding Domain (“**vCD3-Binding Domain**”), which comprises a CDR<sub>H1</sub> Domain, a CDR<sub>H2</sub> Domain, a CDR<sub>H3</sub> Domain, a CDR<sub>L1</sub> Domain, a CDR<sub>L2</sub> Domain, and a CDR<sub>L3</sub> Domain, at least one of which differs in amino acid sequence from the amino acid sequence of the corresponding CDR of a reference CD3-Binding Domain (“**rCD3-Binding Domain**”), and wherein the **DA x CD3 Binding Molecule** comprising such vCD3-Binding Domain exhibits an altered affinity for CD3, relative to a **DA x CD3 Binding Molecule** comprising such rCD3-Binding Domain. The disclosure particularly concerns to such **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domain which exhibit reduced affinity for CD3 and are capable of mediating redirected killing of target cells expressing a DA and exhibit lower levels of cytokine release relative to a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. The disclosure particularly concerns the use of **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domain in the treatment of cancer and pathogen-associated diseases. The present disclosure is also directed to pharmaceutical compositions that comprise such molecule(s).

**[0020]** In one aspect, the present disclosure refers to a Disease Antigen x CD3 (**DA x CD3**) **Binding Molecule** comprising a CD3-Binding Domain capable of binding an epitope of CD3 and a Disease Antigen-Binding Domain capable of binding an epitope of a Disease Antigen, wherein the CD3-Binding Domain comprises:

- (I) (A) a CDR<sub>H1</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:99**, **SEQ ID NO:91**, **SEQ ID NO:93**, **SEQ ID NO:95** and **SEQ ID NO:97**;
- (B) a CDR<sub>H2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:61**; and

- (F) a CDR<sub>L</sub>3 Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (II) (A) a CDR<sub>H</sub>1 Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H</sub>2 Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H</sub>3 Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105 and SEQ ID NO:107**;
- (D) a CDR<sub>L</sub>1 Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L</sub>2 Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR<sub>L</sub>3 Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (III) (A) a CDR<sub>H</sub>1 Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H</sub>2 Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H</sub>3 Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L</sub>1 Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L</sub>2 Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR<sub>L</sub>3 Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:109 or SEQ ID NO:111**; or
- (IV) (A) a CDR<sub>H</sub>1 Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H</sub>2 Domain comprising the amino acid sequence of **SEQ ID NO:58**;

- (C) a CDR<sub>H3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L2</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:113** and **SEQ ID NO:115**; and
- (F) a CDR<sub>L3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:62**.

**[0021]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the CD3-Binding Domain comprises:

- (I) (A) a VL Domain comprising the amino acid sequence of **SEQ ID NO:56**;
- (B) a VH Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:98, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104** and **SEQ ID NO:106**; or
- (II) (A) a VL Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112**; and **SEQ ID NO:114**;
- (B) a VH Domain comprising an amino acid sequence of **SEQ ID NO:55**.

**[0022]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** is a bispecific antibody, a bispecific diabody, a bispecific scFv, a bispecific TandAb, or a trivalent binding molecule.

**[0023]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** is capable of binding more than one Disease Antigen and/or a different cell surface molecule of an effector cell. Particularly,

wherein the different cell surface molecule of an effector cell is CD2, CD8, CD16, TCR, NKp46, or NKG2D.

**[0024]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the Disease Antigen is a Cancer Antigen, or a Pathogen-Associated Antigen.

**[0025]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe<sup>b</sup>/Le<sup>y</sup>, Burkitt's lymphoma antigen-38.13, C14, CA125, Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E<sub>1</sub> series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, Human Papillomavirus-E6/Human Papillomavirus-E7, HMW-MAA, I antigen, IL13R $\alpha$ 2, Integrin  $\beta$ 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6/L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R<sub>24</sub>, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T-cell receptor derived peptide, T<sub>5A7</sub>, TAG-72, TL5, TNF-receptor, TNF- $\gamma$  receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le<sup>y</sup>.

**[0026]** The disclosure particularly concerns the example of such **DA x CD3 Binding Molecule**, wherein the Cancer Antigen is B7-H3, CEACAM5/CEACAM6, EGFR, EphA2, gpA33, HER2/neu, VEGF, 5T4, IL13R $\alpha$ 2, CD123, CD19, or ROR1.

**[0027]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen-Associated Antigens: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, Human

Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

**[0028]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** comprises: a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(A) the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a Domain 1, comprising:

- (1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to the epitope of a Disease Antigen (VL<sub>DA</sub>); and
- (2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to the epitope of CD3 (VH<sub>CD3</sub>);

wherein the sub-Domains 1A and 1B are separated from one another by a peptide Linker; and

(ii) a Domain 2, wherein the Domain 2 is a Heterodimer-Promoting Domain;

(B) the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a Domain 1, comprising

- (1) a sub-Domain (1A), which comprises a VL Domain of the monoclonal antibody capable of binding to the epitope of CD3 (VL<sub>CD3</sub>); and
- (2) a sub-Domain (1B), which comprises a VH Domain of the monoclonal antibody capable of binding to the epitope of a Disease Antigen (VH<sub>DA</sub>);

wherein the sub-Domains 1A and 1B are separated from one another by a peptide Linker;

(ii) a Domain 2, wherein the Domain 2 is a Heterodimer-Promoting Domain, wherein the Heterodimer-Promoting Domain of the first and the second polypeptide chains are different;

and wherein:

- (a) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain associate to form the Disease Antigen-Binding Domain, and the VH Domain of the first polypeptide chain and the VL Domain of the second polypeptide chain associate to form the CD3-Binding Domain; or
- (b) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain associate to form the CD3-Binding Domain, and the VH Domain of the first polypeptide chain and the VL Domain of the second polypeptide chain associate to form the Disease Antigen-Binding Domain.

**[0029]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein:

- (a) said Heterodimer-Promoting Domain of said first polypeptide chain is an E-coil Domain, and said Heterodimer-Promoting Domain of said second polypeptide chain is a K-coil Domain; or
- (b) said Heterodimer-Promoting Domain of said first polypeptide chain is a K-coil Domain, and said Heterodimer-Promoting Domain of said second polypeptide chain is an E-coil Domain.

**[0030]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the first or second polypeptide chain additionally comprises a Domain 3 comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.

**[0031]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** further comprises a third polypeptide chain comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.

**[0032]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** further comprises a CD8-Binding Domain.

**[0033]** The disclosure additionally concerns the example of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** comprises:

- (I) (A) a first polypeptide comprising **SEQ ID NO:179**;
- (B) a second polypeptide comprising **SEQ ID NO:175**; and

- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (II) (A) a first polypeptide comprising **SEQ ID NO:184**;
- (B) a second polypeptide comprising **SEQ ID NO:181**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (III) (A) a first polypeptide comprising **SEQ ID NO:196**;
- (B) a second polypeptide comprising **SEQ ID NO:186**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (IV) (A) a first polypeptide comprising **SEQ ID NO:197**;
- (B) a second polypeptide comprising **SEQ ID NO:192**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (V) (A) a first polypeptide comprising **SEQ ID NO:193**;
- (B) a second polypeptide comprising **SEQ ID NO:194**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (VI) (A) a first polypeptide comprising **SEQ ID NO:179**;
- (B) a second polypeptide comprising **SEQ ID NO:175**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (VII) (A) a first polypeptide comprising **SEQ ID NO:184**;
- (B) a second polypeptide comprising **SEQ ID NO:181**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (VIII) (A) a first polypeptide comprising **SEQ ID NO:196**;
- (B) a second polypeptide comprising **SEQ ID NO:186**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (IX) (A) a first polypeptide comprising **SEQ ID NO:193**;
- (B) a second polypeptide comprising **SEQ ID NO:194**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**.

**[0034]** In another aspect, the present disclosure refers to a pharmaceutical composition that comprises any of the above-described **DA x CD3 Binding Molecules** and a pharmaceutically acceptable carrier.

[0035] The disclosure additionally concerns a method for the treatment of a disease, comprising administering to a subject in need thereof a therapeutically effective amount of any of the above-described **DA x CD3 Binding Molecules** or the above-described pharmaceutical composition.

[0036] The disclosure additionally concerns the example of such method, wherein the disease is cancer. Including examples, wherein the cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, hematological cancer, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

[0037] The disclosure additionally concerns the example of such method, wherein the disease is a pathogen-associated disease; including examples, wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen-Associated Antigens: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

[0038] The disclosure additionally concerns the use of any of the above-described **DA x CD3 Binding Molecules** or the above-described pharmaceutical composition in the treatment of a disease. In one aspect, the present disclosure refers to use of any one of the above-described **DA x CD3 Binding Molecules** or the above-described pharmaceutical composition in the manufacture of a medicament for the treatment of a disease. In another aspect, the present disclosure refers to any one of the above-described **DA x CD3 Binding Molecules** or the above-described pharmaceutical composition for use in the treatment of a disease.

[0039] The disclosure additionally concerns the example of such use, wherein the disease is cancer. Including, examples, wherein the cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, hematological cancer, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

[0040] The disclosure additionally concerns the example of such use, wherein the disease is a pathogen-associated disease. Including examples, wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen-Associated

WHAT IS CLAIMED IS:

Claim 1. A Disease Antigen x CD3 (**DA x CD3**) **Binding Molecule** comprising a CD3-Binding Domain capable of binding an epitope of CD3 and a Disease Antigen-Binding Domain capable of binding an epitope of a Disease Antigen, wherein said CD3-Binding Domain comprises:

- (I) (A) a CDR<sub>H1</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:99, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95** and **SEQ ID NO:97**;
- (B) a CDR<sub>H2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR<sub>L3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (II) (A) a CDR<sub>H1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H3</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105** and **SEQ ID NO:107**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;

- (E) a CDR<sub>L2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR<sub>L3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (III) (A) a CDR<sub>H1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR<sub>L3</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:109** or **SEQ ID NO:111**; or
- (IV) (A) a CDR<sub>H1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR<sub>H2</sub> Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR<sub>H3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR<sub>L1</sub> Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR<sub>L2</sub> Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:113** and **SEQ ID NO:115**; and
- (F) a CDR<sub>L3</sub> Domain comprising the amino acid sequence of **SEQ ID NO:62**.

- Claim 2. The **DA x CD3 Binding Molecule** of claim 1, wherein said CD3-Binding Domain comprises:
- (I) (A) a VL Domain comprising the amino acid sequence of **SEQ ID NO:56**;
  - (B) a VH Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:98, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO: 92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104 and SEQ ID NO:106**; or
  - (II) (A) a VL Domain comprising an amino acid sequence selected from the group consisting of **SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112**; and **SEQ ID NO:114**;
  - (B) a VH Domain comprising an amino acid sequence of **SEQ ID NO:55**.
- Claim 3. The **DA x CD3 Binding Molecule** of claim 1 or 2, wherein said **DA x CD3 Binding Molecule** is a bispecific antibody, a bispecific diabody, a bispecific scFv, a bispecific TandAb, or a trivalent binding molecule.
- Claim 4. The **DA x CD3 Binding Molecule** of any one of claims 1-3, wherein said **DA x CD3 Binding Molecule** is capable of binding more than one Disease Antigen and/or a different cell surface molecule of an effector cell.
- Claim 5. The **DA x CD3 Binding Molecule** of any one of claims 1-4, wherein said Disease Antigen is a Cancer Antigen.
- Claim 6. The **DA x CD3 Binding Molecule** of any one of claims 1-4, wherein said Disease Antigen is a Pathogen-Associated Antigen.

- Claim 7. The **DA x CD3 Binding Molecule** of any one of claims 4-6, wherein said different cell surface molecule of an effector cell is CD2, CD8, CD16, TCR, NKp46, or NKG2D.
- Claim 8. The **DA x CD3 Binding Molecule** of claim 5 or 7, wherein said Cancer Antigen is selected from the group consisting of: 19.9, 4.2, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe<sup>b</sup>/Le<sup>y</sup>, Burkitt's lymphoma antigen-38.13, C14, CA125, Carboxypeptidase M, CD5, CD19, CD20, CD22, CD23, CD25, CD27, CD28, CD33, CD36, CD40/CD154, CD45, CD56, CD46, CD52, CD56, CD79a/CD79b, CD103, CD123, CD317, CDK4, CEA, CEACAM5/CEACAM6, CO17-1A, CO-43, CO-514, CTA-1, CTLA-4, Cytokeratin 8, D1.1, D156-22, DR5, E1 series, EGFR, an Ephrin receptor, EphA2, Erb, GAGE, a GD2/GD3/GM2 ganglioside, GICA 19-9, gp100, Gp37, gp75, gpA33, HER2/neu, HMFG, Human Papillomavirus-E6/Human Papillomavirus-E7, HMW-MAA, I antigen, IL13R $\alpha$ 2, Integrin  $\beta$ 6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6, L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R<sub>24</sub>, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T-cell receptor derived peptide, T<sub>5A7</sub>, TAG-72, TL5, TNF-receptor, TNF- $\gamma$  receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le<sup>y</sup>.
- Claim 9. The **DA x CD3 Binding Molecule** of claim 8, wherein said Disease Antigen is B7-H3, CEACAM5/CEACAM6, EGRF, EphA2, gpA33, HER2/neu, VEGF, 5T4, IL13R $\alpha$ 2, CD123, CD19, or ROR1.
- Claim 10. The **DA x CD3 Binding Molecule** of claim 6 or 7, wherein said Pathogen-Associated Antigen is selected from the group consisting of: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, Human Papillomavirus E6,

Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.

Claim 11. The **DA x CD3 Binding Molecule** of any one of claims 1-10, wherein said **DA x CD3 Binding Molecule** comprises: a first polypeptide chain and a second polypeptide chain, covalently bonded to one another, wherein:

(A) the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a Domain 1, comprising:

- (1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to said epitope of a Disease Antigen (VL<sub>DA</sub>); and
- (2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to said epitope of CD3 (VH<sub>CD3</sub>);

wherein said sub-Domains 1A and 1B are separated from one another by a peptide Linker; and

(ii) a Domain 2, wherein said Domain 2 is a Heterodimer-Promoting Domain;

(B) the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

(i) a Domain 1, comprising:

- (1) a sub-Domain (1A), which comprises a VL Domain of said monoclonal antibody capable of binding to said epitope of CD3 (VL<sub>CD3</sub>); and
- (2) a sub-Domain (1B), which comprises a VH Domain of said monoclonal antibody capable of binding to said epitope of a Disease Antigen (VH<sub>DA</sub>);

wherein said sub-Domains 1A and 1B are separated from one another by a peptide Linker;

(ii) a Domain 2, wherein said Domain 2 is a Heterodimer-Promoting Domain, wherein said Heterodimer-Promoting Domain of said first and said second polypeptide chains are different;

and wherein

the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain associate to form the Disease Antigen-Binding Domain, and the VH Domain of the first polypeptide chain and the VL Domain of the second polypeptide chain associate to form the CD3-Binding Domain.

- Claim 12. The **DA x CD3 Binding Molecule** of claim 11, wherein:
- (a) said Heterodimer-Promoting Domain of said first polypeptide chain is an E-coil Domain, and said Heterodimer-Promoting Domain of said second polypeptide chain is a K-coil Domain; or
  - (b) said Heterodimer-Promoting Domain of said first polypeptide chain is a K-coil Domain, and said Heterodimer-Promoting Domain of said second polypeptide chain is an E-coil Domain.
- Claim 13. The **DA x CD3 Binding Molecule** of claim 11 or 12, wherein the first or second polypeptide chain additionally comprises a Domain 3 comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.
- Claim 14. The **DA x CD3 Binding Molecule** of claim 13, wherein said **DA x CD3 Binding Molecule** further comprises a third polypeptide chain comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.
- Claim 15. The **DA x CD3 Binding Molecule** of any one of claims 11-14, wherein said **DA x CD3 Binding Molecule** further comprises a CD8-Binding Domain.
- Claim 16. The **DA x CD3 Binding Molecule** of any one of claims 11-15, wherein said **DA x CD3 Binding Molecule** comprises:
- (I) (A) a first polypeptide comprising **SEQ ID NO:179**;  
(B) a second polypeptide comprising **SEQ ID NO:175**; and  
(C) a third polypeptide comprising **SEQ ID NO:176**; or
  - (II) (A) a first polypeptide comprising **SEQ ID NO:184**;  
(B) a second polypeptide comprising **SEQ ID NO:181**; and  
(C) a third polypeptide comprising **SEQ ID NO:176**; or
  - (III) (A) a first polypeptide comprising **SEQ ID NO:196**;  
(B) a second polypeptide comprising **SEQ ID NO:186**; and

- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (IV) (A) a first polypeptide comprising **SEQ ID NO:197**;
- (B) a second polypeptide comprising **SEQ ID NO:192**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (V) (A) a first polypeptide comprising **SEQ ID NO:193**;
- (B) a second polypeptide comprising **SEQ ID NO:194**; and
- (C) a third polypeptide comprising **SEQ ID NO:176**; or
- (VI) (A) a first polypeptide comprising **SEQ ID NO:179**;
- (B) a second polypeptide comprising **SEQ ID NO:175**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (VII) (A) a first polypeptide comprising **SEQ ID NO:184**;
- (B) a second polypeptide comprising **SEQ ID NO:181**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (VIII) (A) a first polypeptide comprising **SEQ ID NO:196**;
- (B) a second polypeptide comprising **SEQ ID NO:186**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**; or
- (IX) (A) a first polypeptide comprising **SEQ ID NO:193**;
- (B) a second polypeptide comprising **SEQ ID NO:194**;
- (C) a third polypeptide comprising **SEQ ID NO:187**; and
- (D) a fourth polypeptide comprising **SEQ ID NO:188**.

Claim 17. A pharmaceutical composition that comprises the **DA x CD3 Binding Molecule** of any one of claims 1-16 and a pharmaceutically acceptable carrier.

Claim 18. Use of the **DA x CD3 Binding Molecule** of any one of claims 1-16 or the pharmaceutical composition of claim 17 in the manufacture of a medicament for the treatment of a disease.

Claim 19. The use of claim 18, wherein said disease is cancer.

- Claim 20. The use of claim 19, wherein said cancer is selected from the group consisting of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, hematological cancer, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.
- Claim 21. The use of claim 18, wherein said disease is a pathogen-associated disease.
- Claim 22. The use of claim 21, wherein said Pathogen-Associated Antigen is selected from the group consisting of: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.
- Claim 23. The **DA x CD3 Binding Molecule** of any one of claims 1-16 or the pharmaceutical composition of claim 17 for use in the treatment of a disease.
- Claim 24. The **DA x CD3 Binding Molecule** or pharmaceutical composition of claim 23, wherein said disease is cancer.
- Claim 25. The **DA x CD3 Binding Molecule** or pharmaceutical composition of claim 24, wherein said cancer is selected from the group consisting of: adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, hematological cancer, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.
- Claim 26. The **DA x CD3 Binding Molecule** or pharmaceutical composition of claim 23, wherein said disease is a pathogen-associated disease.

Claim 27. The **DA x CD3 Binding Molecule** or pharmaceutical composition of claim 26, wherein said Pathogen-Associated Antigen is selected from the group consisting of: Herpes Simplex Virus infected cell protein (ICP)47, Herpes Simplex Virus gD, Epstein-Barr Virus LMP-1, Epstein-Barr Virus LMP-2A, Epstein-Barr Virus LMP-2B, Human Immunodeficiency Virus gp160, Human Immunodeficiency Virus gp120, Human Immunodeficiency Virus gp41, Human Papillomavirus E6, Human Papillomavirus E7, human T-cell leukemia virus gp64, human T-cell leukemia virus gp46, and human T-cell leukemia virus gp21.