

TITLE OF THE INVENTION

Variant CD3-Binding Domains and Their Use in Combination Therapies for the Treatment of Disease

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Applications. Serial No. 62/631,043 (filed on February 15, 2018; pending), and 62/738,632 (filed on September 28, 2018; pending), each of which applications are herein incorporated by reference in their entirety.

REFERENCE TO SEQUENCE LISTING

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in computer-readable media (file name: 1301_0150PCT_ST25.txt, created on January 30, 2019, and having a size of 295,037 bytes), which file is incorporated herein in its entirety.

FIELD OF THE INVENTION

[0003] The present invention 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 invention particularly concerns such **DA x CD3 Binding Molecules** comprising a variant CD3-Binding Domain (“**vCD3-Binding Domain**”), which comprises a CDR_{H1} Domain, a CDR_{H2} Domain, a CDR_{H3} Domain, a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} 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 invention 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 invention 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 invention is also directed to pharmaceutical compositions that comprise such molecule(s).

BACKGROUND OF THE INVENTION

I. The Mammalian Immune System

[0004] The mammalian immune system serves as a defense against a variety of conditions, including, *e.g.*, injury, infection and neoplasia. The efficiency with which humans and other mammals develop an immunological response to pathogens, foreign substances and cancer antigens rests on two characteristics: the exquisite specificity of the immune response for antigen recognition, and the immunological memory that allows for faster and more vigorous responses upon re-activation with the same antigen (Portolés, P. *et al.* (2009) “*The TCR/CD3 Complex: Opening the Gate to Successful Vaccination*,” *Current Pharmaceutical Design* 15:3290-3300; Guy, C.S. *et al.* (2009) “*Organization of Proximal Signal Initiation at the TCR:CD3 Complex*,” *Immunol Rev.* 232(1):7-21; Topalian, S.L. *et al.* (2015) “*Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy*,” *Cancer Cell* 27:450-461).

[0005] In healthy individuals, the immune system is in a quiescent state, inhibited by a repertoire of diverse inhibitory receptors and receptor ligands. Upon recognition of a cancer antigen, microbial pathogen, or an allergen, an array of activating receptors and receptor ligands are triggered to induce the activation of the immune system. Such activation leads to the activation of macrophages, Natural Killer (NK) cells and antigen-specific, cytotoxic, T-cells, and promotes the release of various cytokines, all of which act to counter the perceived threat to the health of the subject (Dong, C. *et al.* (2003) “*Immune Regulation by Novel Costimulatory Molecules*,” *Immunolog. Res.* 28(1):39-48; Viglietta, V. *et al.* (2007) “*Modulating Co-Stimulation*,” *Neurotherapeutics* 4:666-675; Korman, A.J. *et al.* (2007) “*Checkpoint Blockade in Cancer Immunotherapy*,” *Adv. Immunol.* 90:297-339). The immune system is capable of returning to its normal quiescent state when the countervailing inhibitory immune signals outweigh the activating immune signals.

[0006] Thus, the disease state of cancer (and indeed the disease states of infectious diseases) may be considered to reflect a failure to adequately activate a subject's immune system. Such failure may reflect an inadequate presentation of activating immune signals, or it may reflect an inadequate ability to alleviate inhibitory immune signals in the subject.

In some instances, researchers have determined that cancer cells can co-opt the immune system to evade being detected by the immune system (Topalian, S.L. *et al.* (2015) “*Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy*,” *Cancer Cell* 27:450-461).

[0007] The mammalian immune system is mediated by two separate but interrelated systems: the humoral immune system and the cellular immune system. Generally speaking, the humoral system is mediated by soluble molecules (antibodies or immunoglobulins) produced by **B Cells**. Such molecules have the ability to combine with and neutralize antigens that have been recognized as being foreign to the body. The cellular immune system involves the mobilization of certain cells, termed “**T-cells**,” that serve a variety of therapeutic roles. T-cells are lymphocytes that mature in the thymus and circulate between the tissues, lymphatic system and the circulatory system. In response to the presence and recognition of foreign structures (antigens), T-cells become “**activated**” to initiate an immune response. In many instances, these foreign antigens are expressed on host cells as a result of neoplasia or infection. Although T-cells do not themselves secrete antibodies, they are usually required for antibody secretion by the second class of lymphocytes, “**B Cells**” (which derive from bone marrow). Critically, T-cells exhibit extraordinary immunological specificity so as to be capable of discerning one antigen from another).

[0008] Two interactions are required for T-cell activation (Viglietta, V. *et al.* (2007) “*Modulating Co-Stimulation*,” *Neurotherapeutics* 4:666-675; Korman, A.J. *et al.* (2007) “*Checkpoint Blockade in Cancer Immunotherapy*,” *Adv. Immunol.* 90:297-339). In the first interaction, a cell must display the relevant target antigen bound to a cell’s Class I or Class II Major Histocompatibility Complex (“**MHC**”) so that it can bind the T-cell Receptor (“**TCR**”) of a naïve T lymphocyte. Although almost all cell types can serve as antigen-presenting cells, some cells, such as **macrophages**, **B cells**, and **dendritic cells**, specialize in presenting foreign antigens and are “professional” “**Antigen-Presenting Cells**.” Immunologic detection of antigen bound to an Antigen-Presenting Cell’s MHC I molecules leads to the production of cytotoxic T-cells. Immunologic detection of antigen bound to an Antigen-Presenting Cell’s MHC II molecules leads to the production of cytotoxic T-cells. In the second interaction, a ligand of the Antigen-Presenting Cell must bind a co-receptor of the T-cell (Dong, C. *et al.* (2003) “*Immune Regulation by Novel Costimulatory Molecules*,” *Immunolog. Res.* 28(1):39-48; Lindley, P.S. *et al.* (2009) “*The Clinical Utility*

Of Inhibiting CD28-Mediated Costimulation,” Immunol. Rev. 229:307-321). T-cells experiencing both stimulatory signals are then capable of responding to cytokines (such as Interleukin-2 and Interleukin-12).

[0009] In the absence of both co-stimulatory signals during TCR engagement, T-cells enter a functionally unresponsive state, referred to as clonal anergy (Khawli, L.A. *et al.* (2008) “*Cytokine, Chemokine, and Co-Stimulatory Fusion Proteins for the Immunotherapy of Solid Tumors,*” Exp. Pharmacol. 181:291-328). In pathologic states, T-cells are the key players of various organ-specific autoimmune diseases, such as type I diabetes, rheumatoid arthritis, and multiple sclerosis (Dong, C. *et al.* (2003) “*Immune Regulation by Novel Costimulatory Molecules,*” Immunolog. Res. 28(1):39-48).

[0010] This immune “checkpoint” pathway is important in maintaining self-tolerance (*i.e.*, in preventing a subject from mounting an immune system attack against his/her own cells (an “autoimmune” reaction) and in limiting collateral tissue damage during anti-microbial or anti-allergic immune responses. Where contact of a T-cell results in the generation of only one of two required signals, the T-cell does not become activated and an adaptive immune response does not occur. The “two signal” mechanism of T-cell activation thus provides a way for the immune system to avoid undesired responses, such as responses to self-antigens that would otherwise result in an immune system attack against a subject’s own cells (an “autoimmune” reaction).

II. Cell Surface Molecules of the Cellular Immune System

A. CD3, CD4 and CD8

[0011] The cells of the immune system are characterized by their expression of specialized glycoprotein cell surface molecules. Interactions between such molecules and molecules of other cells triggers, maintains or dampens the immune response. In particular, all T-cells are characterized by their expression of **CD3**. CD3 is a T-cell co-receptor composed of four distinct chains (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(4):a005140; pages 1-14; Chetty, R. *et al.* (1994) “*CD3: Structure, Function, And Role Of Immunostaining In Clinical Practice,*” J. Pathol. 173(4):303-307; Guy, C.S. *et al.* (2009) “*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex,*” Immunol. Rev. 232(1):7-21).

[0012] In mammals, the complex contains a CD3 γ chain, a CD3 δ chain, and two CD3 ϵ chains. These chains associate with the TCR in order to generate an activation signal in T lymphocytes (Smith-Garvin, J.E. *et al.* (2009) “*T Cell Activation*,” *Annu. Rev. Immunol.* 27:591-619). In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*,” *Immunology* 129(2):170–177). CD3 is found bound to the membranes of all mature T-cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: *IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE*,” 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) “*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The CD3 ϵ : γ Heterodimer*,” *Cell* 105(7):913-923; Kuhns, M.S. *et al.* (2006) “*Deconstructing The Form And Function Of The TCR/CD3 Complex*,” *Immunity*. 2006 Feb;24(2):133-139).

[0013] The invariant CD3 ϵ signaling component of the TCR complex on T-cells, has been used as a target to force the formation of an immunological synapse between T-cells and cancer cells. Co-engagement of CD3 and the tumor antigen activates the T-cells, triggering lysis of cancer cells expressing the tumor antigen (Baeuerle *et al.* (2011) “*Bispecific T-cell Engager For Cancer Therapy*,” In: *BISPECIFIC ANTIBODIES*, Kontermann, R.E. (Ed.) Springer-Verlag; 2011:273-287). This approach allows bispecific antibodies to interact globally with the T-cell compartment with high specificity for cancer cells and is widely applicable to a broad array of cell surface tumor antigens and has also been implemented to target pathogen-infected cells (see, *e.g.*, Sloan *et al.* (2015) “*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells*,” *PLoS Pathog* 11(11): e1005233. doi:10.1371/journal.ppat.1005233; WO 2014/159940; and WO 2016/054101).

[0014] A first subset of T-cells, known as “**helper T-cells**,” is characterized by the expression of the **CD4** (*i.e.*, they are “CD4⁺” as well as CD3⁺). CD4⁺ T-cells are the essential organizers of most mammalian immune and autoimmune responses (Dong, C. *et al.* (2003) “*Immune Regulation by Novel Costimulatory Molecules*,” *Immunolog. Res.* 28(1):39-48). The activation of CD4⁺ T-cells has been found to be mediated through co-stimulatory interactions between an antigen:Major histocompatibility Class II (**MHC II**) molecule complex that is arrayed on the surface of an Antigen-Presenting Cell (such as a B-

Cell, a macrophage or a dendritic cell) and a complex of two molecules, the TCR and a **CD3** cell surface receptor ligand, both of which are arrayed on the surface of a naïve CD4⁺ T-cell. Activated T helper cells are capable of proliferating into Th1 cells that are capable of mediating an inflammatory response to the target cell.

[0015] A second subset of T-cells, known as “**cytotoxic T-cells**,” are characterized by the expression of **CD8** (*i.e.*, they are “CD8⁺” as well as CD3⁺). CD8 is a T-cell co-receptor composed of two distinct chains (Leahy, D.J. (1995) “*A Structural View of CD4 and CD8*,” FASEB J. 9:17-25) that is expressed on cytotoxic T-cells. The activation of CD8⁺ T-cells has been found to be mediated through co-stimulatory interactions between an antigen:major histocompatibility class I (**MHC I**) molecule complex that is arrayed on the surface of a target cell and a complex of CD8 and the T-cell Receptor, that are arrayed on surface of the CD8⁺ T-cell ((Gao, G. *et al.* (2000) “*Molecular Interactions Of Coreceptor CD8 And MHC Class I: The Molecular Basis For Functional Coordination With The T-Cell Receptor*,” Immunol. Today 21:630-636). Unlike major histocompatibility class II (**MHC II**) molecules, which are expressed by only certain immune system cells, MHC I molecules are very widely expressed. Thus, cytotoxic T-cells are capable of binding a wide variety of cell types. Activated cytotoxic T-cells mediate cell killing through their release of the cytotoxins perforin, granzymes, and granulysin. Through the action of perforin, granzymes enter the cytoplasm of the target cell and their serine protease function triggers the caspase cascade, which is a series of cysteine proteases that eventually lead to apoptosis (programmed cell death) of targeted cells.

B. The T-Cell Receptor (“TCR”)

[0016] The T-cell Receptor (“**TCR**”) is natively expressed by CD4⁺ or CD8⁺ T-cells, and permits such cells to recognize antigenic peptides that are bound and presented by class I or class II MHC proteins of antigen-presenting cells. Recognition of a pMHC (peptide–MHC) complex by a TCR initiates the propagation of a cellular immune response that leads to the production of cytokines and the lysis of the Antigen-Presenting Cell (see, *e.g.*, Armstrong, K.M. *et al.* (2008) “*Conformational Changes And Flexibility In T-Cell Receptor Recognition Of Peptide–MHC Complexes*,” Biochem. J. 415(Pt 2):183–196; Willemsen, R. (2008) “*Selection Of Human Antibody Fragments Directed Against Tumor T-Cell Epitopes For Adoptive T-Cell Therapy*,” Cytometry A. 73(11):1093-1099; Beier, K.C. *et al.* (2007) “*Master Switches Of T-Cell Activation And Differentiation*,” Eur. Respir. J. 29:804-812;

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 ζ 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 OF THE INVENTION

[0019] The present invention 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 invention particularly concerns such **DA x CD3 Binding Molecules** comprising a variant CD3-Binding Domain (“**vCD3-Binding Domain**”), which comprises a CDR_{H1} Domain, a CDR_{H2} Domain, a CDR_{H3} Domain, a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} 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 invention 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 invention 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 invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[0020] In detail, the invention provides a **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_{H1} 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_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and

- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (II) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} 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_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (III) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} 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_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;

- (C) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_L2 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_L3 Domain comprising the amino acid sequence of **SEQ ID NO:62**.

[0021] The invention additionally concerns the embodiment 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 invention additionally concerns the embodiment 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 invention additionally concerns the embodiment 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 invention additionally concerns the embodiment of such **DA x CD3 Binding Molecule**, wherein the Disease Antigen is a Cancer Antigen, or a Pathogen-Associated Antigen.

[0025] The invention additionally concerns the embodiment 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^b/Le^y, 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 α 2, Integrin β 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, R24, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T-cell receptor derived peptide, T5A7, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.

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

[0027] The invention additionally concerns the embodiment 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 invention additionally concerns the embodiment 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_{DA}); and
 - (2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to the epitope of CD3 (VH_{CD3});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_{CD3}); 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_{DA});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 invention additionally concerns the embodiment 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 invention additionally concerns the embodiment 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 invention additionally concerns the embodiment 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 invention additionally concerns the embodiment of such **DA x CD3 Binding Molecule**, wherein the **DA x CD3 Binding Molecule** further comprises a CD8-Binding Domain.

[0033] The invention additionally concerns the embodiment 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] The invention additionally concerns a pharmaceutical composition that comprises any of the above-described **DA x CD3 Binding Molecules** and a pharmaceutically acceptable carrier.

[0035] The invention 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 invention additionally concerns the embodiment of such method, wherein the disease is cancer. Including embodiments, 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 invention additionally concerns the embodiment of such method, wherein the disease is a pathogen-associated disease; including embodiments, 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 invention 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.

[0039] The invention additionally concerns the embodiment of such use, wherein the disease is cancer. Including, embodiments, 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 invention additionally concerns the embodiment of such use, wherein the disease is a pathogen-associated disease. Including embodiments, 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.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0041] **Figures 1A-1B** provides schematics of representative covalently bonded diabodies having two Epitope-Binding Domains composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a Linker (**Figure 1A**) and/or in the Heterodimer-Promoting Domain (**Figure 1B**). VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0042] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two Epitope-Binding Domains composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0043] **Figures 3A-3C** provide schematics showing representative covalently bonded tetravalent diabodies having four Epitope-Binding Domains composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments, wherein the two pairs of polypeptide chains are the same and the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3B**), the resulting molecule possesses four Epitope-Binding Domains and is bispecific and bivalent with respect to each bound epitope. In such embodiments, wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule possesses four Epitope-Binding Domains and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments, wherein the two pairs of

polypeptide chains are different and the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown by the different shading and patterns in **Figure 3C**), the resulting molecule possesses four Epitope-Binding Domains and is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc Domain-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc Domain-containing diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a Linker (with an optional cysteine residue). **Figure 3C**, shows an Fc Domain-Containing diabody, which contains antibody CH1 and CL Domains to promote heterdimerization.

[0044] **Figures 4A-4B** provide schematics of a representative covalently bonded diabody molecule having two Epitope-Binding Domains composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0045] **Figure 5** provides the schematics of a representative covalently bonded diabody molecule having four Epitope-Binding Domains composed of five polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Domain that comprises all or part of an Fc Domain. The polypeptide chains comprising the linked VL and VH Domains further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0046] **Figures 6A-6F** provide schematics of representative Fc Domain-containing trivalent Binding Molecules having three Epitope-Binding Domains. **Figures 6A** and **6B**, respectively, illustrate schematically the domains of trivalent Binding Molecules comprising two Diabody-Type Binding Domains and a Fab-Type Binding Domain having different domain orientations in which the Diabody-Type Binding Domains are N-terminal or C-terminal to an Fc Domain. The molecules in **Figures 6A** and **6B** comprise four chains. **Figures 6C** and **6D**, respectively, illustrate schematically the domains of trivalent Binding Molecules comprising two Diabody-Type Binding Domains N-terminal to an Fc Domain, and a Fab-Type Binding Domain in which the Light Chain and Heavy Chain are linked via a polypeptide spacer, or an scFv-Type Binding Domain. The trivalent Binding Molecules in

Figures 6E and 6F, respectively, illustrate schematically the domains of trivalent Binding Molecules comprising two Diabody-Type Binding Domains C-terminal to an Fc Domain, and a Fab-Type Binding Domain in which the Light Chain and Heavy Chain are linked via a polypeptide spacer, or an scFv-Type Binding Domain. The trivalent Binding Molecules in **Figures 6C-6F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0047] **Figure 7A-7D** show the results of CTL and binding assays. **Figure 7A** shows the results of representative redirected cell killing (% cytotoxicity in a CTL assay) mediated by **DART-A**-type diabody constructs containing the VL and VH Domains of **CD3 mAb 1**; **CD3 mAb 1 M1**; **CD3 mAb 1 M2**; **CD3 mAb 1 M15**; **CD3 mAb 1 M17**; **CD3 mAb 1 M18**; **CD3 mAb 1 M19**; and **CD3 mAb 1 M20**. **Figures 7B-7C** plot the correlation between the affinity constants (**Figure 7B**: KD; **Figure 7C**: k_a ; and **Figure 7D**: k_d) and CTL activity (EC_{50} of cytolysis at 18 hours) reported in **Tables 11-12**.

[0048] **Figure 8A-8E** show the results of representative studies of redirected cell killing (CTL assay) mediated **DART A**-type diabody constructs containing the VL and VH Domains of **CD3 mAb 1**; **CD3 mAb 1 M2**; **CD3 mAb 1 M7**; **CD3 mAb 1 M13**; and **CD3 mAb 1 M15**; using Pan-T effector cells and MV-4-11 leukemia target cells. Percent cytotoxicity is plotted in **Figure 8A**. Cytokine responses are plotted in **Figures 8B-8E** (**Figure 8B**: IFN-gamma; **Figure 8C**: TNF-alpha; **Figure 8D**: IL-6; **Figure 8E**: IL-2); NegCtrl: negative control.

[0049] **Figure 9A-9B** show the ability of **DART-B**-type diabodies to bind to Disease Antigens. **Figure 9A** shows the ability of **CD123-WT**, **CD123-M1**, **CD123-M2** and **CD123-M18 DART-B**-type diabodies to bind to CD123-expressing MOLM-13 cells. **Figure 9B** shows the ability of **5T4-WT**, **5T4-M1**, **5T4-M2**, and **5T4-M18 DART-B**-type diabodies to bind to 5T4-expressing A498 cells. Binding was detected using biotinylated antibody specific for the diabodies' E/K coils and streptavidin-phycoerythrin (PE).

[0050] **Figures 10A-10B** show the ability of **CD123-WT**, **CD123-M1**, **CD123-M2** and **CD123-M18 DART-B**-type diabodies to bind to CD8⁺ T-cells (**Figure 10A**) and CD4⁺ T-cells (**Figure 10B**).

[0051] **Figures 11A-11Q** show the results of representative studies of redirected cell killing (CTL assay) mediated by CD123 x CD3 **DART B**-type diabody constructs (possessing Fc Domains): CD123-WT (**Figures 11B, 11F, 11J and 11N**), CD123-M2 (**Figures 11C, 11G, 11K and 11O**), CD123-M18 (**Figures 11D, 11H, 11L and 11P**), HIV-WT (**Figures 11E, 11I, 11M and 11Q**), using Pan-T effector cells and MOLM-13 acute monocytic leukemia (AML) target cells. Percent cytotoxicity is plotted in **Figure 11A**. Cytokine responses and percent cytotoxicity are plotted in **Figures 11B-11Q** (**Figures 11B-11E**: IFN-gamma; **Figures 11F-11I**: TNF-alpha; **Figures 11J-11M**: IL-6; **Figures 11N-11Q**: IL-2).

[0052] **Figures 12A-12E** show the results of representative studies of redirected cell killing (CTL assay) mediated by CD123 x CD3 **DART B**-type diabody constructs (possessing Fc Domains) using PBMC effector cells and MOLM-13 AML target cells. Percent cytotoxicity is plotted in **Figure 12A** (E:T= 15:1, 24 h). Cytokine responses are plotted in **Figures 12B-12E** (**Figure 12B**: IFN-gamma; **Figure 12C**: TNF-alpha; **Figure 12D**: IL-6; **Figure 12E**: IL-2).

[0053] **Figures 13A-13Q** show the results of representative studies of redirected cell killing (CTL assay) mediated by 5T4 x CD3 **DART B**-type diabody constructs (possessing Fc Domains) 5T4-WT (**Figures 13B, 13F, 13J and 13N**), 5T4-M2 (**Figures 13C, 13G, 13K and 13O**), 5T4-M18 (**Figures 13D, 13H, 13L and 13P**), HIV-WT (**Figures 13E, 13I, 13M and 13Q**), using Pan-T effector cells and A498 renal cell carcinoma target cells (E:T= 5:1, 24 h). Cytotoxicity is plotted in **Figure 13A**. Cytokine responses and percent cytotoxicity are plotted in **Figures 13B-13Q** (**Figures 13B-13E**: IFN-gamma; **Figures 13F-13I**: TNF-alpha; **Figures 13J-13M**: IL-6; **Figures 13N-13Q**: IL-2).

[0054] **Figures 14A-14J** show the results of representative studies of redirected cell killing (CTL assay) mediated by CD19 x CD3 **DART B**-type diabody constructs (possessing Fc Domains) using PBMCs (**Figures 14A-14E**) or Pan-T effector cells (**Figures 14F-14J**) (E:T= 30:1 for PBMCs and 10:1 for Pan-T-cells, 24-48 h). Percent cytotoxicity (48 hrs) is plotted in **Figures 14A** (PBMCs) and **Figure 14F** (Pan-T-cells). Cytokine responses at 48 hours using PBMCs are plotted in **Figures 14B-14E** (PBMCs) and **Figures 14G-14J** (Pan T-cells) (**Figures 14B and 14G**: IFN-gamma; **Figures 14C and 14H**: TNF-alpha; **Figures 14D and 14I**: IL-6; **Figures 14E and 14J**: IL-2).

[0055] **Figures 15A-15E** show the ability of representative CD123 x CD3 **DART-B**-type diabodies to mediate T-cell activation. T-cell activation was measured by evaluating the ability of the diabodies to affect expression of CD25 and CD69. Percent cytotoxicity is plotted in **Figure 15A**. Activation of CD4⁺ T-cells as determined by measuring CD25 is plotted in **Figure 15B**. Activation of CD4⁺ T-cells as determined by measuring CD69 is plotted in **Figure 15C**. Activation of CD8⁺ T-cells as determined by measuring CD25 is plotted in **Figure 15D**. Activation of CD8⁺ T-cells as determined by measuring CD69 is plotted in **Figure 15E**.

[0056] **Figures 16A-16E** show the ability of representative 5T4 x CD3 **DART-B**-type diabodies to mediate T-cell activation. T-cell activation was measured by evaluating the ability of the diabodies to affect expression of CD25 and CD69. Percent cytotoxicity is plotted in **Figure 16A**. Activation of CD4⁺ T-cells as determined by measuring CD25 is plotted in **Figure 16B**. Activation of CD4⁺ T-cells as determined by measuring CD69 is plotted in **Figure 16C**. Activation of CD8⁺ T-cells as determined by measuring CD25 is plotted in **Figure 16D**. Activation of CD8⁺ T-cells as determined by measuring CD69 is plotted in **Figure 16E**.

[0057] **Figures 17A-17B** show the results of *in vivo* studies on the ability of exemplary CD123 x CD3 **DART B**-type diabody constructs to mediate the reduction of tumors *in vivo*. **CD123-WT** (50 µg/kg) or **CD123-M18** (at 5 µg/kg or 50 µg/kg) were provided to mice that had received the KG1A cells, and tumor volume was assessed over 35 days. **Figure 17A**: CD4; **Figure 17B**: CD8.

[0058] **Figures 18A-18D** show the results of *in vivo* studies on the ability of CD123 x CD3 **DART B**-type diabody constructs to mediate the reduction of tumors *in vivo*. **CD123-WT**, **CD123-M2** or **CD123-M18** (at 0.5, 5 50, or 500 µg/kg) were provided to mice that had received the KG1A cells, and tumor volume was assessed over 35 days. **Figure 18A**: **CD123-WT**; **Figure 18B**: **CD123-M2**; **Figure 18C**: **CD123-M18**; **Figure 18D**: **CD123-WT** and **CD123-M18** 50 µg/kg and 500 µg/kg treatment groups.

[0059] **Figures 19A-19D** show the results of *in vivo* studies on the ability of CD123 x CD3 **DART B**-type diabody constructs to mediate the reduction of tumors *in vivo*. **CD123-WT**, **CD123-M2** or **CD123-M18** (at 0.5, 5 50, or 500 µg/kg) were provided to mice that had received the MV4-11 cells, and tumor volume was assessed over 35 days. **Figure 19A**:

CD123-WT; Figure 19B: CD123-M18; Figure 19C: CD123-M2; Figure 19D: CD123-WT, CD123-M2 and CD123-M18 500 µg/kg treatment groups.

[0060] **Figures 20A-20B** show the results of *in vivo* studies on the ability of 5T4 x CD3 **DART B**-type diabody constructs to mediate the reduction of tumors *in vivo*. **5T4-WT** (at 10, 50, 100, or 500 µg/kg), **5T4-M18** (at 10, 50, 100, or 500 µg/kg) or **5T4-M2** (at 500 µg/kg) were provided to mice that had received the SKOV3 cells, and tumor volume was assessed over 45 days. **Figure 20A: 5T4-WT; Figure 20B: 5T4-M18 and 5T4-M2.**

[0061] **Figures 21A-21D** show the results of *in vivo* studies on the cytokine release profile induced by CD123 x CD3 **DART-B**-type diabodies. Serum cytokine levels (pg/ml) were evaluated six hours after administration of **CD123-WT, CD123-M2 or CD123-M18** (at 50, or 500 µg/kg) to mice that had received the KG1A cells. **Figure 21A: IFN-γ; Figure 21B: TNF-α; Figure 21C: IL-6; and Figure 21D: IL-2.**

[0062] **Figures 22A-22C** show the ability of CD123 x CD3 x CD8 **TRIVALENT**-type molecules, **T-CD123-WT, T-CD123-M1, T-CD123-M2 and T-CD123-M18**, to bind to cell surface antigens. **Figure 22A: binding to CD123-expressing MOLM-13 cells; Figure 22B: binding to CD4⁺ T-cells; Figure 22C: binding to CD8⁺ T-cells.**

[0063] **Figures 23A-23G** show the results of representative studies of redirected cell killing (CTL assay) mediated by **T-CD123-WT, T-CD123-M1, T-CD123-M2 and T-CD123-M18** CD123 x CD3 x CD8 **TRIVALENT**-type molecules using different T-cell populations. Percent cytotoxicity using CD3⁺ Pan-T-cells (**Figure 23A**); CD4⁺ T-cells (**Figure 23B**) and CD8⁺ T-cells (**Figure 23C**). Cytokine responses using CD3⁺ Pan-T-cells are plotted in **Figures 23D-23G**. **Figure 23D: IFN-γ; Figure 23E: TNF-α; Figure 23F: IL-6; and Figure 23G: IL-2.**

[0064] **Figures 24A-24J** show the serum cytokine levels, Ki67 expression, and clinical pathology marker levels observed in cynomolgus monkeys treated with **CD123-M18** (10 mg/kg and 20 mg/kg) or **CD123-WT** (0.003 mg/kg). **Figure 24A: IFN-γ; Figure 24B: TNF-α; Figure 24C: IL-6; Figure 24D: IL-2; Figure 24E: IL-15; Figure 24F: Ki67 positive CD4⁺ T-cells; Figure 24G: Ki67 positive CD8⁺ T-cells; Figure 24H: platelet; Figure 24I: C-reactive protein; Figure 24J: blood urea nitrogen.**

[0065] **Figures 25A-25G** show the results of a representative study of AML blast depletion mediated by **DART-A-WT**, **CD123-WT**, **CD123-M1** and **CD123-M18** in peripheral blood samples from an AML patient. **Figure 25A**: AML 34⁺ blast cell count as a percent of control; **Figure 25B**: CD4⁺ cell expansion; **Figure 25C**: CD8⁺ cell expansion; **Figures 25D-G**: Cytokine release (**Figure 25D**: IFN- γ ; **Figure 25E**: TNF- α ; **Figure 25F**: IL-6; and **Figure 25G**: IL-2).

[0066] **Figures 26A-26E** show the results of representative studies of redirected cell killing (CTL assay) mediated by CD123 x CD3 diabody constructs **CD123-WT**, **CD123-M1**, **CD123-M13**, **CD123-M17**, **CD123-M18**, and **CD123-M19** using Pan-T effector cells and MOLM-13 AML target cells (E:T= 15:1, 48-96 hr). Cytotoxicity as a function of %LDH released is plotted in **Figure 26A**. Cytokine responses are plotted in **Figures 26B-26E** (**Figure 26B**: IFN-gamma; **Figure 26C**: TNF-alpha; **Figure 26D**: IL-6; **Figure 26E**: IL-2).

[0067] **Figures 27A-27D** present the cumulative results from 4-7 redirected cell killing assays (CTL assay) and cytokine release studies mediated by CD123 x CD3 diabody constructs **CD123-WT**, **CD123-M1**, **CD123-M13**, **CD123-M17**, **CD123-M18**, **CD123-M19**, and **DART-A-WT** using Pan-T effector cells and MOLM-13 AML target cells (E:T= 15:1, 48-96 hr). CTL activity EC₅₀ values in pM are plotted in **Figure 27A**. CTL activity as a multiple of the EC₅₀ value of **CD123-WT** is plotted in **Figure 27B**. CTL activity E_{max} as a percent of **CD123-WT** is plotted in **Figure 27C**. The calculated Therapeutic Index (TI = E_{max} (CTL) : E_{max} (cytokine)) normalized to **CD123-WT** is plotted in **Figure 27D**.

[0068] **Figures 28A-28B** show the results of *in vivo* studies on the ability of CD123 x CD3 diabody constructs to mediate the reduction of tumors *in vivo*. **CD123-WT** (0.5 mg/kg), **CD123-M18** or **CD123-M13** (at 0.005, 0.05, 0.5 and 1 mg/kg) were provided to mice that had received KG1A cells, and tumor volume was assessed over 42 days. **Figure 28A**: **CD123-WT** and **CD123-M18**. **Figure 28B**: **CD123-WT** and **CD123-M13**.

[0069] **Figures 29A-29B** show the results of *in vivo* studies on the ability of CD123 x CD3 diabody constructs to mediate the reduction of tumors *in vivo*. **CD123-WT** (0.05 mg/kg), **CD123-M18** or **CD123-M17** (at 0.005, 0.05, 0.5 and 1 mg/kg) were provided to mice that had received KG1A cells, and tumor volume was assessed over 42 days. **Figure 29A**: **CD123-WT** and **CD123-M18**. **Figure 29B**: **CD123-WT** and **CD123-M17**.

[0070] **Figures 30A-30B** show the results of *in vivo* studies on the interleukin-2 cytokine release profile induced by CD123 x CD3 **DART-B**-type diabodies. Serum cytokine levels (pg/ml) were evaluated six hours after administration of **CD123-WT** (0.5 mg/kg), **CD123-M13**, **CD123-M17** or **CD123-M18** (at 0.05, 0.5 and 1 mg/kg) to mice that had received the KG1A cells. **Figure 30A**: **CD123-WT**, **CD123-M13**, and **CD123-M18**; and **Figure 30B**: **CD123-WT**, **CD123-M17** and **CD123-M18**.

[0071] **Figures 31A-31F** show the results of a representative study of autologous B-cell depletion by **CD19-WT**, **CD19.1-M18**, and **HIV-M18** from human and cynomolgus monkey PBMCs. Depletion of CD20⁺ B-cells is plotted in **Figure 31A** (human PBMCs) and **Figure 31B** (cyno PBMCs). Cytokine release from the treated human PBMCs is plotted in **Figures 31C-F** (**Figure 31C**: IFN- γ ; **Figure 31D**: TNF- α ; **Figure 31E**: IL-6; and **Figure 31F**: IL-2).

[0072] **Figures 32A-32D** show the reduction in B-cells levels observed in the peripheral blood of cynomolgus monkeys treated with **CD19.1-M18** (1 mg/kg and 10 mg/kg) or **CD123-WT** (0.1 mg/kg). The predose B-cell levels are show in **Figure 32A** (the B-cell population is indicated with an oval). The levels at Day 1, Day 8 and Day 15 are shown in **Figures 32B-32C**, respectively.

[0073] **Figures 33A-33C** show the immunohistochemistry staining of B-cells in lymph nodes from cynomolgus monkeys pretreatment and at Day 7 post treatment with the positive control **CD19-WT** (**Figure 33A**: 0.1 mg/kg) or the CD3 variant **CD19.1-M18** (**Figure 33B**: 10 mg/kg; and **Figure 33C**: 30 mg/kg).

[0074] **Figure 34** shows the reduction in B-cells levels observed in the peripheral blood of cynomolgus monkeys treated with **CD19.1-M13** (1 mg/kg), **CD19.1-M17** (1 mg/kg) or **CD19-WT** (0.1 mg/kg).

[0075] **Figures 35A-35E** show the serum cytokine levels observed in cynomolgus monkeys treated with **CD19.1-M13** (1 mg/kg), **CD19.1-M17** (1 mg/kg), or **CD19-WT** (0.1 mg/kg). **Figure 35A**: TNF- α , **Figure 35B**: IFN- γ , **Figure 35C**: IL-2, **Figure 35D**: IL-6; and **Figure 35E**: IL-15.

[0076] **Figures 36A-36B** show the proliferation of T-cells observed in cynomolgus monkeys treated with **CD19.1-M13** (1 mg/kg), **CD19.1-M17** (1 mg/kg), or **CD19-WT** (0.1 mg/kg). **Figure 36A**: Ki67 positive T-cells CD4⁺ T-cells; **Figure 36B**: Ki67 positive CD8⁺ T-cells.

DETAILED DESCRIPTION OF THE INVENTION

[0077] The present invention 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 invention particularly concerns such **DA x CD3 Binding Molecules** comprising a variant CD3-Binding Domain (“**vCD3-Binding Domain**”), which comprises a CDR_{H1} Domain, a CDR_{H2} Domain, a CDR_{H3} Domain, a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} 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 invention 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 invention 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 invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[0078] As indicated above, the therapeutic molecules of the present invention particularly include bispecific Binding Molecules that comprises an Epitope-Binding Domain capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and an Epitope-Binding Domain that is capable of immunospecifically binding an epitope of a target cell that expresses a Disease Antigen. As used herein, the term “**Disease Antigen**” (abbreviated as “**DA**”) denotes an antigen that is expressed on the surface of an abnormal or infected cell and that is characteristic of such abnormality of infection, or that is expressed

on the surface of a foreign cell and that is characteristic of such foreign origin. As used herein, a cell that expresses a Disease Antigen on its cell surface, and that may therefore become bound by the therapeutic molecules of the present invention and thereby targeted for killing by such therapeutic molecules is a “**target cell**.” Of particular relevance to the present invention are Disease Antigens that are “**Cancer Antigens**” or “**Pathogen-Associated Antigens**.”

I. Antibodies and Their Binding Domains

[0079] The **DA x CD3 Binding Molecules** of the present invention may be antibodies or be derivable from antibodies (*e.g.*, by fragmentation, cleavage, *etc.* of antibody polypeptides, or from use of the amino acid sequences of antibody molecules or of polynucleotides (or their sequences) that encode such polynucleotides, *etc.*).

[0080] **Antibodies** are immunoglobulin molecules capable of specific binding to a particular domain or moiety or conformation (an “**epitope**”) of a molecule, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.* An epitope-containing molecule may have immunogenic activity, such that it elicits an antibody production response in an animal; such molecules are termed “**antigens**.” As used herein, the terms “**antibody**” and “**antibodies**” refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab’) fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and Epitope-Binding Domains of any of the above. In particular, the term “antibody” includes immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an Epitope-Binding Domain. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass. Antibodies are capable of “**immunospecifically binding**” to a polypeptide or protein or a non-protein molecule due to the presence on such molecule of a particular domain or moiety or conformation (an “**epitope**”).

[0081] The term “**monoclonal antibody**” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring or non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-

length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv, single-chain (scFv), mutants thereof), fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of "antibody." Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) "*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*," Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) "*Review of Selected Adjuvants Used in Antibody Production*," ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freund's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced, and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the monospecific or multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized, a chimeric

antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody, as detailed below.

[0082] The Binding Molecules of the present invention bind epitopes via their binding domains in an “**immunospecific**” manner. As used herein, an antibody, diabody or other epitope-binding molecule is said to “**immunospecifically**” bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and/or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind a second target. As such, “**immunospecific binding**” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference herein to binding means “immunospecific” binding.

[0083] The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) “*The Use Of Antibodies In The Treatment Of Infectious Diseases*,” Singapore Med. J. 50(7):663-666). Over 200 antibody-based drugs have been approved for use or are under development.

[0084] Natural antibodies (such as IgG antibodies) are composed of two “**Light Chains**” complexed with two “**Heavy Chains**.” Each Light Chain contains a Variable Domain (“**VL**”) and a Constant Domain (“**CL**”). Each Heavy Chain contains a Variable Domain (“**VH**”), three Constant Domains (“**CH1**,” “**CH2**” and “**CH3**”), and a “**Hinge**” Region (“**H**”) located between the **CH1** and **CH2** Domains. In contrast, scFvs are single-chain molecules made by linking Light and Heavy Chain Variable Domains together via a short linking peptide.

[0085] The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two Light Chains and two Heavy Chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal (“**N-terminal**”) portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily

responsible for antigen recognition. The carboxy-terminal (“**C-terminal**”) portion of each chain defines a constant region, with Light Chains having a single Constant Domain and Heavy Chains usually having three Constant Domains and a Hinge Domain. Thus, the structure of the Light Chains of an IgG molecule is n-VL-CL-c and the structure of the IgG Heavy Chains is n-VH-CH1-H-CH2-CH3-c (where n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). The ability of an intact, unmodified antibody (*e.g.*, an IgG antibody) to bind an epitope of an antigen depends upon the presence and sequences of the Variable Domains. Unless specifically noted, the order of domains of the protein molecules described herein is in the “**N-terminal to C-terminal**” direction.

A. Characteristics of Antibody Variable Domains

[0086] The Variable Domains of an IgG molecule consist of three complementarity determining regions (“**CDR**”), which contain the amino acid residues of the antibody that will be in contact with epitope, and four intervening non-CDR segments, referred to as framework regions (“**FR**”), which separate the CDR segments and which in general maintain the structure and determine the positioning of the CDR residues so as to permit them to contact the epitope (although certain framework residues may also play a role in such contact). Thus, the VL and VH Domains have the structure **n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c**, where n and c respectively denote the N-terminal end and the C-terminal end of the domains. The amino acid sequences of the CDRs determine whether an antibody will be able to bind to a particular epitope.

[0087] Amino acids from the Variable Domains of the mature Heavy and Light Chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat *et al.* (SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NIH, MD (1991) (“**Kabat**”), expressly incorporated herein by reference), described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. The CDRs are identified as defined by Kabat (it will be understood that CDR_{H1} as defined by Chothia, C. & Lesk, A. M. ((1987) “*Canonical structures for the hypervariable regions of immunoglobulins*,” J. Mol. Biol. 196:901-917) begins five residues earlier). Kabat’s numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in

the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody Light Chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody Light Chain.

[0088] Polypeptides that are (or may serve as) the first, second and third CDR of the Light Chain of an antibody are herein respectively designated as: **CDR_L1 Domain**, **CDR_L2 Domain**, and **CDR_L3 Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of the Heavy Chain of an antibody are herein respectively designated as: **CDR_H1 Domain**, **CDR_H2 Domain**, and **CDR_H3 Domain**. Thus, the terms CDR_L1 Domain, CDR_L2 Domain, CDR_L3 Domain, CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind a specific epitope regardless of whether such protein is an antibody having light and Heavy Chains or is a diabody or a single-chain binding molecule (e.g., an scFv, a BiTe, *etc.*), or is another type of protein. Accordingly, as used herein, the term “**Epitope-Binding Domain**” denotes a domain comprising a fragment or portion of a binding molecule (or a polypeptide having the amino acid sequence of such a fragment or portion) that contributes to the ability of the binding molecule to immunospecifically bind an epitope. An Epitope-Binding Domain may contain any 1, 2, 3, 4, or 5 the CDR Domains of an antibody, or may contain all 6 of the CDR Domains of an antibody and, although capable of immunospecifically binding such epitope, may exhibit an immunospecificity, affinity or selectivity towards such epitope that differs from that of such antibody. An Epitope-Binding Domain may contain only part of a CDR, namely the subset of CDR residues required for binding (termed “**Specificity-Determining Residues**,” or “**SDRs**,” Kim, J.H. *et al.* (2012) “*Humanization By CDR Grafting And Specificity-Determining Residue Grafting*,” *Methods Mol. Biol.* 907:237-245; Kim, K.S. *et al.* (2010) “*Construction Of A Humanized Antibody To Hepatitis B Surface Antigen By Specificity-Determining Residues (SDR)-Grafting And De-Immunization*,” *Biochem. Biophys. Res. Commun.* 396(2):231-237; Kashmiri, S.V. *et al.* (2005) “*SDR Grafting – A New Approach To Antibody Humanization*,” *Methods* 36(1):25-34; Gonzales, N.R. *et al.* (2004) “*SDR Grafting Of A Murine Antibody Using Multiple Human Germline Templates To Minimize Its Immunogenicity*,” *Mol. Immunol.* 41:863-872). Preferably, however, an Epitope-Binding Domain will contain all 6 of the CDR Domains of such antibody. An Epitope-Binding Domain of an antibody may be a single polypeptide chain (e.g., an scFv), or may comprise two or more polypeptide chains,

each having an amino terminus and a carboxy terminus (*e.g.*, a diabody, a Fab fragment, an Fab₂ fragment, *etc.*).

[0089] The invention also particularly encompasses Binding Molecules that comprise a VL and/or VH Domain of a humanized antibody. The term “**humanized antibody**” refers to a chimeric molecule, generally prepared using recombinant techniques, having an Epitope-Binding Domain of an immunoglobulin from a non-human species and a remaining immunoglobulin structure of the molecule that is based upon the structure and /or sequence of a human immunoglobulin. The polynucleotide sequence of the Variable Domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the Epitope-Binding Domain of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy Variable Domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415

[0090] The Epitope-Binding Domain may comprise either a complete Variable Domain fused onto Constant Domains or only the complementarity determining regions (CDRs) of such Variable Domain grafted to appropriate framework regions. Epitope-binding domains may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign Variable Domain remains (LoBuglio, A.F. *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the Variable Domains as well so as to reshape them as closely as possible to human form. It is known that the Variable Domains of both Heavy and Light Chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a

given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the Variable Domains can be “reshaped” or “humanized” by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) *Cancer Res* 53:851-856. Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” *Nature* 332:323-327; Verhoeven, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” *Science* 239:1534-1536; Kettleborough, C. A. *et al.* (1991) “*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*,” *Protein Engineering* 4:773-3783; Maeda, H. *et al.* (1991) “*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*,” *Human Antibodies Hybridoma* 2:124-134; Gorman, S. D. *et al.* (1991) “*Reshaping A Therapeutic CD4 Antibody*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 88:4181-4185; Tempest, P.R. *et al.* (1991) “*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*,” *Bio/Technology* 9:266-271; Co, M. S. *et al.* (1991) “*Humanized Antibodies For Antiviral Therapy*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 88:2869-2873; Carter, P. *et al.* (1992) “*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 89:4285-4289; and Co, M.S. *et al.* (1992) “*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*,” *J. Immunol.* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

[0091] A number of humanized antibody molecules comprising an Epitope-Binding Domain derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent Variable Domain and their associated complementarity determining regions (CDRs) fused to human Constant Domains (see, for example, Winter *et al.* (1991) “*Man-made Antibodies*,” *Nature* 349:293-299; Lobuglio *et al.* (1989) “*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 86:4220-4224 (1989), Shaw *et al.* (1987) “*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*,” *J. Immunol.* 138:4534-4538, and Brown *et al.* (1987) “*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*,”

Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody Constant Domain (see, for example, Riechmann, L. *et al.* (1988) “*Reshaping Human Antibodies for Therapy*,” Nature 332:323-327; Verhoeyen, M. *et al.* (1988) “*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*,” Science 239:1534-1536; and Jones *et al.* (1986) “*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*,” Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These “humanized” molecules are designed to minimize unwanted immunological response towards rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) “*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*,” Nucl. Acids Res. 19:2471-2476 and in U.S. Patent Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

B. Characteristics of Antibody Constant Regions

[0092] Throughout the present specification, the numbering of the residues in the constant region of an IgG is that of the EU index as in Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NH1, MD (1991) (“**Kabat**”), expressly incorporated herein by reference. The term “**EU index as in Kabat**” refers to the numbering of the Constant Domains of human IgG1 EU antibody.

[0093] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Heavy Chain allotypes (“Gm allotypes”) are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, “*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation*,” Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated

that the antibodies of the present invention may incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue in the Binding Molecules of the invention. Specifically encompassed by the instant invention are Binding Molecules lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

1. Constant Regions of the Heavy Chain

[0094] The CH1 Domains of the two Heavy Chains of an antibody complex with the antibody's Light Chain's "CL" constant region, and are attached to the Heavy Chains CH2 Domains via an intervening Hinge Domain.

[0095] An exemplary CH1 Domain is a human IgG1 CH1 Domain. The amino acid sequence of an exemplary human IgG1 CH1 Domain is (**SEQ ID NO:1**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV

[0096] An exemplary CH1 Domain is a human IgG2 CH1 Domain. The amino acid sequence of an exemplary human IgG2 CH1 Domain is (**SEQ ID NO:2**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTV

[0097] An exemplary CH1 Domain is a human IgG3 CH1 Domain. The amino acid sequence of an exemplary human IgG3 CH1 Domain is (**SEQ ID NO:3**):

ASTKGPSVFP LAPCSRSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YTCNVNHKPS NTKVDKRV

[0098] An exemplary CH1 Domain is a human IgG4 CH1 Domain. The amino acid sequence of an exemplary human IgG4 CH1 Domain is (**SEQ ID NO:4**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[0099] One exemplary Hinge Domain is a human IgG1 Hinge Domain. The amino acid sequence of an exemplary human IgG1 Hinge Domain is (**SEQ ID NO:5**):
EPKSCDKTHTCPPCP.

[00100] Another exemplary Hinge Domain is a human IgG2 Hinge Domain. The amino acid sequence of an exemplary human IgG2 Hinge Domain is (SEQ ID NO:6): ERKCCVECPPCP.

[00101] Another exemplary Hinge Domain is a human IgG3 Hinge Domain. The amino acid sequence of an exemplary human IgG2 Hinge Domain is (SEQ ID NO:7):

ELKTPLGDDT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
SCDTPPPCPR CP

[00102] Another exemplary Hinge Domain is a human IgG4 Hinge Domain. The amino acid sequence of an exemplary human IgG4 Hinge Domain is (SEQ ID NO:8): ESKYGPPCPSCP. As described herein, an IgG4 Hinge Domain may comprise a stabilizing mutation such as the S228P substitution. The amino acid sequence of an exemplary S228P-stabilized human IgG4 Hinge Domain is (SEQ ID NO:9): ESKYGPPCPPCP.

[00103] The CH2 and CH3 Domains of the two Heavy Chains of an IgG antibody interact to form an “Fc Domain,” of IgG antibodies that is recognized by cellular **Fc Receptors**, including but not limited to Fc gamma Receptors (**FcγRs**). As used herein, the term “Fc Domain” is used to define a C-terminal region of an IgG Heavy Chain. An Fc Domain is said to be of a particular IgG isotype, class or subclass if its amino acid sequence is most homologous to that isotype relative to other IgG isotypes. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents.

[00104] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (SEQ ID NO:10):

231 240 250 260 270 280
APELLGGPSV FLFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD

 290 300 310 320 330
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

 340 350 360 370 380
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

 390 400 410 420 430
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSV MHE

 440 447
ALHNHYTQKS LSLSPGX

as numbered by the EU index as set forth in Kabat, wherein X is lysine (K) or is absent.

[00105] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (SEQ ID NO:11):

```

231      240      250      260      270      280
APPVA-GPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD

      290      300      310      320      330
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA

      340      350      360      370      380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

      390      400      410      420      430
WESNGQPENN YKTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

      440      447
ALHNHYTQKS LSLSPGX

```

as numbered by the EU index as set forth in Kabat, wherein X is lysine (K) or is absent.

[00106] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (SEQ ID NO:12):

```

231      240      250      260      270      280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD

      290      300      310      320      330
GVEVHNAKTK PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

      340      350      360      370      380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

      390      400      410      420      430
WESSGQPENN YNTTPMLDS DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE

      440      447
ALHNRFTQKS LSLSPGX

```

as numbered by the EU index as set forth in Kabat, wherein X is lysine (K) or is absent.

[00107] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (SEQ ID NO:13):

```

231      240      250      260      270      280
APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD

      290      300      310      320      330
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS

      340      350      360      370      380
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE

      390      400      410      420      430
WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE

      440      447
ALHNHYTQKS LSLSLGX

```

as numbered by the EU index as set forth in Kabat, wherein X is lysine (K) or is absent.

2. Constant Regions of the Light Chain

[00108] As indicated above, each Light Chain of an antibody contains a Variable Domain (“VL”) and a Constant Domain (“CL”).

[00109] A preferred CL Domain is a human IgG CL Kappa Domain. The amino acid sequence of an exemplary human CL Kappa Domain is (SEQ ID NO:14):

```

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

```

[00110] Alternatively, an exemplary CL Domain is a human IgG CL Lambda Domain. The amino acid sequence of an exemplary human CL Lambda Domain is (SEQ ID NO:15):

```

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

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II. Multispecific Binding Molecules

[00111] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody’s VL and VH Domains. Interaction of an antibody’s Light Chain and Heavy Chain and, in particular, interaction of its VL and VH Domains forms one of the two Epitope-Binding Domains of a natural antibody, such as an IgG. Natural antibodies are capable of binding only one epitope species (*i.e.*, they are

monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bivalency or multivalency).

[00112] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (*i.e.*, more than two Epitope-Binding Domains) for the same epitope and/or antigen.

[00113] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use Linker peptides either to fuse a further Epitope-Binding Domain (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple Epitope-Binding Domains (*e.g.*, two Fab fragments or scFvs). Alternative formats use Linker peptides to fuse Epitope-Binding Domains (*e.g.*, an scFv, VL, VH, *etc.*) to a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786 WO 2006/107617, WO 2007/046893). PCT Publication Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind more than one antigen. PCT Publication Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. PCT Publication Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Domains have been replaced with additional VL and VH Domains, so as to form trivalent Binding Molecules. PCT Publication Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publication Nos. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. PCT Publication Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional

binding domains or functional groups to an antibody or an antibody portion (e.g., adding a diabody to the antibody's Light Chain, or adding additional VL and VH Domains to the antibody's light and Heavy Chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another).

[00114] The art has additionally noted the capability to produce diabodies that differ from such natural antibodies in being capable of binding two or more different epitope species (i.e., exhibiting bispecificity or multispecificity in addition to, or in exchange of, bivalency or multivalency) (see, e.g., Holliger *et al.* (1993) “*Diabodies’: Small Bivalent And Bispecific Antibody Fragments,*” Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 / WO 02/02781 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) “*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,*” J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) “*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,*” Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) “*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange,*” Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) “*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain,*” Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) “*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*” Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) “*Bispecific T-Cell Engaging Antibodies For Cancer Therapy,*” Cancer Res. 69(12):4941-4944).

[00115] In particular, stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART®** diabodies have been developed; see, e.g., Sloan, D.D. *et al.* (2015) “*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells,*” PLoS Pathog. 11(11):e1005233. doi: 10.1371/journal.ppat.1005233; Al Hussaini, M. *et al.* (2015) “*Targeting CD123 In AML Using A T-Cell Directed Dual-Affinity Re-Targeting (DART®) Platform,*” Blood pii: blood-2014-05-575704; Chichili, G.R. *et al.* (2015) “*A CD3xCD123 Bispecific DART For Redirecting Host T Cells To Myelogenous Leukemia: Preclinical*

Activity And Safety In Nonhuman Primates,” Sci. Transl. Med. 7(289):289ra82; Moore, P.A. *et al.* (2011) “*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma,*” Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) “*Therapeutic Control Of B-Cell Activation Via Recruitment Of Fcγ Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold,*” Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) “*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion,*” J. Mol. Biol. 399(3):436-449; US Patent Nos. 8,044,180; 8,133,982; 8,187,593; 8,193,318; 8,530,627; 8,669,349; 8,778,339; 8,784,808; 8,795,667; 8,802,091; 8,802,093; 8,946,387; 8,968,730; and 8,993,730; US Patent Publication Nos. 2009/0060910; 2010/0174053; 2011/0081347; 2011/0097323; 2011/0117089; 2012/0009186; 2012/0034221; 2012/0141476; 2012/0294796; 2013/0149236; 2013/0295121; 2014/0017237; and 2014/0099318; European Patent Documents No. EP 1868650; EP 2158221; EP 2247304; EP 2252631; EP 2282770; EP 2328934; EP 2376109; EP 2542256; EP 2601216; EP 2714079; EP 2714733; EP 2786762; EP 2839842; EP 2840091; and PCT Publication Nos. WO 2006/113665; WO 2008/157379; WO 2010/027797; WO 2010/033279; WO 2010/080538; WO 2011/109400; WO 2012/018687; WO 2012/162067; WO 2012/162068; WO 2014/159940; WO 2015/021089; WO 2015/026892; and WO 2015/026894). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond one or more pairs of such polypeptide chains to one another. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the involved polypeptide chains, stabilizing the resulting diabody without interfering with the diabody’s binding characteristics.

[00116] The simplest **DART®** diabody comprises two polypeptide chains each comprising three Domains (**Figures 1A-1B**). The first polypeptide chain comprises: (i) a Domain that comprises an Epitope-Binding Domain of a Light Chain Variable Domain of the a first immunoglobulin (VL1), (ii) a second Domain that comprises an Epitope-Binding Domain of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), and (iii) a third Domain that serves to promote heterodimerization (a “**Heterodimer-Promoting Domain**”) with the second polypeptide chain and to covalently bond the first polypeptide to the second polypeptide chain of the diabody. The second polypeptide chain contains a complementary

first Domain (a VL2 Domain), a complementary second Domain (a VH1 Domain) and a third Domain that complexes with the third Domain of the first polypeptide chain in order to promote heterodimerization (a “**Heterodimer-Promoting Domain**”) and covalent bonding with the first polypeptide chain. Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. In one embodiment, the Third Domains of the first and second polypeptide chains each contain a cysteine residue (denoted as “©” in the Figures), which serves to bind the polypeptides together via a covalent disulfide bond. The third Domain of one or both of the polypeptide chains may additionally possess the sequence of a CH2-CH3 Domain, such that complexing of one diabody polypeptide to another diabody polypeptide forms an Fc Domain. Such Fc Domains may serve to alter the biological half-life of the diabody, decrease its immunogenicity, and/or be capable of binding to an Fc Receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells) to enhance or inhibit effector function. Many variations of such molecules have been described (see, *e.g.*, United States Patent Publication Nos. 2015/0175697; 2014/0255407; 2014/0099318; 2013/0295121; 2010/0174053; 2009/0060910; 2007/0004909; European Patent Publication Nos. EP 2714079; EP 2601216; EP 2376109; EP 2158221; EP 1868650; and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538; WO 2006/113665), and are provided herein.

[00117] Recently, trivalent structures incorporating two Diabody-Type Binding Domains and one Non-Diabody-type Domain, and an Fc Domain have been described (see, *e.g.*, PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such trivalent Binding Molecules may be utilized to generate monospecific, bispecific or trispecific molecules as provided in more detail below. The ability to bind three different epitopes provides enhanced capabilities.

[00118] Alternative constructs are known in the art for applications where a bispecific or tetravalent molecule is desirable but an Fc is not required including, but not limited to, Bispecific T-cell Engager molecules, also referred to as “**BiTEs**” (see, *e.g.*, PCT Publication Nos: WO 1993/11161; and WO 2004/106381) and tetravalent tandem antibodies, also referred to as “**TandAbs**” (see, *e.g.* United States Patent Publication No: 2011-0206672; European Patent Publication No. EP 2371866, and; PCT Publication Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700). BiTEs are formed from a single

polypeptide chain comprising tandem linked scFvs, while TandAbs are formed by the homo-dimerization of two identical polypeptide chains, each possessing a VH1, VL2, VH2, and VL2 Domain.

[00119] The ability to produce multispecific Binding Molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent molecules, *etc.*) has led to their use (in “**trans**”) to co-ligate two cells together, for example, by co-ligating receptors that are present on the surface of different cells (*e.g.*, cross-linking cytotoxic T-cells to target cells, such as cancer cells or pathogen-infected cells, that express a Disease Antigen) (Staerz *et al.* (1985) “*Hybrid Antibodies Can Target Sites For Attack By T Cells*,” *Nature* 314:628-631, and Holliger *et al.* (1996) “*Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody*,” *Protein Eng.* 9:299-305; Marvin *et al.* (2005) “*Recombinant Approaches To IgG-Like Bispecific Antibodies*,” *Acta Pharmacol. Sin.* 26:649-658; Sloan *et al.* (2015) “*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells*,” *PLoS Pathog* 11(11): e1005233. doi:10.1371/journal.ppat.1005233)). Alternatively (or additionally), multispecific molecules can be used (in “**cis**”) to co-ligate molecules, such as receptors, *etc.*, that are present on the surface of the same cell. Co-ligation of different cells and/or receptors is useful to modulate effector functions and/or immune cell signaling. Multispecific molecules (*e.g.*, bispecific diabodies) comprising Epitope-Binding Domains may be directed to a surface determinant of any immune cell such as CD2, CD3, CD8, CD16, TCR, the Natural Killer Group 2, Member D Receptor (NKG2D), *etc.*, which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-Presenting Cells or other mononuclear cells. In particular, Epitope-Binding Domains directed to a cell surface receptor that is present on immune effector cells, are useful in the generation of multispecific Binding Molecules capable of mediating redirected cell killing.

[00120] The present invention provides Binding Molecules that are capable of mediating the redirected killing of a target cell (*e.g.*, a cancer cell or a pathogen-infected cell, *etc.*) expressing a Disease Antigen (“**DA**”). Such Binding Molecules are capable of binding a “**first epitope**” and a “**second epitope**,” wherein one of such epitopes is an epitope of CD3 and the other of such epitopes is an epitope of a Disease Antigen. It is irrelevant whether a particular epitope is designated as the first vs. the second epitope; such notation having relevance only with respect to the presence and orientation of the domains of the polypeptide

chains of the Binding Molecules of the present invention. Thus, the bispecific molecules of the present invention comprise “VL_{CD3}” / “VH_{CD3}” Domains that are capable of binding an epitope of CD3, and “VL_{DA}” / “VH_{DA}” Domains that are capable of binding an epitope of a Disease Antigen. The instant invention particular encompasses bispecific diabodies, bispecific scFvs, BiTEs, antibodies, TandAbs, and trivalent Binding Molecules produced using any of the methods provided herein.

A. Bispecific Diabodies Lacking Fc Domains

[00121] In one embodiment, the **DA x CD3 Binding Molecule** of the invention are bispecific diabodies and comprises domains capable of binding both a first and a second epitope, but will lack an Fc Domain, and thus will be unable to bind FcγR molecules via an Fc-FcγR interaction. The first polypeptide chain of such an embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, the VL Domain of a monoclonal antibody capable of binding either the first or second epitope (*i.e.*, either VL_{CD3} or VL_{DA}), a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding the epitope of the Disease Antigen (if such first polypeptide chain contains VL_{CD3}) or a VH Domain of a monoclonal antibody capable of binding CD3 (if such first polypeptide chain contains VL_{DA}), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figures 1A-1B**).

[00122] The second polypeptide chain of this embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, the VL Domain of a monoclonal antibody capable of binding the first or second epitope (*i.e.*, VL_{CD3} or VL_{DA}, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding either the first or second epitope (*i.e.*, VH_{CD3} or VH_{DA}, and being the VH Domain not selected for inclusion in the first polypeptide chain of the diabody), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figures 1A-1B**). The employed VL and VH Domains specific for a particular epitope are preferably obtained or derived from the same monoclonal antibody. However, such domains may be derived from different monoclonal antibodies provided that they associate to form a functional binding site capable

of immunospecifically binding such epitope. Such different antibodies are referred to herein as being “**corresponding**” antibodies.

[00123] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional Epitope-Binding Domain that is specific for one of the epitopes (*e.g.*, the first epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional Epitope-Binding Domain that is specific for the other epitope (*i.e.*, the second epitope). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is “**coordinated**,” such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding both the first epitope and the second epitope (*i.e.*, they collectively comprise VL_{CD3}/VH_{CD3} and VL_{DA}/VH_{DA}).

[00124] Most preferably, the length of the intervening spacer peptide (*i.e.*, “**Linker 1**,” which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding one another (for example consisting of from 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 intervening Linker amino acid residues). Thus, the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding one another. Likewise, the VL and VH Domains of the second polypeptide chain are substantially or completely incapable of binding one another. A preferred intervening spacer peptide (Linker 1) has the sequence (SEQ ID NO:16): GGGSGGGG.

[00125] The length and composition of the second intervening spacer peptide (“**Linker 2**”) is selected based on the choice of one or more polypeptide domains that promote such dimerization (*i.e.*, a “**Heterodimer-Promoting Domain**”). Typically, the second intervening spacer peptide (Linker 2) will comprise 3-20 amino acid residues. In particular, where the employed Heterodimer-Promoting Domain(s) do/does not comprise a cysteine residue a cysteine-containing second intervening spacer peptide (Linker 2) is utilized. A cysteine-containing second intervening spacer peptide (Linker 2) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence GGCGGG (SEQ ID NO:17). Alternatively, Linker 2 does not comprise a cysteine (*e.g.*, GGG, GGGS (SEQ ID NO:18), LGGGSG (SEQ ID NO:19), GGGSGGGSGGG (SEQ ID NO:20), ASTKG (SEQ ID NO:21), LEPKSS (SEQ ID NO:22), APSSS (SEQ ID NO:23),

etc.) and a cysteine-containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00126] The Heterodimer-Promoting Domains may comprise or consist of GVEPKSC (SEQ ID NO:24) or VEPKSC (SEQ ID NO:25) or AEPKSC (SEQ ID NO:26) on one polypeptide chain and GFNRGEC (SEQ ID NO:27) or FNRGEC (SEQ ID NO:28) on the other polypeptide chain (US2007/0004909).

[00127] In a preferred embodiment, the Heterodimer-Promoting Domains will comprise tandemly repeated coil domains of opposing charge for example, an “E-coil” Heterodimer-Promoting Domain (SEQ ID NO:29: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, or a “K-coil” Heterodimer-Promoting Domain (SEQ ID NO:30: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Heterodimer-Promoting Domains that comprise modifications of the above-described E-coil and K-coil sequences so as to include one or more cysteine residues may be utilized. The presence of such cysteine residues permits the coil present on one polypeptide chain to become covalently bonded to a complementary coil present on another polypeptide chain, thereby covalently bonding the polypeptide chains to one another and increasing the stability of the diabody. Examples of such particularly preferred are Heterodimer-Promoting Domains include a Modified E-Coil having the amino acid sequence EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:31), and a modified K-coil having the amino acid sequence KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:32).

[00128] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of a polypeptide chain of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind other proteins and thereby extend their serum half-

lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) “*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Modules*,” J. Biol. Chem. 277(10):8114-8120). Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:33**): LAEAKVLANR ELDKYGVSDY YKNLINNAKT VEGVKALIDE ILAALP.

[00129] As disclosed in WO 2012/162068 (herein incorporated by reference), “deimmunized” variants of **SEQ ID NO:33** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized ABD: 66D/70S +71A; 66S/70S +71A; 66S/70S +79A; 64A/65A/71A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLID₆₆NAKS₇₀ A₇₁EGVKALIDE ILAALP
(**SEQ ID NO:34**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNA₆₄A₆₅NNAKT VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO:35**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLIS₆₆NAKS₇₀ VEGVKALIAA₇₉E ILAALP
(**SEQ ID NO:36**),

are particularly preferred as such deimmunized ABD exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an ABD contains a third Linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the ABD (which is preferably a deimmunized ABD). A preferred sequence for such Linker 3 is **SEQ ID NO:18**: GGGS.

B. Diabodies Comprising Fc Domains

[00130] One embodiment of the present invention relates to multispecific diabodies (*e.g.*, bispecific, trispecific, tetraspecific, *etc.*) that comprise an Fc Domain and that are capable of simultaneously binding an epitope of CD3 and an epitope of a Disease Antigen. The Fc Domain of such molecules may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4). The molecules may further comprise a CH1 Domain and/or a Hinge Domain. When present, the CH1 Domain and/or Hinge Domain may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4), and is preferably of the same isotype as the desired Fc Domain.

[00131] The addition of an IgG CH2-CH3 Domain to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Domain, increases the biological half-life and/or alters the valency of the diabody. Such diabodies comprise, two or more polypeptide chains whose sequences permit the polypeptide chains to covalently bind each other to form a covalently associated diabody that is capable of simultaneously binding the first epitope and the second epitope. Incorporating an IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain bispecific Fc Domain-containing diabody to form (**Figure 2**).

[00132] Alternatively, incorporating IgG CH2-CH3 Domains onto only one of the diabody polypeptides will permit a more complex four-chain bispecific Fc Domain-containing diabody to form (**Figures 3A-3C**). **Figure 3C** shows a representative four-chain diabody possessing the Constant Light (CL) Domain and the Constant Heavy CH1 Domain, however fragments of such domains as well as other polypeptides may alternatively be employed (see, *e.g.*, **Figures 3A and 3B**, United States Patent Publication Nos. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538). Thus, for example, in lieu of the CH1 Domain, one may employ a peptide having the amino acid sequence GVEPKSC (**SEQ ID NO:24**), VEPKSC (**SEQ ID NO:25**), or AEPKSC (**SEQ ID NO:26**), derived from the Hinge Domain of a human IgG, and in lieu of the CL Domain, one may employ the C-terminal 6 amino acids of the human kappa Light Chain, GFNRGEC (**SEQ ID NO:27**) or FNRGEC (**SEQ ID NO:28**). A representative peptide containing four-chain diabody is shown in **Figure 3A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the “E-coil” helical domains (**SEQ ID NO:29**: EVAALEK-EVAALEK-

EVAALEK-EVAALEK or **SEQ ID NO:31**: EVAACEK-EVAALEK-EVAALEK-EVAALEK); and the “K-coil” domains (**SEQ ID NO:30**: KVAALEK-KVAALEK-KVAALEK-KVAALEK or **SEQ ID NO:32**: KVAACEK-KVAALEK-KVAALEK-KVAALEK). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00133] Fc Domain-containing diabody molecules of the present invention may include additional intervening spacer peptides (Linkers), generally such Linkers will be incorporated between a Heterodimer-Promoting Domain (*e.g.*, an E-coil or K-coil) and a CH2-CH3 Domain and/or between a CH2-CH3 Domain and a Variable Domain (*i.e.*, VH or VL). Typically, the additional Linkers will comprise 3-20 amino acid residues and may optionally contain all or a portion of an IgG Hinge Domain (preferably a cysteine-containing portion of an IgG Hinge Domain possessing 1, 2, 3 or more cysteine residues). Linkers that may be employed in the bispecific Fc Domain-containing diabody molecules of the present invention include: GGGG (**SEQ ID NO:18**), LGGGSG (**SEQ ID NO:19**), GGGSGGGSGGG (**SEQ ID NO:20**), ASTKG (**SEQ ID NO:21**), LEPKSS (**SEQ ID NO:22**), APSSS (**SEQ ID NO:23**), APSSSPME (**SEQ ID NO:37**), VEPKSADKTHTCPPCP (**SEQ ID NO:38**), LEPKSADKTHTCPPCP (**SEQ ID NO:39**), DKTHTCPPCP (**SEQ ID NO:40**), the scFv Linker: GGGGSGGGGSGGGG (**SEQ ID NO:41**); the “long” Linker: GGGGSGGGGSGGG (**SEQ ID NO:42**), GGC, and GGG. LEPKSS (**SEQ ID NO:22**) may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or LEPKSS (**SEQ ID NO:22**) may be immediately followed by DKTHTCPPCP (**SEQ ID NO:40**) to form the alternate Linkers: GGDKTHTCPPCP (**SEQ ID NO:43**); and LEPKSSDKTHTCPPCP (**SEQ ID NO:44**). Bispecific Fc Domain-containing molecules of the present invention may incorporate an IgG Hinge Domain in addition to or in place of a Linker. Exemplary Hinge Domains include: EPKSCDKTHTCPPCP (**SEQ ID NO:5**) from IgG1, ERKCCVECPPCP (**SEQ ID NO:6**) from IgG2, ELKTPGLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCP (**SEQ ID NO:7**) from IgG3, ESKYGPPCPSCP (**SEQ ID NO:8**) from IgG4, and ESKYGPPCPPCP (**SEQ ID NO:9**) an IgG4 Hinge variant comprising a stabilizing S228P substitution (as numbered by the EU index as set forth in Kabat) to reduce strand exchange.

[00134] As provided in **Figure 3A-3C**, Fc Domain-containing diabodies of the invention may comprise four chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a

Heterodimer-Promoting Domain, and (iv) a Domain containing a CH₂-CH₃ sequence. The second and fourth polypeptide chains contain: (i) a VL₂-containing Domain, (ii) a VH₁-containing Domain, and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third polypeptide chains with the second/fourth polypeptide chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. The notation “VL₃” and “VH₃” denote respectively, the Light Chain Variable Domain and Variable Heavy Chain Domain that bind a “third” epitope of such diabody. Similarly, the notation “VL₄” and “VH₄” denote respectively, the Light Chain Variable Domain and Variable Heavy Chain Domain that bind a “fourth” epitope of such diabody. The general structure of the polypeptide chains of a representative four-chain bispecific Fc Domain-containing diabodies of invention is provided in **Table 1**:

Table 1		
Bispecific	2 nd Chain	NH ₂ -VL ₂ -VH ₁ -HPD-COOH
	1 st Chain	NH ₂ -VL ₁ -VH ₂ -HPD-CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -VL ₁ -VH ₂ -HPD-CH ₂ -CH ₃ -COOH
	2 nd Chain	NH ₂ -VL ₂ -VH ₁ -HPD-COOH
Tetraspecific	2 nd Chain	NH ₂ -VL ₂ -VH ₁ -HPD-COOH
	1 st Chain	NH ₂ -VL ₁ -VH ₂ -HPD-CH ₂ -CH ₃ -COOH
	3 rd Chain	NH ₂ -VL ₃ -VH ₄ -HPD-CH ₂ -CH ₃ -COOH
	4 th Chain	NH ₂ -VL ₄ -VH ₃ -HPD-COOH

HPD = Heterodimer-Promoting Domain

[00135] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four Epitope-Binding Domains), Fc-containing diabodies that are composed of four total polypeptide chains (**Figures 3A-3C**). The bispecific, tetravalent, Fc-containing diabodies of the invention comprise two first Epitope-Binding Domains and two second Epitope-Binding Domains.

[00136] In a further embodiment, the Fc Domain-containing diabodies of the present invention may comprise three polypeptide chains. The first polypeptide of such a diabody contains three domains: (i) a VL₁-containing Domain, (ii) a VH₂-containing Domain and (iii) a Domain containing a CH₂-CH₃ sequence. The second polypeptide of such a diabody contains: (i) a VL₂-containing Domain, (ii) a VH₁-containing Domain and (iii) a Domain

that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such a diabody comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such a diabody associate together to form a VL1/VH1 Epitope-Binding Domain that is capable of binding either the first or second epitope, as well as a VL2/VH2 Epitope-Binding Domain that is capable of binding the other of such epitopes. The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain that is stabilized via a disulfide bond. Such bispecific diabodies have enhanced potency. **Figures 4A** and **4B** illustrate the structures of such diabodies. Such Fc Domain-containing diabodies may have either of two orientations (**Table 2**):

Table 2		
First Orientation	3 rd Chain	NH ₂ -CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -VL1-VH2-HPD-CH ₂ -CH ₃ -COOH
	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
Second Orientation	3 rd Chain	NH ₂ -CH ₂ -CH ₃ -COOH
	1 st Chain	NH ₂ -CH ₂ -CH ₃ -VL1-VH2-HPD-COOH
	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00137] In a specific embodiment, diabodies of the present invention are bispecific, bivalent (*i.e.*, possess two Epitope-Binding Domains), Fc-containing diabodies that are composed of three total polypeptide chains (**Figures 4A-4B**). The bispecific, bivalent Fc-containing diabodies of the invention comprise one Epitope-Binding Domain immunospecific for either the first or second epitope, as well as a VL2/VH2 Epitope-Binding Domain that is capable of binding the other of such epitopes.

[00138] In a further embodiment, the Fc Domain-containing diabodies may comprise a total of five polypeptide chains. In a particular embodiment, two of the five polypeptide chains have the same amino acid sequence. The first polypeptide chain of such a diabody contains: (i) a VH1-containing Domain, (ii) a CH1-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence. The first polypeptide chain may be the Heavy Chain of an antibody that contains a VH1 and a Heavy Chain constant region. The second and fifth polypeptide chains of such a diabody contain: (i) a VL1-containing Domain, and (ii) a CL-

containing Domain. The second and/or fifth polypeptide chains of such a diabody may be Light Chains of an antibody that contains a VL1 complementary to the VH1 of the first/third polypeptide chain. The first, second and/or fifth polypeptide chains may be isolated from a naturally occurring antibody. Alternatively, they may be constructed recombinantly. The third polypeptide chain of such a diabody contains: (i) a VH1-containing Domain, (ii) a CH1-containing Domain, (iii) a Domain containing a CH2-CH3 sequence, (iv) a VL2-containing Domain, (v) a VH3-containing Domain and (vi) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the third chain with the fourth chain. The fourth polypeptide of such diabodies contains: (i) a VL3-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's third polypeptide chain.

[00139] Thus, the first and second, and the third and fifth, polypeptide chains of such diabodies associate together to form two VL1/VH1 Epitope-Binding Domains capable of binding a first epitope. The third and fourth polypeptide chains of such diabodies associate together to form a VL2/VH2 Epitope-Binding Domain that is capable of binding a second epitope, as well as a VL3/VH3 binding site that is capable of binding a third epitope. The first and third polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective constant regions. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain. Such multispecific diabodies have enhanced potency. **Figure 5** illustrates the structure of such diabodies. It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific.

[00140] The VL and VH Domains of the polypeptide chains are selected so as to form VL/VH binding sites specific for a desired epitope. The VL/VH binding sites formed by the association of the polypeptide chains may be the same or different so as to permit tetravalent binding that is monospecific, bispecific, trispecific or tetraspecific. In particular, the VL and VH Domains maybe selected such that a multivalent diabody may comprise two binding sites for a first epitope and two binding sites for a second epitope, or three binding sites for a first epitope and one binding site for a second epitope, or two binding sites for a first epitope, one binding site for a second epitope and one binding site for a third epitope (as depicted in **Figure 5**). The general structure of the polypeptide chains of representative five-chain Fc Domain-containing diabodies of invention is provided in **Table 3**:

Table 3		
Bispecific (2x2)	2 nd Chain	NH ₂ -VL1-CL-COOH
	1 st Chain	NH ₂ -VH1-CH1-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VH1-CH1-CH2-CH3-VL2-VH2-HPD-COOH
	5 nd Chain	NH ₂ -VL1-CL-COOH
	4 th Chain	NH ₂ -VL2-VH2-HPD-COOH
Bispecific (3x1)	2 nd Chain	NH ₂ -VL1-CL-COOH
	1 st Chain	NH ₂ -VH1-CH1-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VH1-CH1-CH2-CH3-VL1-VH2-HPD-COOH
	5 nd Chain	NH ₂ -VL1-CL-COOH
	4 th Chain	NH ₂ -VL2-VH1-HPD-COOH
Trispecific (2x1x1)	2 nd Chain	NH ₂ -VL1-CL-COOH
	1 st Chain	NH ₂ -VH1-CH1-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VH1-CH1-CH2-CH3-VL2-VH3-HPD-COOH
	5 nd Chain	NH ₂ -VL1-CL-COOH
	4 th Chain	NH ₂ -VL3-VH2-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00141] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four Epitope-Binding Domains), Fc-containing diabodies that are composed of five total polypeptide chains having two Epitope-Binding Domains immunospecific for the first epitope, and two Epitope-Binding Domains specific for the second epitope. In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise three Epitope-Binding Domains immunospecific for the first epitope and one Epitope-Binding Domain specific for the second epitope. As provided above, the VL and VH Domains may be selected to permit trispecific binding. Accordingly, the invention also encompasses trispecific, tetravalent, Fc-containing diabodies. The trispecific, tetravalent, Fc-containing diabodies of the invention comprise two Epitope-Binding Domains immunospecific for the first epitope, one Epitope-Binding Domain immunospecific for the second molecule, and one Epitope-Binding Domain immunospecific for the third epitope.

[00142] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody-dependent cytotoxicity, mast cell degranulation, and

phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Domain of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc Receptors: Fc γ RI (CD64), Fc γ RII (CD32), and Fc γ RIII (CD16). Fc γ RI (CD64), Fc γ RIIA (CD32A) and Fc γ RIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; Fc γ RIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. In addition, interaction with the neonatal Fc Receptor (FcRn) mediates the recycling of IgG molecules from the endosome to the cell surface and release into the blood. The amino acid sequence of exemplary wild-type IgG1 (SEQ ID NO:10), IgG2 (SEQ ID NO:11), IgG3 (SEQ ID NO:12), and IgG4 (SEQ ID NO:13) are presented above.

[00143] Modification of the Fc Domain may lead to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may therefore be desirable to modify an Fc Domain-containing binding molecule of the present invention with respect to effector function, for example, so as to enhance the effectiveness of such molecule in treating cancer. Reduction or elimination of Fc Domain-mediated effector function is desirable in certain cases, for example in the case of antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the Fc γ Rs are expressed at low levels, for example, tumor-specific B cells with low levels of Fc γ RIIB (*e.g.*, non-Hodgkin's lymphoma, CLL, and Burkitt's lymphoma). Molecules of the invention possessing such conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection in which an enhanced efficacy of effector function activity is desired.

[00144] Accordingly, in certain embodiments, the Fc Domain of the Fc Domain-containing molecules of the present invention may be an engineered variant Fc Domain. Although the Fc Domain of the bispecific Fc Domain-containing molecules of the present invention may possess the ability to bind one or more Fc Receptors (*e.g.*, Fc γ R(s)), more preferably such variant Fc Domain have altered binding Fc γ RIA (CD64), Fc γ RIIA (CD32A), Fc γ RIIB (CD32B), Fc γ RIIIA (CD16a) or Fc γ RIIIB (CD16b) (relative to the binding exhibited by a

wild-type Fc Domain), *e.g.*, will have enhanced binding an activating receptor and/or will have substantially reduced or no ability to bind inhibitory receptor(s). Thus, the Fc Domain of the Fc Domain-containing molecules of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Domain, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 Domains of a complete Fc Domain). Such Fc Domains may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Domains, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as, for example, two CH2 Domains or two CH3 Domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00145] Fc Domain modifications identified as altering effector function are known in the art, including modifications that increase binding activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) “Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcγgamma Receptors,” Cancer Res. 57(18):8882-8890). **Table 4** lists exemplary single, double, triple, quadruple and quintuple substitutions (numbering (according to the EU index) and substitutions are relative to the amino acid sequence of **SEQ ID NO:10** as presented above) of exemplary modification that increase binding activating receptors and/or reduce binding inhibitory receptors.

Table 4			
Variations of Preferred Activating Fc Domains†			
Single-Site Variations			
F243L	R292G	D270E	R292P
Y300L	P396L		
Double-Site Variations			
F243L and R292P	F243L and Y300L	F243L and P396L	R292P and Y300L
D270E and P396L	R292P and V305I	P396L and Q419H	P247L and N421K
R292P and P396L	Y300L and P396L	R255L and P396L	R292P and P305I
K392T and P396L			
Triple-Site Variations			
F243L, P247L and N421K		P247L, D270E and N421K	
F243L, R292P and Y300L		R255L, D270E and P396L	
F243L, R292P and V305I		D270E, G316D and R416G	
F243L, R292P and P396L		D270E, K392T and P396L	
F243L, Y300L and P396L		D270E, P396L and Q419H	
V284M, R292L and K370N		R292P, Y300L and P396L	

Table 4
Variations of Preferred Activating Fc Domains[†]

Quadruple-Site Variations	
L234F, F243L, R292P and Y300L	F243L, P247L, D270E and N421K
L234F, F243L, R292P and Y300L	F243L, R255L, D270E and P396L
L235I, F243L, R292P and Y300L	F243L, D270E, G316D and R416G
L235Q, F243L, R292P and Y300L	F243L, D270E, K392T and P396L
P247L, D270E, Y300L and N421K	F243L, R292P, Y300L, and P396L
R255L, D270E, R292G and P396L	F243L, R292P, V305I and P396L
R255L, D270E, Y300L and P396L	F243L, D270E, P396L and Q419H
D270E, G316D, P396L and R416G	
Quintuple-Site Variations	
L235V, F243L, R292P, Y300L and P396L	F243L, R292P, V305I, Y300L and P396L
L235P, F243L, R292P, Y300L and P396L	
[†] numbering is according to the EU index as in Kabat	

[00146] Exemplary variants of human IgG1 Fc Domains with reduced binding CD32B and/or increased binding CD16A contain F243L, R292P, Y300L, V305I or P396L substitutions, wherein the numbering is that of the EU index as in Kabat. These amino acid substitutions may be present in a human IgG1 Fc Domain in any combination. In one embodiment, the variant human IgG1 Fc Domain contains a F243L, R292P and Y300L substitution. In another embodiment, the variant human IgG1 Fc Domain contains a F243L, R292P, Y300L, V305I and P396L substitution.

[00147] In certain embodiments, it is preferred for the Fc Domains of the Fc Domain-containing Binding Molecules of the present invention to exhibit decreased (or substantially no) binding FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:10**)). In a specific embodiment, the Fc Domain-containing Binding Molecules of the present invention comprise an IgG Fc Domain that exhibits reduced antibody-dependent cell-mediated cytotoxicity (ADCC) effector function. In a preferred embodiment, the CH2-CH3 Domains of such Binding Molecules include any 1, 2, 3, or 4 of the substitutions: L234A, L235A, D265A, N297Q, and N297G, wherein the numbering is that of the EU index as in Kabat. In another embodiment, the CH2-CH3 Domains contain an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 Domain of a naturally occurring Fc Domain that inherently exhibits decreased (or substantially no) binding FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding and effector function exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:10**)) is utilized.

In a specific embodiment, the Fc Domain-containing Binding Molecules of the present invention comprise an IgG2 Fc Domain (**SEQ ID NO:11**), an IgG3 Fc Domain (**SEQ ID NO:12**) or an IgG4 Fc Domain (**SEQ ID NO:13**). When an IgG4 Fc Domain is utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such as the Hinge Region S228P substitution described above (see, *e.g.*, **SEQ ID NO:9**). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00148] A preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Domain-containing molecules of the present invention having reduced or abolished effector function will comprise the substitutions L234A/L235A (**SEQ ID NO:45**):

```
APEAAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGX
```

wherein, X is a lysine (K) or is absent.

[00149] The serum half-life of proteins comprising Fc Domains may be increased by increasing the binding affinity of the Fc Domain for FcRn. The term “half-life” as used herein means a pharmacokinetic property of a molecule that is a measure of the mean survival time of the molecules following their administration. Half-life can be expressed as the time required to eliminate fifty percent (50%) of a known quantity of the molecule from a subject’s body (*e.g.*, a human patient or other mammal) or a specific compartment thereof, for example, as measured in serum, *i.e.*, circulating half-life, or in other tissues. In general, an increase in half-life results in an increase in mean residence time (MRT) in circulation for the molecule administered.

[00150] In some embodiments, the Fc Domain-containing Binding Molecules of the present invention comprise a variant Fc Domain that comprises at least one amino acid modification relative to a wild-type Fc Domain, such that the molecule has an increased half-life (relative to such molecule if comprising a wild-type Fc Domain). In some embodiments, the Fc Domain-containing Binding Molecules of the present invention comprise a variant IgG Fc Domain that comprises a half-life extending amino acid substitution at one or more positions selected from the group consisting of 238, 250, 252, 254, 256, 257, 258, 265, 272, 286, 288, 303, 305, 307, 308, 309, 311, 312, 317, 340, 356,

360, 362, 376, 378, 380, 382, 413, 424, 428, 433, 434, 435, and 436, wherein the numbering is that of the EU index as in Kabat. Numerous mutations capable of increasing the half-life of an Fc Domain-containing molecule are known in the art and include, for example M252Y, S254T, T256E, and combinations thereof. For example, see the mutations described in U.S. Patent Nos. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and PCT Publication Nos. WO 98/23289; WO 2009/058492; and WO 2010/033279, which are herein incorporated by reference in their entireties.

[00151] In some embodiments, the Fc Domain-containing Binding Molecules of the present invention exhibiting enhanced half-life possess a variant Fc Domain comprising substitutions at two or more of Fc Domain residues 250, 252, 254, 256, 257, 288, 307, 308, 309, 311, 378, 428, 433, 434, 435 and 436. In particular, two or more substitutions selected from: T250Q, M252Y, S254T, T256E, K288D, T307Q, V308P, A378V, M428L, N434A, H435K, and Y436I. In a specific embodiment, such molecules may possess a variant IgG Fc Domain comprising the substitution:

- (A) M252Y, S254T and T256E;
- (B) M252Y and S254T;
- (C) M252Y and T256E;
- (D) T250Q and M428L;
- (E) T307Q and N434A;
- (F) A378V and N434A;
- (G) N434A and Y436I;
- (H) V308P and N434A; or
- (I) K288D and H435K.

[00152] In a preferred embodiment, an Fc Domain-containing binding molecule of the present invention possesses a variant IgG Fc Domain comprising any 1, 2, or 3 of the substitutions: M252Y, S254T and T256E. The invention further encompasses such Binding Molecules that possess a variant Fc Domain comprising:

- (A) one or more mutations which alter effector function and/or Fc γ R binding;
and
- (B) one or more mutations which extend serum half-life.

[00153] An IgG1 sequence for the CH2 and CH3 Domains of the Fc Domain-containing molecules of the present invention that provides an increased half-life (and that has a 10-fold increase in binding to both cynomolgus monkey and human FcRn) (Dall'Acqua, W.F. *et al.* (2006) “*Properties of Human IgG1s Engineered for Enhanced Binding to the Neonatal Fc Receptor (FcRn)*,” J. Biol. Chem. 281(33):23514-23524) will comprise the substitutions M252Y/S254T/T256E (**SEQ ID NO:46**):

```
APELLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME
ALHNHYTQKS LSLSPGX
```

wherein, X is a lysine (K) or is absent.

[00154] An alternative IgG1 sequence for the CH2 and CH3 Domains of the Fc Domain-containing molecules of the present invention combining the reduced or abolished effector function provided by the substitutions L234A/L235A and the increased serum half-life provided by the substitutions M252Y/S254T/T256E is provided by **SEQ ID NO: 47**:

```
APEAAGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME
ALHNHYTQKS LSLSPGX
```

wherein, X is a lysine (K) or is absent.

[00155] For certain antibodies, diabodies and trivalent Binding Molecules that are desired to have Fc-Domain-containing polypeptide chains of differing amino acid sequence (*e.g.*, whose Fc Domain-containing polypeptide chains are desired to not be identical), it is desirable to reduce or prevent homodimerization from occurring between the CH2-CH3 Domains of identical chains (*e.g.*, two first polypeptide chains or between the CH2-CH3 Domains of two third polypeptide chains). The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a “knob”, *e.g.*, tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, “the hole” (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising

CH2-CH3 Domains that forms an Fc Domain to foster heterodimerization. Methods of protein engineering to favor heterodimerization over homodimerization are well-known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) “‘Knobs-Into-Holes’ Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,” Protein Engr. 9:617-621, Atwell *et al.* (1997) “Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,” J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) “A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,” J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety).

[00156] A preferred knob is created by modifying an IgG Fc Domain to contain the modification T366W. A preferred hole is created by modifying an IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying a hole-bearing polypeptide chain homodimer from the final bispecific heterodimeric Fc Domain-containing molecule, the protein A binding site of the hole-bearing CH2 and CH3 Domains a polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the hole-bearing polypeptide chain homodimer will not bind protein A, whereas the bispecific heterodimer will retain its ability to bind protein A via the protein A binding site on the knob-bearing polypeptide chain. In an alternative embodiment, the hole-bearing polypeptide chain may incorporate amino acid substitutions at positions 434 and 435 (N434A/N435K).

[00157] A preferred IgG1 amino acid sequence for the CH2 and CH3 Domains of one Fc Domain-containing polypeptide chain of an Fc Domain-containing molecule of the present invention will have the “**knob-bearing**” sequence (SEQ ID NO:48):

```
APEAAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGX
```

wherein X is a lysine (K) or is absent.

[00158] An alternative IgG1 amino acid sequence for the CH2 and CH3 Domains of one Fc Domain-containing polypeptide chain of an Fc Domain-containing molecule of the present invention having a M252Y/S254T/T256E substitution and a “knob-bearing” sequence is **SEQ ID NO:49**:

APEAAGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
 WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME
 ALHNHYTQKS LSLSPGX

wherein X is a lysine (K) or is absent.

[00159] A preferred IgG1 amino acid sequence for the CH2 and CH3 Domains of the other Fc Domain-containing polypeptide chain of an Fc Domain-containing molecule of the present invention will have the “hole-bearing” sequence (**SEQ ID NO:50**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
 WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVME
 ALHNRYTQKS LSLSPGX

wherein X is a lysine (K) or is absent.

[00160] An alternative IgG1 amino acid sequence for the CH2 and CH3 Domains of the other Fc Domain-containing polypeptide chain of an Fc Domain-containing molecule of the present invention having a M252Y/S254T/T256E substitution and a “hole-bearing” sequence is **SEQ ID NO:51**:

APEAAGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
 WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVME
 ALHNRYTQKS LSLSPGX

wherein X is a lysine (K) or is absent.

[00161] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:48**, **SEQ ID NO:49**, **SEQ ID NO:50** and **SEQ ID NO:51** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain exhibit decreased (or substantially no) binding FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIA (CD16a) or FcγRIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (**SEQ ID NO:10**)). The invention also encompasses such CH2-CH3 Domains, which comprise the wild-type alanine residues, alternative and/or additional substitutions which modify effector function and/or FcγR binding activity of the Fc Domain. The invention also encompasses such CH2-CH3 Domains, which further comprise one or more half-live extending amino

acid substitutions. In particular, the invention encompasses such hole-bearing and such knob-bearing CH2-CH3 Domains which further comprise the M252Y/S254T/T256E.

[00162] An IgG4 amino acid sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Domain-containing molecule of the present invention has enhanced serum half-life (relative to IgG1 CH2 and CH3 Domains) due to its possession of Y252/T254/E256 (SEQ ID NO:52):

```
APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSV MHE
ALHNHYTQKS LSLSLGX
```

wherein X is a lysine (K) or is absent.

[00163] A “knob-bearing” variant of such an IgG4 CH2-CH3 amino acid sequence has the amino acid sequence of SEQ ID NO:53:

```
APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSV MHE
ALHNHYTQKS LSLSLGX
```

wherein X is a lysine (K) or is absent.

[00164] A “hole-bearing” variant of such an IgG4 CG2-CH3 amino acid sequence has the amino acid sequence of SEQ ID NO:54:

```
APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLSCAVK GFYPSDIAVE
WESNGQPENN YKTTTPVLDS DGSFFLVSRL TVDKSRWQEG NVFSCSV MHE
ALHNRYTQKS LSLSLGX
```

wherein X is a lysine (K) or is absent.

[00165] It is preferred that the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of SEQ ID NO:48 or SEQ ID NO:49. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, SEQ ID NO:50 or SEQ ID NO:51) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, SEQ ID NO:48 or SEQ ID NO:49) would be employed in the second polypeptide chain of an Fc Domain-containing molecule of the present invention having two polypeptide chains (or in the third polypeptide chain of an Fc Domain-containing molecule having three, four, or five polypeptide chains).

[00166] In other embodiments, the invention encompasses Fc Domain-containing Binding Molecules comprising CH2 and/or CH3 Domains that have been engineered to favor heterodimerization over homodimerization using mutations known in the art, such as those disclosed in PCT Publication No. WO 2007/110205; WO 2011/143545; WO 2012/058768; WO 2013/06867, all of which are incorporated herein by reference in their entirety.

III. Trivalent Binding Molecules Containing Fc Domains

[00167] A further embodiment of the present invention relates to trivalent Binding Molecules comprising an Fc Domain capable of simultaneously binding a first epitope, a second epitope and a third epitope, wherein at least one of such epitopes is not identical to another. Such trivalent Binding Molecules comprise three Epitope-Binding Domains, two of which are Diabody-Type Binding Domains, which provide binding Site A and binding Site B, and one of which is a Fab-Type Binding Domain, or an scFv-Type Binding Domain, which provides binding Site C (see, *e.g.*, **Figures 6A-6F**, PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such trivalent Binding Molecules thus comprise “**VL1**” / “**VH1**” domains that are capable of binding the first epitope and “**VL2**” / “**VH2**” domains that are capable of binding the second epitope and “**VL3**” and “**VH3**” domains that are capable of binding the “third” epitope of such trivalent binding molecule. A “Diabody-Type Binding Domain” is the type of Epitope-Binding Domain present in a diabody, as described above. Each of a “Fab-Type Binding Domain” and an “scFv-Type Binding Domain” are Epitope-Binding Domains that are formed by the interaction of the VL Domain of an immunoglobulin Light Chain and a complementing VH Domain of an immunoglobulin Heavy Chain. Fab-Type Binding Domains differ from Diabody-Type Binding Domains in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single Epitope-Binding Domain, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two Epitope-Binding Domains. Similarly, scFv-Type Binding Domains also differ from Diabody-Type Binding Domains in that they comprise only a single Epitope-Binding Domain. Thus, as used herein Fab-Type, and scFv-Type Binding Domains are distinct from Diabody-Type Binding Domains.

[00168] Typically, the trivalent Binding Molecules of the present invention will comprise four different polypeptide chains (see **Figures 6A-6B**), however, the molecules may comprise fewer or greater numbers of polypeptide chains, for example by fusing such polypeptide chains to one another (*e.g.*, via a peptide bond) or by dividing such polypeptide

chains to form additional polypeptide chains, or by associating fewer or additional polypeptide chains via disulfide bonds. **Figures 6C-6F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 6A-6F**, the trivalent Binding Molecules of the present invention may have alternative orientations in which the Diabody-Type Binding Domains are N-terminal (**Figures 6A, 6C and 6D**) or C-terminal (**Figures 6B, 6E and 6F**) to an Fc Domain. CH2 and CH3 Domains useful for the generation of trivalent Binding Molecules are provided above and include knob-bearing and hole-bearing domains.

[00169] In certain embodiments, the first polypeptide chain of such trivalent Binding Molecules of the present invention contains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The VL1 and VL2 Domains are located N-terminal or C-terminal to the CH2-CH3-containing domain as presented in **Table 4** (also see, **Figures 6A and 6B**). The second polypeptide chain of such embodiments contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain. The third polypeptide chain of such embodiments contains: (i) a VH3-containing Domain, (ii) a CH1-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The third polypeptide chain may be the Heavy Chain of an antibody that contains a VH3 and a Heavy Chain constant region, or a polypeptide that contains such domains. The fourth polypeptide of such embodiments contains: (i) a VL3-containing Domain and (ii) a CL-containing Domain. The fourth polypeptide chains may be a Light Chain of an antibody that contains a VL3 complementary to the VH3 of the third polypeptide chain, or a polypeptide that contains such domains. The third or fourth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly, synthetically or by other means.

[00170] The Light Chain Variable Domain of the first and second polypeptide chains are separated from the Heavy Chain Variable Domains of such polypeptide chains by an intervening spacer peptide having a length that is too short to permit their VL1/VH2 (or their VL2/VH1) Domains to associate together to form Epitope-Binding Domain capable of binding either the first or second epitope. A preferred intervening spacer peptide (Linker 1) for this purpose has the sequence (**SEQ ID NO:16**): GGGSGGGG. Other Domains of the trivalent Binding Molecules may be separated by one or more intervening spacer peptides

(Linkers), optionally comprising a cysteine residue. In particular, as provided above, such Linkers will typically be incorporated between Variable Domains (*i.e.*, VH or VL) and peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and between such peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and CH2-CH3 Domains. Exemplary Linkers useful for the generation of trivalent Binding Molecules are provided above and are also provided in PCT Application Nos: PCT/US15/33081; and PCT/US15/33076. Thus, the first and second polypeptide chains of such trivalent Binding Molecules associate together to form a VL1/VH1 binding site capable of binding a first epitope, as well as a VL2/VH2 binding site that is capable of binding a second epitope. The third and fourth polypeptide chains of such trivalent Binding Molecules associate together to form a VL3/VH3 binding site that is capable of binding a third epitope.

[00171] As described above, the trivalent Binding Molecules of the present invention may comprise three polypeptides. Trivalent Binding Molecules comprising three polypeptide chains may be obtained by linking the domains of the fourth polypeptide N-terminal to the VH3-containing Domain of the third polypeptide (*e.g.*, using an intervening spacer peptide (**Linker 4**)). Alternatively, a third polypeptide chain of a trivalent binding molecule of the invention containing the following domains is utilized: (i) a VL3-containing Domain, (ii) a VH3-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence, wherein the VL3 and VH3 are spaced apart from one another by an intervening spacer peptide that is sufficiently long (at least 9 or more amino acid residues) so as to allow the association of these domains to form an Epitope-Binding Domain. One preferred intervening spacer peptide for this purpose has the sequence: GGGGSGGGGSGGGGS (**SEQ ID NO:41**).

[00172] It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains of such trivalent Binding Molecules may be different so as to permit binding that is monospecific, bispecific or trispecific. In particular, the VL and VH Domains may be selected such that a trivalent binding molecule comprises two binding sites for a first epitope and one binding sites for a second epitope, or one binding site for a first epitope and two binding sites for a second epitope, or one binding site for a first epitope, one binding site for a second epitope and one binding site for a third epitope.

[00173] The general structure of the polypeptide chains of representative trivalent Binding Molecules of invention is provided in **Figures 6A-6F** and in **Table 5**:

Table 5		
Four-Chain 1 st Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -VL1-VH2-HPD-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VH3-CH1-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL3-CL-COOH
Four-Chain 2 nd Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -CH2-CH3-VL1-VH2-HPD-COOH
	3 rd Chain	NH ₂ -VH3-CH1-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL3-CL-COOH
Three Chain 1 st Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -VL1-VH2-HPD-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2 nd Orientation	2 nd Chain	NH ₂ -VL2-VH1-HPD-COOH
	1 st Chain	NH ₂ -CH2-CH3-VL1-VH2-HPD-COOH
	3 rd Chain	NH ₂ -VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[00174] As provided above, such trivalent Binding Molecules may comprise three, four, five, or more polypeptide chains.

IV. Embodiments of the Invention

[00175] As stated above, the present invention is directed to **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domain that comprises a CDR_{H1} Domain, a CDR_{H2} Domain, a CDR_{H3} Domain, a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, at least one of which differs in amino acid sequence from the amino acid sequence of the corresponding CDR of an rCD3-Binding Domain. The rCD3-Binding Domain that is to be employed in such comparison with a particular vCD3-Binding Domain is the CD3-Binding Domain of an isolated CD3-binding antibody that exhibits the greatest identity of CDR sequence with such particular vCD3-Binding Domain. The rCD3-Binding Domain preferably also exhibits at least 95% to 100% identity in the framework regions. A preferred rCD3-Binding Domain comprises the CDR_{H1} Domain, CDR_{H2} Domain, CDR_{H3} Domain, CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of **CD3 mAb-1**. The **DA x CD3 Binding Molecules** of the present invention that comprise such vCD3-Binding Domain

exhibit an altered affinity for CD3, relative to a **DA x CD3 Binding Molecule** comprising such rCD3-Binding Domain. The invention 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 Disease Antigen, and exhibit reduced levels of cytokine release relative to a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. The invention 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 invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[00176] The invention thus encompasses **DA x CD3 Binding Molecules** comprising one or more of the VH and/or VL Domains of a vCD3-Binding Domain, or more preferably, the CDR_{H1}, CDR_{H2}, and CDR_{H3}, and the CDR_{L1}, CDR_{L2} and CDR_{L3} portions of such Domains. In a preferred embodiment of the invention, such **DA x CD3 Binding Molecules** Binding Molecules will additionally contain binding domains sufficient to permit such molecules to bind to epitope(s) of one, two, or more Disease Antigens. In another preferred embodiment of the invention, such **DA x CD3 Binding Molecules** will additionally contain binding domains sufficient to permit such molecules to bind to epitope(s) of another molecule expressed on the surface of an effector cell, such as CD2, CD8, CD16, T-cell Receptor (TCR), NKp46, NKG2D, *etc.*, which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-Presenting Cells or other mononuclear cells).

[00177] The present invention is also directed to pharmaceutical compositions that comprise such **DA x CD3 Binding Molecule(s)**.

[00178] By possessing binding domains sufficient to immunospecifically bind CD3 and a Disease Antigen, the molecules of the present invention have the ability to mediate the redirected killing of a target cell (*e.g.*, a cancer cell or a pathogen-infected cell) that arrays the Disease Antigen on its surface. The combined presence of both such binding affinities serves to localize the a CD3-expressing effector cell to the site of the target cell (*i.e.*, to “redirect” the effector cell) so that it may mediate the killing of the target cell. As discussed above, such molecules may be bispecific, or may be capable of binding more than two epitopes (*e.g.*, trispecific).

[00179] Efforts to employ CD3 Binding Molecules have been encumbered by the high magnitude of immune activation caused by such therapies and the attendant and adverse production of high levels of cytokines in some patients. Thus, although anti-CD3 therapies have resulted in a significant degree of immune activation in recipient patients, which has correlated with greatly increased efficacy, the use of such molecules has been associated with notable toxicity (Frey, N.V. *et al.* (2016) “*Cytokine Release Syndrome With Novel Therapeutics For Acute Lymphoblastic Leukemia*,” Hematol. Am. Soc. Hematol. Educ Program. (1):567-572; Teachey, D.T. *et al.* (2013) “*Cytokine Release Syndrome After Blinatumomab Treatment Related To Abnormal Macrophage Activation And Ameliorated With Cytokine-Directed Therapy*,” Blood 121(26):5154-5157; Le Jeune, C. *et al.* (2016) “*Potential For Bispecific T-Cell Engagers: Role Of Blinatumomab In Acute Lymphoblastic Leukemia*,” Drug Des. Devel. Ther. 10:757-765; Newman, M.J. *et al.* (2016) “*A Review Of Blinatumomab, A Novel Immunotherapy*,” J. Oncol. Pharm. Pract. 22(4):639-645; Fitzgerald, J.C. *et al.* (2017) “*Cytokine Release Syndrome After Chimeric Antigen Receptor T-Cell Therapy for Acute Lymphoblastic Leukemia*,” Crit. Care Med. 45(2):e124-e131; Teachey, D.T. *et al.* (2016) “*Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia*,” Cancer Discov. 6(6):664-679; Goebeler, M.E. *et al.* (2016) “*Blinatumomab: A CD19/CD3 Bispecific T Cell Engager (Bite) With Unique Anti-Tumor Efficacy*,” Leuk. Lymphoma 57(5):1021-1032; Barrett, D.M. *et al.* (2014) “*Toxicity Management For Patients Receiving Novel T-Cell Engaging Therapies*,” Curr. Opin. Pediatr. 26(1):43-49).

[00180] The present invention addresses such encumbrance by demonstrating that parental CD3-Binding Domains (*i.e.*, rCD3-Binding Domains) that exhibit both high cytotoxicity and high cytokine release when incorporated into **DA x CD3 Binding Molecules** may be engineered to produce variants (*i.e.*, vCD3-Binding Domains) having altered affinity for CD3 that are capable of mediating redirected killing and exhibit reduced levels of cytokine release relative to a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. In particular, **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domains of the invention exhibit reduced levels of release of any one or more of: IFN- γ , TNF- α , IL-2, and/or IL-6.

[00181] The present invention stems, in part, from the recognition that cytotoxicity and cytokine release are separable properties of **DA x CD3 Binding Molecules**. The present

invention encompasses variant CD3-Binding Domains (*i.e.*, vCD3-Binding Domains) that retain high levels of cytotoxicity while exhibiting reduced levels of cytokine release, and the use of **DA x CD3 Binding Molecules** comprising such vCD3-Binding Domains in the treatment of disease. As used herein, the term “**variant**” with respect to such CD3-Binding Domains is intended to refer to CD3-Binding Domains having at least one CDR_H, and/or at least one CDR_L, that differs from the “**corresponding**” CDR_H and/ CDR_L of a “**reference**” CD3-Binding Domain (*i.e.*, rCD3-Binding Domain). As used herein the term “corresponding” CDR_H and/ CDR_L denotes a comparison between two CDR sequences in which both such CDRs are CD_H1 Domains, both such CDRs are CD_H2 Domains, both such CDRs are CD_H3 Domains, both such CDRs are CD_L1 Domains, both such CDRs are CD_L2 Domains, or both such CDRs are CD_L3 Domains. A preferred rCD3-binding domain for the exemplary vCD3-binding domains described herein is a CD3-Binding Domain having at least 5, at least 4, at least 3, at least 2 or at least 1 of the CDRs: CD_H1, CDR_H2, CDR_H3 and CD_L1, CDR_L2, and CDR_L3 of **CD3 mAb 1**. Preferably, such exemplary vCD3-binding domains will possess at least 5 of the CDRs: CD_H1, CDR_H2, CDR_H3 and CD_L1, CDR_L2, and CDR_L3 of **CD3 mAb 1**. vCD3-binding domains may be obtained through the chemical modification of one or more CDRs of the rCD3-Binding Domain, but will more preferably be obtained by forming one or more polynucleotides that encode such one or more CDRs of the rCD3-binding Domain, except being altered to encode the desired vCD3-Binding Domain, and then expressing such polynucleotide in an appropriate protein expression system (*e.g.*, a cell, or in vitro translation system). Cytotoxicity may be measured in any suitable manner (*e.g.*, a CTL assay to determine the EC₅₀, maximum, *etc.*). Cytokine release may be measured by assaying for any one or more of: IFN-gamma, TNF-alpha, IL-6 or IL-2 in any suitable manner (*e.g.*, a CTL assay to determine the EC₅₀, maximum, *etc.*).

[00182] Notably, the absolute levels of maximal cytotoxicity and cytokine release are not the only criteria used to assess whether a candidate CD3-Binding Domain is a suitable vCD3-Binding Domain encompassed by the present invention. In addition, or alternatively, EC₅₀ values may be employed. As provided herein, a suitable vCD3-Binding Domain is one that, when incorporated into a **DA x CD3 Binding Molecule**, is capable of mediating high levels of cytotoxicity (*i.e.*, a low EC₅₀ concentration) while exhibiting reduced levels of cytokine release.

[00183] In certain embodiments, the instant invention provides a vCD3-Binding Domain that, when incorporated into a **DA x CD3 Binding Molecule**, mediates cell redirected cell killing to a maximum cytotoxicity (*e.g.*, as measured in a CTL assay at 18-48 hours) that is at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, of that mediated by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. Additionally, or alternatively, a **DA x CD3 Binding Molecule** comprising a vCD3-Binding Domains of the invention exhibits an EC₅₀ of cytotoxicity (*e.g.*, a measured in a CTL assay at 18-48 hours) that is increased by less than about 10%, less than about 20%, less than about 30%, less than about 40%, less than about 50%, less than about 60%, less than about 70%, less than about 80%, less than about 90%, less than about 100%, less than about 200%, less than about 300%, less than about 400%, or less than about 500% of that exhibited by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. Additionally, or alternatively the ratio of the EC₅₀ of cytotoxicity (*e.g.*, as measured in a CTL assay at 18-24 hours) of a **DA x CD3 Binding Molecule** comprising a vCD3-Binding Domain of the invention to a **DA x CD3 Binding Molecule** comprising the rCD3-Binding Domain (EC₅₀ variant/ EC₅₀ reference) is less than about 2, is less than about 5, is less than about 10, is less than about 20, is less than about 40, is less than about 60, is less than about 80, is less than about 100, or is less than about 200.

[00184] In certain embodiments, a **DA x CD3 Binding Molecule** comprising a vCD3-Binding Domains of the invention exhibits a maximum release of one or more cytokine (*e.g.*, as measured in a CTL assay at 18-24 hours) that is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more of that exhibited by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. Additionally, or alternatively, **DA x CD3 Binding Molecules** comprising the vCD3-Binding Domains of the invention exhibit an EC₅₀ of release of one or more cytokine (*e.g.*, as measured in a CTL assay at 18-48 hours) that is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80, at least about 90%, or more of that exhibited by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. In particular embodiments, the cytokine released is selected from the group consisting of: IFN- γ , TNF- α , IL-2, and IL-6. Additionally, or alternatively the ratio of the EC₅₀ of release of one or more cytokine (*e.g.*, as measured in a CTL assay at 18-24 hours) of a **DA x CD3 Binding Molecule** comprising

a vCD3-Binding Domain of the invention to a **DA x CD3 Binding Molecule** comprising the rCD3-Binding Domain (EC₅₀ variant/ EC₅₀ reference) is more than about 1, is more than about 2, is more than about 5, is more than about 10, is more than about 20, is more than about 40, is more than about 60, is more than about 80, is more than about 100, or is more than about 200.

[00185] Additionally, **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domain of the invention retain at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, of an *in vivo* activity (*e.g.*, anti-tumor, anti-pathogen activity) exhibited by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain. In view of the instant disclosure it will be understood that **DA x CD3 Binding Molecules** comprising a vCD3-Binding Domain may be administered at a higher dose to achieve an *in vivo* activity that is at least about 50% or more of that exhibited by a **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain, but that such higher dose will exhibit reduced levels of cytokine release as compared to the **DA x CD3 Binding Molecule** comprising a rCD3-Binding Domain.

[00186] In one embodiment, such **DA x CD3 Binding Molecules** of the present invention will be monospecific so as to possess the ability to bind to only a single epitope of CD3 and only a single epitope of the Disease Antigen.

[00187] Alternatively, such **DA x CD3 Binding Molecules** may be multispecific, *i.e.*, capable of binding 1, 2, 3, 4, or more than 4 epitopes, which may be apportioned in any manner to bind 1, 2, or more epitope(s) of CD3 and 1, 2, 3, 4, or more than 4 epitope(s) of one or more Disease Antigen(s).

[00188] In certain embodiments, where such **DA x CD3 Binding Molecules** are capable of immunospecifically binding to only a single Disease Antigen, they may be capable of immunospecifically binding to only one CD3 epitope and to one, two epitope(s) of such Disease Antigen (which two Disease Antigen epitopes may be the same or different), or they may be capable of immunospecifically binding to only one CD3 epitope and to three epitope(s) of such Disease Antigen (which three Disease Antigen epitopes may be the same, or may be different, or may be two epitopes that are the same and one epitope that is different).

[00189] In other embodiments, where such **DA x CD3 Binding Molecules** are capable of immunospecifically binding to two different Disease Antigens (*e.g.*, a First Disease Antigen and a Second Disease Antigen), they may be capable of immunospecifically binding to only one CD3 epitope and to one or two epitope(s) of the First Disease Antigen (which two First Disease Antigen epitopes may be the same or different) and two or one epitope(s) of the Second Disease Antigen (which two Second Disease Antigen epitopes may be the same or different).

[00190] In still other embodiments, such **DA x CD3 Binding Molecules** may be capable of immunospecifically binding to three different Disease Antigens (*e.g.*, a First Disease Antigen, a Second Disease Antigen and a Third Disease Antigen) and only one CD3 epitope.

[00191] In still other embodiments, such **DA x CD3 Binding Molecules** may be capable of immunospecifically binding to one or two different Disease Antigens (*e.g.*, a First Disease Antigen and a Second Disease Antigen), only one CD3 epitope, and one or two different cell surface molecules (which may be the same cell surface molecule or may be different surface molecules) of an effector cell (which may be the same type of effector cell or may be a different type of effector cell).

[00192] Thus, for example, such **DA x CD3 Binding Molecules** may bind:

- (1) a single epitope of CD3 and a single epitope of a Disease Antigen that is arrayed on the surface of the target cell;
- (2) a single epitope of CD3 and two epitopes of the same Disease Antigen that is arrayed on the surface of the target cell;
- (3) a single epitope of CD3, an epitope of a First Disease Antigen that is arrayed on the surface of the target cell and an epitope of a Second Disease Antigen that is arrayed on the surface of the target cell;
- (4) a single epitope of CD3 and three epitopes of the same Disease Antigen that is arrayed on the surface of the target cell;
- (5) a single epitope of CD3, two epitopes of a First Disease Antigen that is arrayed on the surface of the target cell, and one epitope of a Second Disease Antigen that is arrayed on the surface of the target cell;
- (6) a single epitope of CD3, an epitope of a First Disease Antigen that is arrayed on the surface of the target cell, and an epitope of a Second Disease Antigen that is arrayed on the surface of the target cell;

- (7) a single epitope of CD3, a single epitope of a Disease Antigen that is arrayed on the surface of the target cell and a single epitope of a cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell);
- (8) a single epitope of CD3, two epitopes of a Disease Antigen that is arrayed on the surface of the target cell and a single epitope of a cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell);
- (9) a single epitope of CD3, an epitope of a First Disease Antigen that is arrayed on the surface of the target cell, an epitope of a Second Disease Antigen that is arrayed on the surface of the target cell and a single epitope of a cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell);
- (10) a single epitope of CD3, an epitope of a Disease Antigen that is arrayed on the surface of the target cell, and two epitopes of a cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell); or
- (11) a single epitope of CD3, an epitope of a Disease Antigen that is arrayed on the surface of the target cell, an epitope of a first cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell), and an epitope of a second cell surface molecule other than CD3 that is arrayed on the surface of an effector cell (which may be the same type of effector cell as that arraying CD3 or may be a different type of effector cell).

[00193] The invention thus contemplates **DA x CD3 Binding Molecules** that comprise a first Epitope-Binding Domain capable of immunospecifically binding an epitope of CD3 and a second Epitope-Binding Domain that is capable of immunospecifically binding an epitope of a Disease Antigen that is arrayed on the surface of such target cell and a third

Epitope-Binding Domain capable of immunospecifically binding an epitope of a different cell surface molecule of an effector cell (which may be the same type of effector cell or may be a different type of effector cell). In a specific embodiment, the different cell surface molecule of an effector cell is CD8. **Table 6** illustrates possible combination binding specificities of exemplary molecules of the invention.

Table 6					
Number of Epitopes Recognized by Exemplary Molecules of the Invention Capable of Mediating the Redirected Killing of a Target Cell					
Total Number of Binding Domains	CD3 Epitope	Other Effector Cell Epitope	1 st Disease Antigen Epitope	2 nd Disease Antigen Epitope	3 rd Disease Antigen Epitope
2	1	0	1	0	0
3	1	0	1	1	0
3	1	1	1	0	0
3	1	0	1	1	0
3	2	0	1	0	0
4	1	0	1	1	1
4	1	0	1	2	0
4	1	0	2	1	0
4	1	1	2	0	0
4	1	1	1	1	0
4	1	2	1	0	0
4	2	0	1	1	0
4	2	0	1	1	0
4	2	1	1	0	0

[00194] By forming more complex molecules, one may obtain **DA x CD3 Binding Molecules** that are capable of binding CD3 and one or more Disease Antigens and optionally a different cell surface molecule of an effector cell that possess more than four Epitope-Binding Domains. No limitation is placed on the nature or number of epitopes or additional epitopes that may be bound by the molecules of the present invention other than that such additional binding capability does not prevent the molecule or Binding Domain thereof that is capable of binding to an epitope of CD3 from such binding and does not prevent the molecule or Binding Domain thereof that is capable of binding to an epitope of a Disease Antigen from such binding, so that the molecule(s) may mediate the redirected killing of the target cell.

V. Exemplary Binding Molecules

[00195] The present invention is directed to **DA x CD3 Binding Molecules** (*e.g.*, a diabody, a bispecific antibody, a bispecific, a trivalent molecule, a BiTe, a TandAb, *etc.*)

capable of binding to CD3 and a Disease Antigen, such as a Cancer Antigen or a Pathogen-Associated Antigen. Such Binding Molecules can be readily produced from the CDRs of antibodies and from the VL and VH Domains of antibodies. Listed below are exemplary antibodies that may be used to produce the Binding Molecules and combination therapy of the present invention.

A. Anti-CD3 Antibody CD3 mAb 1

[00196] The present invention employs variant CD3-Binding Domains (*i.e.*, vCD3-Binding Domains) that comprise the Light Chain Variable (VL) Domain and the Heavy Chain Variable (VH) Domain of anti-human CD3 antibodies, or CD3-binding portions thereof, and that mediate variant binding to CD3. As used herein, the term “variant binding” is intended to refer to the comparative binding exhibited by the CD3-Binding Domains of a reference antibody whose CDRs exhibit the highest sequence identity to the CDRs of the variant CD3-Binding Domain. The CD3-binding reference antibody for the illustrative vCD3-Binding Domains of the present invention is **CD3 mAb 1**, whose rCD3-Binding Domain is capable of binding human CD3 and CD3 of non-human primates (*e.g.*, cynomolgus monkey).

[00197] The amino acid sequence of the VH Domain of **CD3 mAb 1** (SEQ ID NO:55) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00198] The amino acid sequence of the VL Domain of **CD3 mAb 1** (SEQ ID NO:56) is shown below (CDR_L residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG

CD3 mAb 1		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

[00199] The rCD3-Binding Domain of “CD3 mAb 1” comprises a CD3 mAb 1 VH Domain having either aspartate (D) or glycine (G) at Kabat position 65, corresponding to residue 68 of SEQ ID NO:55) (*i.e.*, X in SEQ ID NO:55 is aspartate (D) or glycine (G)) and the VL Domain of CD3 mAb 1 (SEQ ID NO:56). Thus, for example, when such CD3 mAb 1 VH Domain has a glycine (G) as its residue 68, its sequence is SEQ ID NO:63, shown below (CDR_H residues are shown underlined, Kabat position 65 is shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKGR TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS

[00200] CD3-Binding Molecules that possess a vCD3-Binding Domain of the present invention may be recognized using a CTL assay in which:

- (1) a bispecific Cancer Antigen x CD3 diabody (for example, a CD123 x CD3 diabody or a 5T4 x CD3 diabody) potentially having a vCD3-Binding Domain, and
- (2) a bispecific Cancer Antigen x CD3 diabody having a corresponding rCD3-Binding Domain (*e.g.*, the rCD3-Binding Domain of CD3 mAb 1),

are separately incubated with effector Pan-T-cells (or PBMCs) and target tumor cells (*e.g.*, MOLM-13 or A498 cells), for example, at an effector:target cell ratio of 5:1 (or 15:1 for PBMCs) for 18, 24, or 42 hours, and the percentage cytotoxicity (*i.e.*, cell killing) and/or EC₅₀ is determined (for example, by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells using the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega)). In one embodiment, the release of IFN- γ , TNF- α , IL-6, and IL-2 cytokines may be determined at the end of the CTL assay. CD4⁺ and CD8⁺ T lymphocyte

populations may also be assessed for up-regulation of the activation markers CD69 and CD25 at the end of the CTL assay. A comparison of the percentage cytotoxicity and/or EC₅₀ for the bispecific Cancer Antigen x CD3 diabody potentially having a vCD3-Binding Domain with that of the Cancer Antigen x CD3 diabody having the rCD3-Binding Domains identifies vCD3-binding domains that exhibit the desired variant CD3 binding and/or reduced level of cytokine release.

[00201] CD3-Binding Molecules that possess a vCD3-Binding Domain of the present invention may alternatively be recognized using a binding assay in which:

- (1) a bispecific Cancer Antigen x CD3 diabody potentially having a vCD3-Binding Domain, and
- (2) a bispecific Cancer Antigen x CD3 diabody having an rCD3-Binding Domain (*e.g.*, the rCD3-Binding Domain of **CD3 mAb 1**),

are separately evaluated for their ability to bind to the surface of cells of tumor antigen-expressing cell lines (MOLM-13 or A498 cells) by FACS analysis. Briefly, cells are incubated with the diabody molecules (in FACS buffer containing 10% human AB serum) in microtiter plates. The cells are then washed and incubated with a labeled anti-human Fc secondary antibody or with a biotin-conjugated mouse anti-EK-coil antibody that recognizes the E-coil/K-coil (EK) Heterodimer-Promoting Domain of the diabodies, mixed with streptavidin-phycoerythrin. The cells are then washed and resuspended with FACS buffer and analyzed by flow cytometry and compared.

[00202] CD3-Binding Molecules that possess a vCD3-Binding Domain of the present invention may alternatively be recognized using, for example, a Co-Mix Xenograft Model such as NOD/SCID mice. In such an assay, the mice are injected with tumor cells (*e.g.*, KG1A (AML) cells) co-mixed with activated human CD4⁺ or CD8⁺ T-cells (E:T = 1:5). The bispecific Cancer Antigen x CD3 diabody potentially having a vCD3-Binding Domain or the Cancer Antigen x CD3 diabody having the rCD3-Binding Domain is injected into the animals and the extent of tumor growth is monitored and compared.

[00203] Alternatively, any one, two, or more than two of the exemplary variants of **CD3 mAb 1**, designated herein as “**CD3 mAb 1 M3**” – “**CD3 mAb 1 M26**” may be employed to provide the vCD3-Binding Domain of the **DA x CD3 Binding Molecules** of the present invention. The invention fully contemplates anti-CD3 antibodies having the VL and VH Domains of ant of **CD3 mAb 1 M3** – **CD3 mAb 1 M26** wherein the VH Domain possesses

an aspartate (D) at Kabat position 65 or a glycine (G) at Kabat position 65. The exemplary variants of **CD3 mAb 1**, **CD3 mAb 1 M3 – CD3 mAb 1 M26** possess vCD3-Binding Domains that comprise a CDR_{H1} Domain, a CDR_{H2} Domain, a CDR_{H3} Domain, a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, at least one of which differs in amino acid sequence from the amino acid sequence of the corresponding CDR of the rCD3-Binding Domain (**CD3 mAb 1**); and relative to a DA x CD3 Binding Domain comprising said rCD3-Binding Domain, a **DA x CD3 Binding Molecule** comprising said vCD3-Binding Domain binds CD3 with an altered affinity and is capable of mediating redirected killing and exhibit lower levels of cytokine release.

[00204] The amino acid sequences of preferred variant anti-CD3 VH Domains of the present invention are variants of **SEQ ID NO:55** and are represented by **SEQ ID NO:207** (CDR_H residues are shown underlined):

EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	<u>X₁X₂X₃</u> MNWVRQA
PGKGLEWVGR	<u>IRSKYNNYAT</u>	<u>YYADSVKX₄</u> RF	TISRDDSKNS
LYLQMNSLKT	EDTAVYYCVR	<u>HX₅NX₆X₇NSX₈ST</u>	<u>X₉FAX₁₀</u> WGQGTL
VTVSS			

wherein: **X₁** is T, D, or E; **X₂** is Y, D or T; **X₃** is A or G; **X₄** is D or G; **X₅** is G, D, E, or K; **X₆** is F or I; **X₇** is G or I; **X₈** is Y, A, G, or Q; **X₉** is W, F, or Y; and **X₁₀** is Y or E.

[00205] The amino acid sequences of preferred variant anti-CD3 VL Domains of the present invention are variants of **SEQ ID NO:56** and are represented by **SEQ ID NO:208** (CDR_L residues are shown underlined):

QAVVTQEPSL	TVSPGGTVTL	TC <u>RSSTGAVT</u>	<u>TSNYANWVQQ</u>	KPGQAPRGLI
G <u>X₁TNX₂RAP</u> WT	PARFSGSLLG	GKAALTITGA	QAEDEADYYC	<u>AX₃WYSNLWVF</u>
GGGTKLTVLG				

wherein: **X₁** is G or D; **X₂** is K or G; and **X₃** is L, E or Q.

B. Variant Anti-CD3 Antibodies

1. CD3 mAb 1 M1

[00206] **CD3 mAb 1 M1** is a low affinity variant of **CD3 mAb 1**, and is thus also referred to as “**CD3 mAb 1 Low**.” The amino acid sequence of the VH Domain of **CD3 mAb 1 M1** is shown below as **SEQ ID NO:64** (CDR_H residues are shown underlined). Relative to **SEQ ID NO:55**, **SEQ ID NO:64** contains an S100dT substitution (shown in double underline,

and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of **SEQ ID NO:64**, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVT WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00207] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M1** is **SEQ ID NO:56**.

CD3 mAb 1 M1		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYV <u>T</u> WFAY	SEQ ID NO:65
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

2. CD3 mAb 1 M2

[00208] **CD3 mAb 1 M2** has a faster off-rate than **CD3 mAb 1**, and is thus also referred to as “**CD3 mAb 1 Fast**.” The amino acid sequence of the VH Domain of **CD3 mAb 1 M2** is shown below as **SEQ ID NO:66** (CDR_H residues are shown underlined). Relative to **SEQ ID NO:55**, **SEQ ID NO:66** contains G96K and S100dT substitutions, numbered as in Kabat (sequence residue 110, shown in double underline); additionally, position 65, numbered as in Kabat, of **SEQ ID NO:66**, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSYVT WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00209] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M2** is **SEQ ID NO:56**.

CD3 mAb 1 M2		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>K</u> NFGNSYV <u>T</u> WFAY	SEQ ID NO:67
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

3. CD3 mAb 1 M3

[00210] The amino acid sequence of the VH Domain of CD3 mAb 1 M3 (SEQ ID NO:68) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:68 contains a G99I substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:68, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFINSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00211] A preferred amino acid sequence of the VL Domain of CD3 mAb 1 M3 is SEQ ID NO:56.

CD3 mAb 1 M3		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNF <u>I</u> NSYVSWFAY	SEQ ID NO:69
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

4. CD3 mAb 1 M4

[00212] The amino acid sequence of the VH Domain of **CD3 mAb 1 M4** (SEQ ID NO:70) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:70 contains a Y100bA substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:70, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSAVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00213] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M4** is SEQ ID NO:56.

CD3 mAb 1 M4		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNS <u>AVS</u> WFAY	SEQ ID NO:71
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

5. CD3 mAb 1 M5

[00214] The amino acid sequence of the VH Domain of **CD3 mAb 1 M5** (SEQ ID NO:72) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:72 contains a Y100bG substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:72, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSGVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00215] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M5** is SEQ ID NO:56.

CD3 mAb 1 M5		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNS <u>G</u> VSWFAY	SEQ ID NO:73
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

6. CD3 mAb 1 M6

[00216] The amino acid sequence of the VH Domain of **CD3 mAb 1 M6** (SEQ ID NO:74) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:74 contains a Y100bQ substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:74, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSQVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00217] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M6** is SEQ ID NO:56.

CD3 mAb 1 M6		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNS <u>Q</u> VSWFAY	SEQ ID NO:75
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

7. CD3 mAb 1 M7

[00218] The amino acid sequence of the VH Domain of **CD3 mAb 1 M7** (SEQ ID NO:76) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:76 contains a G96D substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:76, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HDNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00219] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M7** is SEQ ID NO:56.

CD3 mAb 1 M7		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	<u>H</u> <u>D</u> NFGNSYVSWFAY	SEQ ID NO:77
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

8. CD3 mAb 1 M8

[00220] The amino acid sequence of the VH Domain of **CD3 mAb 1 M8** (SEQ ID NO:78) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:78 contains a G99E substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:78, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HENFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00221] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M8** is SEQ ID NO:56.

CD3 mAb 1 M8		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>EN</u> FGNSYVSWFAY	SEQ ID NO:79
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

9. CD3 mAb 1 M9

[00222] The amino acid sequence of the VH Domain of CD3 mAb 1 M9 (SEQ ID NO:80) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:80 contains a G99K substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:80, also shown in double underline, may be aspartate (D) or glycine (G)):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSYVS WFAYWGQGTL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00223] A preferred amino acid sequence of the VL Domain of CD3 mAb 1 M9 is SEQ ID NO:56.

CD3 mAb 1 M9		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>KN</u> FGNSYVSWFAY	SEQ ID NO:81
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

10. CD3 mAb 1 M10

[00224] The amino acid sequence of the VH Domain of **CD3 mAb 1 M10** (SEQ ID NO:82) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:82 contains a F98I substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:82, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNIGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00225] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M10** is SEQ ID NO:56.

CD3 mAb 1 M10		
CDR	Sequence	SEQ ID NO
CDR _{H1}	TYAMN	SEQ ID NO:57
CDR _{H2}	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _{H3}	HGN <u>I</u> GNSYVSWFAY	SEQ ID NO:83
CDR _{L1}	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _{L2}	GTNKRAP	SEQ ID NO:61
CDR _{L3}	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

11. CD3 mAb 1 M11

[00226] The amino acid sequence of the VH Domain of **CD3 mAb 1 M11** (SEQ ID NO:84) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:84 contains a W100eF substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:84, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS FFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00227] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M11** is SEQ ID NO:56.

CD3 mAb 1 M11		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVS <u>F</u> FAY	SEQ ID NO:85
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

12. CD3 mAb 1 M12

[00228] The amino acid sequence of the VH Domain of **CD3 mAb 1 M12** (SEQ ID NO:86) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:86 contains a W100eY substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:86, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS YFAYWGQGTL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00229] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M12** is SEQ ID NO:56.

CD3 mAb 1 M12		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVS <u>Y</u> FAY	SEQ ID NO:87
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

13. CD3 mAb 1 M13

[00230] The amino acid sequence of the VH Domain of **CD3 mAb 1 M13** (SEQ ID NO:88) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:88 contains a Y102E substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:88, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAEWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00231] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M13** is SEQ ID NO:56.

CD3 mAb 1 M13		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFA <u>E</u>	SEQ ID NO:89
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

14. CD3 mAb 1 M14

[00232] The amino acid sequence of the VH Domain of **CD3 mAb 1 M14** (SEQ ID NO:90) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:90 contains a T31D substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:90, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00233] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M14** is SEQ ID NO:56.

CD3 mAb 1 M14		
CDR	Sequence	SEQ ID NO
CDR _H 1	<u>D</u> YAMN	SEQ ID NO:91
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

15. CD3 mAb 1 M15

[00234] The amino acid sequence of the VH Domain of **CD3 mAb 1 M15** (SEQ ID NO:92) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:92 contains a T31E substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:92, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS EYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00235] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M15** is SEQ ID NO:56.

CD3 mAb 1 M15		
CDR	Sequence	SEQ ID NO
CDR _H 1	<u>E</u> YAMN	SEQ ID NO:93
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

16. CD3 mAb 1 M16

[00236] The amino acid sequence of the VH Domain of **CD3 mAb 1 M16** (SEQ ID NO:94) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:94 contains a Y32D substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:94, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TDAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00237] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M16** is SEQ ID NO:56.

CD3 mAb 1 M16		
CDR	Sequence	SEQ ID NO
CDR _H 1	<u>T</u> DAMN	SEQ ID NO:95
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

17. CD3 mAb 1 M17

[00238] The amino acid sequence of the VH Domain of **CD3 mAb 1 M17** (SEQ ID NO:96) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:96 contains a Y32T substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:96, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TTAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00239] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M17** is SEQ ID NO:56.

CD3 mAb 1 M17		
CDR	Sequence	SEQ ID NO
CDR _H 1	T <u>T</u> AMN	SEQ ID NO:97
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

18. CD3 mAb 1 M18

[00240] The amino acid sequence of the VH Domain of **CD3 mAb 1 M18** (SEQ ID NO:98) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:98 contains a A33G substitution (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:98, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYGMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGT L VTVSS
 wherein X is aspartate (D) or glycine (G)

[00241] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M18** is SEQ ID NO:56.

CD3 mAb 1 M18		
CDR	Sequence	SEQ ID NO
CDR _H 1	TY <u>G</u> MN	SEQ ID NO:99
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

19. CD3 mAb 1 M19

[00242] The amino acid sequence of the VH Domain of **CD3 mAb 1 M19** (SEQ ID NO:100) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:100 contains G96K and F98I substitutions (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:100, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNIGNSYVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00243] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M19** is SEQ ID NO:56.

CD3 mAb 1 M19		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	<u>HKNIGNSYVS</u> WFAY	SEQ ID NO:101
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

20. CD3 mAb 1 M20

[00244] The amino acid sequence of the VH Domain of **CD3 mAb 1 M20** (SEQ ID NO:102) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:102 contains G96K and Y100bG substitutions (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:102, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSGVS WFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00245] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M20** is SEQ ID NO:56.

CD3 mAb 1 M20		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>K</u> NFGNS <u>G</u> VSWFAY	SEQ ID NO:103
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

21. CD3 mAb 1 M21

[00246] The amino acid sequence of the VH Domain of **CD3 mAb 1 M21** (SEQ ID NO:104) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:104 contains G96K and W100eF substitutions (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:104, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSYVS FFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00247] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M21** is SEQ ID NO:56.

CD3 mAb 1 M21		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>K</u> NFGNSYVS <u>F</u> FAY	SEQ ID NO:105
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

22. CD3 mAb 1 M22

[00248] The amino acid sequence of the VH Domain of **CD3 mAb 1 M22** (SEQ ID NO:106) is shown below (CDR_H residues are shown underlined). Relative to SEQ ID NO:55, SEQ ID NO:106 contains G96K and W100Y substitutions (shown in double underline, and numbered as in Kabat); additionally, position 65, numbered as in Kabat, of SEQ ID NO:106, also shown in double underline, may be aspartate (D) or glycine (G):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKXRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSYVS YFAYWGQGTLL VTVSS
 wherein X is aspartate (D) or glycine (G)

[00249] A preferred amino acid sequence of the VL Domain of **CD3 mAb 1 M22** is SEQ ID NO:56.

CD3 mAb 1 M22		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	H <u>K</u> NFGNSYVS <u>Y</u> FAY	SEQ ID NO:107
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTNKRAP	SEQ ID NO:61
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

23. CD3 mAb 1 M23

[00250] A preferred amino acid sequence of the VH Domain of **CD3 mAb 1 M23** is SEQ ID NO:55 or SEQ ID NO:63.

[00251] The amino acid sequence of the VL Domain of **CD3 mAb 1 M23** (SEQ ID NO:108) is shown below (CDR_L residues are shown underlined). Relative to SEQ ID NO:56, SEQ ID NO:108 contains an L95E substitution (shown in double underline, and numbered as in Kabat):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNKRAPWT PARFSGSLLG GKAAALTITGA QAEDADYYC AEWYSNLWVF
 GGGTKLTVLG

CD3 mAb 1 M23		
CDR	Sequence	SEQ ID NO
CDR _{H1}	TYAMN	SEQ ID NO:57
CDR _{H2}	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _{H3}	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _{L1}	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _{L2}	GTNKRAP	SEQ ID NO:61
CDR _{L3}	A <u>E</u> WYSNLWV	SEQ ID NO:109
wherein <u>X</u> is aspartate (D) or glycine (G)		

24. CD3 mAb 1 M24

[00252] A preferred amino acid sequence of the VH Domain of **CD3 mAb 1 M24** is **SEQ ID NO:55** or **SEQ ID NO:63**.

[00253] The amino acid sequence of the VL Domain of **CD3 mAb 1 M24** (**SEQ ID NO:110**) is shown below (CDR_L residues are shown underlined). Relative to **SEQ ID NO:56**, **SEQ ID NO:110** contains an L95Q substitution (shown in double underline, and numbered as in Kabat):

QAVVTQEPSL TVSPGGTVTL TCRRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC AQWYSNLWVF
 GGGTKLTVLG

CD3 mAb 1 M24		
CDR	Sequence	SEQ ID NO
CDR _{H1}	TYAMN	SEQ ID NO:57
CDR _{H2}	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _{H3}	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _{L1}	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _{L2}	GTNKRAP	SEQ ID NO:61
CDR _{L3}	A <u>Q</u> WYSNLWV	SEQ ID NO:111
wherein <u>X</u> is aspartate (D) or glycine (G)		

25. CD3 mAb 1 M25

[00254] A preferred amino acid sequence of the VH Domain of **CD3 mAb 1 M25** is **SEQ ID NO:55** or **SEQ ID NO:63**.

[00255] The amino acid sequence of the VL Domain of **CD3 mAb 1 M25** (**SEQ ID NO:112**) is shown below (CDRL residues are shown underlined). Relative to **SEQ ID NO:56**, **SEQ ID NO:112** contains a G50D substitution (shown in double underline, and numbered as in Kabat):

QAVVTQEPSL TVSPGGTVTL TCRRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GDTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG

CD3 mAb 1 M25		
CDR	Sequence	SEQ ID NO
CDRH1	TYAMN	SEQ ID NO:57
CDRH2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDRH3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDRL1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDRL2	<u>D</u> TNKRAP	SEQ ID NO:113
CDRL3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

26. CD3 mAb 1 M26

[00256] A preferred amino acid sequence of the VH Domain of **CD3 mAb 1 M26** is **SEQ ID NO:55** or **SEQ ID NO:63**.

[00257] The amino acid sequence of the VL Domain of **CD3 mAb 1 M26** (**SEQ ID NO:114**) is shown below (CDRL residues are shown underlined). Relative to **SEQ ID NO:56**, **SEQ ID NO:114** contains a K53G substitution (shown in double underline):

QAVVTQEPSL TVSPGGTVTL TCRRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNGRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG

CD3 mAb 1 M26		
CDR	Sequence	SEQ ID NO
CDR _H 1	TYAMN	SEQ ID NO:57
CDR _H 2	RIRSKYNNYATYYADSVK <u>X</u>	SEQ ID NO:58
CDR _H 3	HGNFGNSYVSWFAY	SEQ ID NO:59
CDR _L 1	RSSTGAVTTSNYAN	SEQ ID NO:60
CDR _L 2	GTN <u>G</u> RAP	SEQ ID NO:115
CDR _L 3	ALWYSNLWV	SEQ ID NO:62
wherein <u>X</u> is aspartate (D) or glycine (G)		

C. Exemplary Antibodies That Bind to Cell Surface Molecules of An Effector Cell

[00258] As used herein, the term “**effector cell**” denotes a cell that directly or indirectly mediates the killing of target cells (e.g., foreign cells, infected cells or cancer cells). Examples of effector cells include helper T-cells, cytotoxic T-cells, Natural Killer (NK) cells, plasma cells (antibody-secreting B cells), macrophages and granulocytes. Preferred cell surface molecules of such cells include CD2, CD3, CD8, CD16, TCR, and the NKG2D receptor. Accordingly, molecules capable of immunospecifically binding an epitope of such molecules, or to other effector cell surface molecules may be used in accordance with the principles of the present invention. Exemplary antibodies, whose VH and VL Domains may be used to construct molecules capable of mediating the redirected killing of a target cell are provided below.

1. Exemplary Anti-CD2 Antibodies

[00259] In one embodiment, the molecules of the present invention that are capable of mediating the redirected killing of a target cell will bind an effector cell by immunospecifically binding an epitope of CD2 present on the surface of such effector cell. Molecules that specifically bind CD2 include the anti-CD2 antibody “**CD2 mAb Lo-CD2a.**”

[00260] The amino acid sequence of the VH Domain of **CD2 mAb Lo-CD2a** (ATCC Accession No: 11423); **SEQ ID NO:116**) is shown below (CDR_H residues are shown underlined):

EVQLQQSGPE LQRPGLASVKL SCKASGYIFT EYYMYWVKQR PKQGLELVGR
IDPEDGSIDY VEKFKKKATL TADTSSNTAY MQLSSLTSED TATYFCARGK
FNYRFAYWGQ GTLVTVSS

[00261] The amino acid sequence of the VL Domain of **CD2 mAb Lo-CD2a** (ATCC Accession No: 11423; **SEQ ID NO:117**) is shown below (CDRL residues are shown underlined):

DVVLITQTPPT LLATIGQSVS ISCRSSQSLL HSSGNTYLNW LLQRTGQSPQ
 PLIYLVSKLE SGVPNRFSGS GSGTDFTLKI SGVEAEDLGV YYCMQFTHYP
YTFGAGTKLE LK

2. Exemplary Anti-CD8 Antibodies

[00262] In one embodiment, the molecules of the present invention that are capable of mediating the redirected killing of a target cell will bind an effector cell by immunospecifically binding an epitope of CD8 present on the surface of such effector cell. Antibodies that specifically bind CD8 include the anti-CD8 antibodies “**OKT8**” and “**TRX2**.”

[00263] The amino acid sequence of the VH Domain of **OKT8** (**SEQ ID NO:118**) is shown below (CDRH residues are shown underlined):

QVQLLESQPE LLKPGASVKM SCKASSGYTFT DYNMHWVKQS HGKSLEWIGY
IYPYTGGTGY NQKFKNKATL TVDSSSSTAY MELRSLTSED SAVYYCARNF
 RYTYWYFDVW GQGTTVTSS

[00264] The amino acid sequence of the VL Domain of **OKT8** (**SEQ ID NO:119**) is shown below (CDRL residues are shown underlined):

DIVMTQSPAS LAVSLGQRAT ISCRASESVD SYDNSLMHWY QQKPGQPPKV
 LIYLASNLES GVPARFSGSG SRTDFTLTID PVEADDAATY YCQQNNEDPY
TFGGGTKLEI KR

[00265] The amino acid sequence of the VH Domain of **TRX2** (**SEQ ID NO:120**) is shown below (CDRH residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS DEGMNWVRQA PGKGLEWVAL
IYYDGSNKFY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKPH
YDGYHFFDS WGQGLVTVS S

[00266] The amino acid sequence of the VL Domain of **TRX2** (**SEQ ID NO:121**) is shown below (CDRL residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCKGSQDIN NYLAWYQQK GKAPKLLIYN
TDILHTGVPS RFGSGSGTD FTFTISLQP EDIATYYCYQ YNNGYTFFGQ
 TKVEIK

VI. Exemplary Cancer and Pathogen-Associated Antigens

A. Exemplary Cancer Antigens Arrayed on the Surface of Cancer Cells

[00267] As used herein, the term “**Cancer Antigen**” denotes an antigen that is characteristically expressed on the surface of a cancer cell, and that may thus be treated with an Antibody-Based Molecule or an Immunomodulatory Molecule. Examples of Cancer Antigens include, but are not limited to: **19.9** as found in colon cancer, gastric cancer mucins; **4.2**; **ADAM-9** (United States Patent Publication No. 2006/0172350; PCT Publication No. WO 06/084075); **AH6** as found in gastric cancer; **ALCAM** (PCT Publication No. WO 03/093443); **APO-1 (malignant human lymphocyte antigen)** (Trauth, B.C. *et al.* (1989) “*Monoclonal Antibody-Mediated Tumor Regression By Induction Of Apoptosis*,” *Science* 245:301-304); **B1** (Egloff, A.M. *et al.* (2006) “*Cyclin B1 And Other Cyclins As Tumor Antigens In Immunosurveillance And Immunotherapy Of Cancer*,” *Cancer Res.* 66(1):6-9); **B7-H3** (Collins, M. *et al.* (2005) “*The B7 Family Of Immune-Regulatory Ligands*,” *Genome Biol.* 6:223.1-223.7). Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- γ Production*,” *Nature Immunol.* 2:269–274; Sun, M. *et al.* (2002) “*Characterization of Mouse and Human B7-H3 Genes*,” *J. Immunol.* 168:6294-6297); **BAGE** (Bodey, B. (2002) “*Cancer-Testis Antigens: Promising Targets For Antigen Directed Antineoplastic Immunotherapy*,” *Expert Opin. Biol. Ther.* 2(6):577-584); **beta-catenin** (Prange W. *et al.* (2003) “*Beta-Catenin Accumulation In The Progression Of Human Hepatocarcinogenesis Correlates With Loss Of E-Cadherin And Accumulation Of P53, But Not With Expression Of Conventional WNT-1 Target Genes*,” *J. Pathol.* 201(2):250-259); **blood group ALe^b/Le^y** as found in colonic adenocarcinoma; **Burkitt’s lymphoma antigen-38.13**; **C14** as found in colonic adenocarcinoma; **CA125 (ovarian carcinoma antigen)** (Bast, R.C. Jr. *et al.* (2005) “*New Tumor Markers: CA125 And Beyond*,” *Int. J. Gynecol. Cancer* 15(Suppl 3):274-281; Yu *et al.* (1991) “*Coexpression Of Different Antigenic Markers On Moieties That Bear CA 125 Determinants*,” *Cancer Res.* 51(2):468-475); **Carboxypeptidase M** (United States Patent Publication No. 2006/0166291); **CD5** (Calin, G.A. *et al.* (2006) “*Genomics Of Chronic Lymphocytic Leukemia MicroRNAs As New Players With Clinical Significance*,” *Semin. Oncol.* 33(2):167-173; **CD19** (Ghetie *et al.* (1994) “*Anti-CD19 Inhibits The Growth Of Human B-Cell Tumor Lines In Vitro And Of Daudi Cells In SCID Mice By Inducing Cell Cycle Arrest*,” *Blood* 83:1329-1336; Troussard, X. *et al.* 1998 *Hematol Cell Ther.*

40(4):139-48); **CD20** (Reff *et al.* (1994) “*Depletion Of B Cells In Vivo By A Chimeric Mouse Human Monoclonal Antibody To CD20*,” Blood 83:435-445; Thomas, D.A. *et al.* 2006 Hematol Oncol Clin North Am. 20(5):1125-36); **CD22** (Kreitman, R.J. (2006) “*Immunotoxins For Targeted Cancer Therapy*,” AAPS J. 8(3):E532-51); **CD23** (Rosati, S. *et al.* (2005) “*Chronic Lymphocytic Leukaemia: A Review Of The Immuno-Architecture*,” Curr. Top. Microbiol. Immunol. 294:91-107); **CD25** (Troussard, X. *et al.* (1998) “*Hairy Cell Leukemia. What Is New Forty Years After The First Description?*” Hematol. Cell. Ther. 40(4):139-148); **CD27** (Bataille, R. (2006) “*The Phenotype Of Normal, Reactive And Malignant Plasma Cells. Identification Of "Many And Multiple Myelomas" And Of New Targets For Myeloma Therapy*,” Haematologica 91(9):1234-1240); **CD28** (Bataille, R. (2006) “*The Phenotype Of Normal, Reactive And Malignant Plasma Cells. Identification Of "Many And Multiple Myelomas" And Of New Targets For Myeloma Therapy*,” Haematologica 91(9):1234-1240); **CD33** (Sgouros *et al.* (1993) “*Modeling And Dosimetry Of Monoclonal Antibody M195 (Anti-CD33) In Acute Myelogenous Leukemia*,” J. Nucl. Med. 34:422-430); **CD36** (Ge, Y. (2005) “*CD36: A Multiligand Molecule*,” Lab Hematol. 11(1):31-7); **CD40/CD154** (Messmer, D. *et al.* (2005) “*CD154 Gene Therapy For Human B-Cell Malignancies*,” Ann. N. Y. Acad. Sci. 1062:51-60); **CD45** (Jurcic, J.G. (2005) “*Immunotherapy For Acute Myeloid Leukemia*,” Curr. Oncol. Rep. 7(5):339-346); **CD56** (Bataille, R. (2006) “*The Phenotype Of Normal, Reactive And Malignant Plasma Cells. Identification Of "Many And Multiple Myelomas" And Of New Targets For Myeloma Therapy*,” Haematologica 91(9):1234-1240); **CD46** (United States Patent No. 7,148,038; PCT Publication No. WO 03/032814); **CD52** (Eketorp, S.S. *et al.* (2014) “*Alemtuzumab (Anti-CD52 Monoclonal Antibody) As Single-Agent Therapy In Patients With Relapsed/Refractory Chronic Lymphocytic Leukaemia (CLL)-A Single Region Experience On Consecutive Patients*,” Ann Hematol. 93(10):1725-1733; Suresh, T. *et al.* (2014) “*New Antibody Approaches To Lymphoma Therapy*,” J. Hematol. Oncol. 7:58; Hoelzer, D. (2013) “*Targeted Therapy With Monoclonal Antibodies In Acute Lymphoblastic Leukemia*,” Curr. Opin. Oncol. 25(6):701-706); **CD56** (Bataille, R. (2006) “*The Phenotype Of Normal, Reactive And Malignant Plasma Cells. Identification Of "Many And Multiple Myelomas" And Of New Targets For Myeloma Therapy*,” Haematologica 91(9):1234-1240); **CD79a/CD79b** (Troussard, X. *et al.* (1998) “*Hairy Cell Leukemia. What Is New Forty Years After The First Description?*” Hematol. Cell. Ther. 40(4):139-148; Chu, P.G. *et al.* (2001) “*CD79: A Review*,” Appl. Immunohistochem. Mol. Morphol. 9(2):97-106); **CD103** (Troussard, X. *et al.* (1998) “*Hairy Cell Leukemia. What Is New Forty Years After The First*

Description?" Hematol. Cell. Ther. 40(4):139-148); **CD317** (Kawai, S. *et al.* (2008) "Interferon- α Enhances CD317 Expression And The Antitumor Activity Of Anti-CD317 Monoclonal Antibody In Renal Cell Carcinoma Xenograft Models," Cancer Science 99(12):2461-2466; Wang, W. *et al.* (2009) *HM1.24 (CD317) Is A Novel Target Against Lung Cancer For Immunotherapy Using Anti-HM1.24 Antibody*," Cancer Immunology, Immunotherapy 58(6):967-976; Wang, W. *et al.* (2009) "Chimeric And Humanized Anti-HM1.24 Antibodies Mediate Antibody-Dependent Cellular Cytotoxicity Against Lung Cancer Cells. Lung Cancer," 63(1):23-31; Sayeed, A. *et al.* (2013) "Aberrant Regulation Of The BST2 (Tetherin) Promoter Enhances Cell Proliferation And Apoptosis Evasion In High Grade Breast Cancer Cells," PLoS ONE 8(6):e67191, pp. 1-10); **CDK4** (Lee, Y.M. *et al.* (2006) "Targeting Cyclins And Cyclin-Dependent Kinases In Cancer: Lessons From Mice, Hopes For Therapeutic Applications In Human," Cell Cycle 5(18):2110-2114); **CEA** (carcinoembryonic antigen; Foon *et al.* (1995) "Immune Response To The Carcinoembryonic Antigen In Patients Treated With An Anti-Idiotypic Antibody Vaccine," J. Clin. Invest. 96(1):334-42); Mathelin, C. (2006) "Circulating Proteinic Biomarkers And Breast Cancer," Gynecol. Obstet. Fertil. 34(7-8):638-646; Tellez-Avila, F.I. *et al.* (2005) "The Carcinoembryonic Antigen: Apropos Of An Old Friend," Rev. Invest. Clin. 57(6):814-819); **CEACAM5/CEACAM6** (Zheng, C. *et al.* (2011) "A Novel Anti-CEACAM5 Monoclonal Antibody, CC4, Suppresses Colorectal Tumor Growth and Enhances NK Cells-Mediated Tumor Immunity," PLoS One 6(6):e21146, pp. 1-11); **CO17-1A** (Ragnhammar *et al.* (1993) "Effect Of Monoclonal Antibody 17-1A And GM-CSF In Patients With Advanced Colorectal Carcinoma - Long-Lasting, Complete Remissions Can Be Induced," Int. J. Cancer 53:751-758); **CO-43** (blood group Le^b); **CO-514** (blood group Le^a) as found in adenocarcinoma; **CTA-1**; **CTLA-4** (Peggs, K.S. *et al.* (2006) "Principles And Use Of Anti-CTLA4 Antibody In Human Cancer Immunotherapy," Curr. Opin. Immunol. 18(2):206-13); **Cytokeratin 8** (PCT Publication No. WO 03/024191); **D1.1**; **D156-22**; **DR5** (Abdulghani, J. *et al.* (2010) "TRAIL Receptor Signaling And Therapeutics," Expert Opin. Ther. Targets 14(10):1091-1108; Andera, L. (2009) "Signaling Activated By The Death Receptors Of The TNFR Family," Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 153(3):173-180; Carlo-Stella, C. *et al.* (2007) "Targeting TRAIL Agonistic Receptors for Cancer Therapy," Clin. Cancer 13(8):2313-2317; Chaudhari, B.R. *et al.* (2006) "Following the TRAIL to Apoptosis," Immunologic Res. 35(3):249-262); **E1 series** (blood group B) as found in pancreatic cancer; **EGFR** (Epidermal Growth Factor Receptor; Adenis, A. *et al.* (2003) "Inhibitors Of Epidermal Growth Factor Receptor And Colorectal Cancer," Bull.

Cancer. 90 Spec No:S228-S232); **Ephrin receptors** (and in particular **EphA2** (United States Patent No. 7,569,672; PCT Publication No. WO 06/084226); **Erb** (ErbB1; ErbB3; ErbB4; Zhou, H. *et al.* (2002) “*Lung Tumorigenesis Associated With Erb-B-2 And Erb-B-3 Overexpression In Human Erb-B-3 Transgenic Mice Is Enhanced By Methylnitrosourea*,” *Oncogene* 21(57):8732-8740; Rimon, E. *et al.* (2004) “*Gonadotropin-Induced Gene Regulation In Human Granulosa Cells Obtained From IVF Patients: Modulation Of Genes Coding For Growth Factors And Their Receptors And Genes Involved In Cancer And Other Diseases*,” *Int. J. Oncol.* 24(5):1325-1338); **GAGE** (GAGE-1; GAGE-2; Akcakanat, A. *et al.* (2006) “*Heterogeneous Expression Of GAGE, NY-ESO-1, MAGE-A and SSX Proteins In Esophageal Cancer: Implications For Immunotherapy*,” *Int. J. Cancer.* 118(1):123-128); **GD2/GD3/GM2** (Livingston, P.O. *et al.* (2005) “*Selection Of GM2, Fucosyl GM1, Globo H And Polysialic Acid As Targets On Small Cell Lung Cancers For Antibody-Mediated Immunotherapy*,” *Cancer Immunol. Immunother.* 54(10):1018-1025); **ganglioside GD2** (GD₂; Saleh *et al.* (1993) “*Generation Of A Human Anti-Idiotypic Antibody That Mimics The GD2 Antigen*,” *J. Immunol.*, 151, 3390-3398); **ganglioside GD3** (GD₃; Shitara *et al.* (1993) “*A Mouse/Human Chimeric Anti-(Ganglioside GD3) Antibody With Enhanced Antitumor Activities*,” *Cancer Immunol. Immunother.* 36:373-380); **ganglioside GM2** (GM₂; Livingston *et al.* (1994) “*Improved Survival In Stage III Melanoma Patients With GM2 Antibodies: A Randomized Trial Of Adjuvant Vaccination With GM2 Ganglioside*,” *J. Clin. Oncol.* 12:1036-1044); **ganglioside GM3** (GM₃; Hoon *et al.* (1993) “*Molecular Cloning Of A Human Monoclonal Antibody Reactive To Ganglioside GM3 Antigen On Human Cancers*,” *Cancer Res.* 53:5244-5250); **GICA 19-9** (Herlyn *et al.* (1982) “*Monoclonal Antibody Detection Of A Circulating Tumor-Associated Antigen. I. Presence Of Antigen In Sera Of Patients With Colorectal, Gastric, And Pancreatic Carcinoma*,” *J. Clin. Immunol.* 2:135-140); **gp100** (Lotem, M. *et al.* (2006) “*Presentation Of Tumor Antigens By Dendritic Cells Genetically Modified With Viral And Nonviral Vectors*,” *J. Immunother.* 29(6):616-27); **Gp37** (human leukemia T-cell antigen; Bhattacharya-Chatterjee *et al.* (1988) “*Idiotypic Vaccines Against Human T Cell Leukemia. II. Generation And Characterization Of A Monoclonal Idiotypic Cascade (Ab1, Ab2, and Ab3)*,” *J. Immunol.* 141:1398-1403); **gp75** (melanoma antigen; Vijayasardahl *et al.* (1990) “*The Melanoma Antigen Gp75 Is The Human Homologue Of The Mouse B (Brown) Locus Gene Product*,” *J. Exp. Med.* 171(4):1375-1380); **gpA33** (Heath, J.K. *et al.* (1997) “*The Human A33 Antigen Is A Transmembrane Glycoprotein And A Novel Member Of The Immunoglobulin Superfamily*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 94(2):469-474; Ritter, G. *et*

al. (1997) "Characterization Of Posttranslational Modifications Of Human A33 Antigen, A Novel Palmitoylated Surface Glycoprotein Of Human Gastrointestinal Epithelium," Biochem. Biophys. Res. Commun. 236(3):682-686; Wong, N.A. *et al.* (2006) "EpCAM and gpA33 Are Markers Of Barrett's Metaplasia," J. Clin. Pathol. 59(3):260-263; Almqvist, Y. (2006) "In vitro and in vivo Characterization of 177Lu-huA33: A Radioimmunoconjugate Against Colorectal Cancer," Nucl. Med. Biol. 33(8):991-998); **HER2 antigen** (HER2/neu, p185^{HER2}; Pal, S.K. *et al.* (2006) "Targeting HER2 Epitopes," Semin. Oncol. 33(4):386-391); **HMFG** (human milk fat globule antigen; WO1995015171); **Human Papillomavirus-E6/Human Papillomavirus-E7** (DiMaio, D. *et al.* (2006) "Human Papillomaviruses And Cervical Cancer," Adv. Virus Res. 66:125-59; **HMW-MAA** (high molecular weight melanoma antigen; Natali *et al.* (1987) "Immunohistochemical Detection Of Antigen In Human Primary And Metastatic Melanomas By The Monoclonal Antibody 140.240 And Its Possible Prognostic Significance," Cancer 59:55-63; Mittelman *et al.* (1990) "Active Specific Immunotherapy In Patients With Melanoma. A Clinical Trial With Mouse Antiidiotypic Monoclonal Antibodies Elicited With Syngeneic Anti-High-Molecular-Weight-Melanoma-Associated Antigen Monoclonal Antibodies," J. Clin. Invest. 86:2136-2144); **I antigen** (differentiation antigen; Feizi (1985) "Demonstration By Monoclonal Antibodies That Carbohydrate Structures Of Glycoproteins And Glycolipids Are Onco-Developmental Antigens," Nature 314:53-57); **IL13Rα2** (PCT Publication No. WO 2008/146911; Brown, C.E. *et al.* (2013) "Glioma IL13Rα2 Is Associated With Mesenchymal Signature Gene Expression And Poor Patient Prognosis," PLoS One. 18;8(10):e77769; Barderas, R. *et al.* (2012) "High Expression Of IL-13 Receptor A2 In Colorectal Cancer Is Associated With Invasion, Liver Metastasis, And Poor Prognosis," Cancer Res. 72(11):2780-2790; Kasaian, M.T. *et al.* (2011) "IL-13 Antibodies Influence IL-13 Clearance In Humans By Modulating Scavenger Activity Of IL-13Rα2," J. Immunol. 187(1):561-569; Bozinov, O. *et al.* (2010) "Decreasing Expression Of The Interleukin-13 Receptor IL-13Rα2 In Treated Recurrent Malignant Gliomas," Neurol. Med. Chir. (Tokyo) 50(8):617-621; Fujisawa, T. *et al.* (2009) "A novel role of interleukin-13 receptor alpha2 in pancreatic cancer invasion and metastasis," Cancer Res. 69(22):8678-8685); **Integrin β6** (PCT Publication No. WO 03/087340); **JAM-3** (PCT Publication No. WO 06/084078); **KID3** (PCT Publication No. WO 05/028498); **KID31** (PCT Publication No. WO 06/076584); **KS 1/4 pan-carcinoma antigen** (Perez *et al.* (1989) "Isolation And Characterization Of A cDNA Encoding The Ks1/4 Epithelial Carcinoma Marker," J. Immunol. 142:3662-3667; Möller *et al.* (1991) "Bi-specific-Monoclonal-Antibody-Directed

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phosphate (Tailor *et al.* (1990) "Nucleotide Sequence Of Human Prostatic Acid Phosphatase Determined From A Full-Length cDNA Clone," Nucl. Acids Res. 18(16):4928); **R₂₄** as found in melanoma; **ROR1** (United States Patent No. 5,843,749); **sphingolipids**; **SSEA-1**; **SSEA-3**; **SSEA-4**; **sTn** (Holmberg, L.A. (2001) "Theratope Vaccine (sTn-KLH)," Expert Opin. Biol. Ther. 1(5):881-91); **T-cell receptor derived peptide** from a cutaneous T-cell lymphoma (see Edelson (1998) "Cutaneous T-Cell Lymphoma: A Model For Selective Immunotherapy," Cancer J. Sci. Am. 4:62-71); **T_{5A7}** found in myeloid cells; **TAG-72** (Yokota *et al.* (1992) "Rapid Tumor Penetration Of A Single-Chain Fv And Comparison With Other Immunoglobulin Forms," Cancer Res. 52:3402-3408); **TL5** (blood group A); **TNF-receptor** (TNF- α receptor, TNF- β receptor; **TNF- γ receptor** (van Horssen, R. *et al.* (2006) "TNF-Alpha In Cancer Treatment: Molecular Insights, Antitumor Effects, And Clinical Utility," Oncologist 11(4):397-408; Gardnerova, M. *et al.* (2000) "The Use Of TNF Family Ligands And Receptors And Agents Which Modify Their Interaction As Therapeutic Agents," Curr. Drug Targets 1(4):327-364); **TRA-1-85** (blood group H); **Transferrin Receptor** (United States Patent No. 7,572,895; PCT Publication No. WO 05/121179); **5T4** (TPBG, trophoblast glycoprotein; Boghaert, E.R. *et al.* (2008) "The Oncofetal Protein, 5T4, Is A Suitable Target For Antibody-Guided Anti-Cancer Chemotherapy With Calicheamicin," Int. J. Oncol. 32(1):221-234; Eisen, T. *et al.* (2014) "Naptumomab Estafenatox: Targeted Immunotherapy with a Novel Immunotoxin," Curr. Oncol. Rep. 16:370, pp. 1-6); **TSTA (tumor-specific transplantation antigen)** such as virally-induced tumor antigens including T-antigen DNA tumor viruses and envelope antigens of RNA tumor viruses, oncofetal antigen-alpha-fetoprotein such as CEA of colon, bladder tumor oncofetal antigen (Hellström *et al.* (1985) "Monoclonal Antibodies To Cell Surface Antigens Shared By Chemically Induced Mouse Bladder Carcinomas," Cancer. Res. 45:2210-2188); **VEGF** (Pietrantonio, F. *et al.* (2015) "Bevacizumab-Based Neoadjuvant Chemotherapy For Colorectal Cancer Liver Metastases: Pitfalls And Helpful Tricks In A Review For Clinicians," Crit. Rev. Oncol. Hematol. 95(3):272-281; Grabowski, J.P. (2015) "Current Management Of Ovarian Cancer," Minerva Med. 106(3):151-156; Field, K.M. (2015) "Bevacizumab And Glioblastoma: Scientific Review, Newly Reported Updates, And Ongoing Controversies," Cancer 121(7):997-1007; Suh, D.H. *et al.* (2015) "Major Clinical Research Advances In Gynecologic Cancer In 2014," J. Gynecol. Oncol. 26(2):156-167; Liu, K.J. *et al.* (2015) "Bevacizumab In Combination With Anticancer Drugs For Previously Treated Advanced Non-Small Cell Lung Cancer," Tumour Biol. 36(3):1323-1327; Di Bartolomeo, M. *et al.*

(2015) “Bevacizumab Treatment In The Elderly Patient With Metastatic Colorectal Cancer,” Clin. Interv. Aging 10:127-133); **VEGF Receptor** (O’Dwyer. P.J. (2006) “The Present And Future Of Angiogenesis-Directed Treatments Of Colorectal Cancer,” Oncologist 11(9):992-998); **VEP8**; **VEP9**; **VIM-D5**; and **Y hapten, Le^y** as found in embryonal carcinoma cells. Additional Cancer Antigens, and molecules (e.g., antibodies) that bind them are disclosed in **Table 7**. 5T4, B7-H3, CEACAM5/CEACAM6, CD123, DR5, EGFR, an Ephrin receptor, gpA33, HER2/neu, IL13R α 2, ROR1, and VEGF are particularly preferred “**Cancer Antigens**” of the present invention.

Table 7 Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
3F8	Gd2	Neuroblastoma
8H9	B7-H3	Neuroblastoma, Sarcoma, Metastatic Brain Cancers
Abagovomab	CA-125	Ovarian Cancer
Adecatumumab	Epcam	Prostate and Breast Cancer
Afutuzumab	CD20	Lymphoma
Alacizumab	VEGFR2	Cancer
Altumomab	CEA	Colorectal Cancer
Amatuximab	Mesothelin	Cancer
Anatumomab Mafenatox	TAG-72	Non-Small Cell Lung Carcinoma
Anifrolumab	Interferon A/B Receptor	Systemic Lupus Erythematosus
Anrukinzumab	IL-13	Cancer
Apolizumab	HLA-DR	Hematological Cancers
Arcitumomab	CEA	Gastrointestinal Cancer
Atinumab	RTN4	Cancer
Bectumomab	CD22	Non-Hodgkin's Lymphoma (Detection)
Belimumab	BAFF	Non-Hodgkin Lymphoma
Bevacizumab	VEGF-A	Metastatic Cancer, Retinopathy of Prematurity
Bivatuzumab	CD44 V6	Squamous Cell Carcinoma
Blinatumomab	CD19	Cancer
Brentuximab	CD30 (TNFRSF8)	Hematologic Cancers
Cantuzumab	MUC1	Cancers
Cantuzumab Mertansine	Mucin Canag	Colorectal Cancer
Caplacizumab	VWF	Cancers
Capromab	Prostatic Carcinoma Cells	Prostate Cancer (Detection)
Carlumab	MCP-1	Oncology/Immune Indications
Catumaxomab	Epcam, CD3	Ovarian Cancer, Malignant Ascites, Gastric Cancer

Table 7		
Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
Cc49	Tag-72	Tumor Detection
Cetuximab	EGFR	Metastatic Colorectal Cancer and Head and Neck Cancer
Ch.14.18	Undetermined	Neuroblastoma
Citatumumab	Epcam	Ovarian Cancer and other Solid Tumors
Cixutumumab	IGF-1 Receptor	Solid Tumors
Clivatuzumab	MUC1	Pancreatic Cancer
Conatumumab	TRAIL-R2	Cancer
Dacetuzumab	CD40	Hematologic Cancers
Dalotuzumab	Insulin-Like Growth Factor I Receptor	Cancer
Daratumumab	CD38	Cancer
Demcizumab	DLL4	Cancer
Detumomab	B-Lymphoma Cell	Lymphoma
Drozitumab	DR5	Cancer
Duligotumab	HER3	Cancer
Dusigitumab	ILGF2	Cancer
Ecromeximab	GD3 Ganglioside	Malignant Melanoma
Eculizumab	C5	Paroxysmal Nocturnal Hemoglobinuria
Edrecolomab	Epcam	Colorectal Carcinoma
Elotuzumab	SLAMF7	Multiple Myeloma
Elsilimomab	IL-6	Cancer
Enavatuzumab	TWEAK Receptor	Cancer
Enlimomab	ICAM-1 (CD54)	Cancer
Enokizumab	IL9	Asthma
Enoticumab	DLL4	Cancer
Ensituximab	5AC	Cancer
Epitumomab Cituxetan	Episialin	Cancer
Epratuzumab	CD22	Cancer, SLE
Ertumaxomab	HER2/Neu, CD3	Breast Cancer
Etaracizumab	Integrin $\text{A}\alpha\beta_3$	Melanoma, Prostate Cancer, Ovarian Cancer
Faralimomab	Interferon Receptor	Cancer
Farletuzumab	Folate Receptor 1	Ovarian Cancer
Fasinumab	HNGF	Cancer
Fbta05	CD20	Chronic Lymphocytic Leukaemia
Ficlatuzumab	HGF	Cancer
Figitumumab	IGF-1 Receptor	Adrenocortical Carcinoma, Non-Small Cell Lung Carcinoma
Flanvotumab	TYRP1 (Glycoprotein 75)	Melanoma
Fontolizumab	IFN- γ	Crohn's Disease

Table 7		
Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
Fresolimumab	TGF-B	Idiopathic Pulmonary Fibrosis, Focal Segmental Glomerulosclerosis, Cancer
Futuximab	EGFR	Cancer
Galiximab	CD80	B-Cell Lymphoma
Ganitumab	IGF-I	Cancer
Gemtuzumab Ozogamicin	CD33	Acute Myelogenous Leukemia
Gevokizumab	IL-1 β	Diabetes
Girentuximab	Carbonic Anhydrase 9 (CA-IX)	Clear Cell Renal Cell Carcinoma
Glembatumumab Vedotin	GPNCB	Melanoma, Breast Cancer
Golimimumab	TNF-A	Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis
Ibritumomab Tiuxetan	CD20	Non-Hodgkin's Lymphoma
Icrucumab	VEGFR-1	Cancer
Igovomab	CA-125	Ovarian Cancer (Diagnosis)
Imab362	Clon18.2	Gastrointestinal Adenocarcinomas and Pancreatic Tumor
Imgatuzumab	EGFR	Cancer
Inclacumab	Selectin P	Cancer
Indatuximab Ravtansine	SDC1	Cancer
Inotuzumab Ozogamicin	CD22	Cancer
Intetumumab	CD51	Solid Tumors (Prostate Cancer, Melanoma)
Ipilimumab	CD152	Melanoma
Iratumumab	CD30 (TNFRSF8)	Hodgkin's Lymphoma
Itolizumab	CD6	Cancer
Labetuzumab	CEA	Colorectal Cancer
Lambrolizumab	PDCD1	Antineoplastic Agent
Lampalizumab	CFD	Cancer
Lexatumumab	TRAIL-R2	Cancer
Libivirumab	Hepatitis B Surface Antigen	Hepatitis B
Ligelizumab	IGHE	Cancer
Lintuzumab	CD33	Cancer
Lirilumab	KIR2D	Cancer
Lorvotuzumab	CD56	Cancer
Lucatumumab	CD40	Multiple Myeloma, Non-Hodgkin's Lymphoma, Hodgkin's Lymphoma
Lumiliximab	CD23	Chronic Lymphocytic Leukemia
Mapatumumab	TRAIL-R1	Cancer

Table 7		
Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
Margetuximab	Ch4d5	Cancer
Matuzumab	EGFR	Colorectal, Lung and Stomach Cancer
Milatuzumab	CD74	Multiple Myeloma and Other Hematological Malignancies
Minretumomab	TAG-72	Cancer
Mitumomab	GD3 Ganglioside	Small Cell Lung Carcinoma
Mogamulizumab	CCR4	Cancer
Morolimumab	Rhesus Factor	Cancer
Moxetumomab Pasudotox	CD22	Cancer
Nacolomab Tafenatox	C242 Antigen	Colorectal Cancer
Namilumab	CSF2	Cancer
Naptumomab Estafenatox	5T4	Non-Small Cell Lung Carcinoma, Renal Cell Carcinoma
Narnatumab	RON	Cancer
Nebacumab	Endotoxin	Sepsis
Necitumumab	EGFR	Non-Small Cell Lung Carcinoma
Nerelimomab	TNF-A	Cancer
Nesvacumab	Angiopoietin 2	Cancer
Nimotuzumab	EGFR	Squamous Cell Carcinoma, Head and Neck Cancer, Nasopharyngeal Cancer, Glioma
Nivolumab	PD-1	Cancer
Nofetumomab Merpentan	Undetermined	Cancer
Ocaratuzumab	CD20	Cancer
Ofatumumab	CD20	Chronic Lymphocytic Leukemia
Olaratumab	PDGF-R A	Cancer
Olokizumab	IL6	Cancer
Onartuzumab	Human Scatter Factor Receptor Kinase	Cancer
Ontuxizumab	TEM1	Cancer
Oportuzumab Monatox	Epcam	Cancer
Oregovomab	CA-125	Ovarian Cancer
Orticumab	Oxldl	Cancer
Otlertuzumab	CD37	Cancer
Panitumumab	EGFR	Colorectal Cancer
Pankomab	Tumor-Specific Glycosylation of MUC1	Ovarian Cancer
Parsatuzumab	EGFL7	Cancer

Table 7 Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
Patritumab	HER3	Cancer
Pembrolizumab	PD-1	Cancer
Pemtumomab	MUC1	Cancer
Perakizumab	IL17A	Arthritis
Pertuzumab	HER2/Neu	Cancer
Pidilizumab	PD-1	Cancer and Infectious Diseases
Pinatuzumab Vedotin	CD22	Cancer
Pintumomab	Adenocarcinoma Antigen	Adenocarcinoma
Placulumab	Human TNF	Cancer
Polatuzumab Vedotin	CD79B	Cancer
Pritoxaximab	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Pritumumab	Vimentin	Brain Cancer
Quilizumab	IGHE	Cancer
Racotumomab	N- Glycolylneuraminic Acid	Cancer
Radretumab	Fibronectin Extra Domain-B	Cancer
Ramucirumab	VEGFR2	Solid Tumors
Rilotumumab	HGF	Solid Tumors
Rituximab	CD20	Lymphomas, Leukemias, Some Autoimmune Disorders
Robatumumab	IGF-1 Receptor	Cancer
Roledumab	RHD	Cancer
Samalizumab	CD200	Cancer
Satumomab Pendetide	TAG-72	Cancer
Seribantumab	ERBB3	Cancer
Setoxaximab	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Sgn-CD19a	CD19	Acute Lymphoblastic Leukemia and B- Cell Non-Hodgkin Lymphoma
Sgn-CD33a	CD33	Acute Myeloid Leukemia
Sibrotuzumab	FAP	Cancer
Siltuximab	IL-6	Cancer
Solitomab	Epcam	Cancer
Sontuzumab	Episialin	Cancer
Tabalumab	BAFF	B-Cell Cancers
Tacatuzumab Tetraxetan	Alpha-Fetoprotein	Cancer

Table 7		
Antibody and Antibody-Based Molecules		
Antibody Name	Cancer Antigens	Therapeutic Target Application
Taplitumomab Paptox	CD19	Cancer
Telimomab	Undetermined	Cancer
Tenatumomab	Tenascin C	Cancer
Teneliximab	CD40	Cancer
Teprotumumab	CD221	Hematologic Tumors
Ticilimumab	CTLA-4	Cancer
Tigatuzumab	TRAIL-R2	Cancer
Tnx-650	Il-13	Hodgkin's Lymphoma
Tositumomab	CD20	Follicular Lymphoma
Tovetumab	CD140a	Cancer
Trastuzumab	HER2/Neu	Breast Cancer
Trbs07	Gd2	Melanoma
Tremelimumab	CTLA-4	Cancer
Tucotuzumab Celmoleukin	Epcam	Cancer
Ublituximab	MS4A1	Cancer
Urelumab	4-1BB	Cancer
Vantictumab	Frizzled Receptor	Cancer
Vapaliximab	AOC3 (VAP-1)	Cancer
Vatelizumab	ITGA2	Cancer
Veltuzumab	CD20	Non-Hodgkin's Lymphoma
Vesencumab	NRP1	Cancer
Volociximab	Integrin A5β1	Solid Tumors
Vorsetuzumab	CD70	Cancer
Votumumab	Tumor Antigen CTAA16.88	Colorectal Tumors
Zalutumumab	EGFR	Squamous Cell Carcinoma of The Head And Neck
Zatuximab	HER1	Cancer
Ziralimumab	CD147	Cancer

[00268] Exemplary antibodies, whose VH and VL Domains may be used to construct the Binding Molecules of the present invention that are capable of binding a Cancer Antigen arrayed on the surface of a cancer cell and mediating the redirected killing of such cancer cells are listed in **Table 7** above, additional antibodies that may be used to construct molecules capable of binding a Cancer Antigen arrayed on the surface of a cancer cell and mediating the redirected killing of such cancer cells are provided below.

1. Exemplary Anti-B7-H3 Antibodies

[00269] **B7-H3** is a Cancer Antigen that is overexpressed on a wide variety of solid tumor types and is a member of the B7 family of molecules that are involved in immune regulation (see, US Patent No. 8,802,091; US 2014/0328750; US 2013/0149236; Loo, D. *et al.* (2012) “*Development Of An Fc-Enhanced Anti-B7-H3 Monoclonal Antibody With Potent Antitumor Activity*,” Clin. Cancer Res. 18(14):3834-3845). In particular, several independent studies have shown that human malignant cancer cells (*e.g.*, cancer cells of neuroblastomas and gastric, ovarian, pancreatic, and non-small cell lung cancers) exhibit a marked increase in expression of B7-H3 protein and that this increased expression was associated with increased disease severity (Zang, X. *et al.* (2007) “*The B7 Family And Cancer Therapy: Costimulation And Coinhibition*,” Clin. Cancer Res. 13:5271-5279), suggesting that B7-H3 is exploited by tumors as an immune evasion pathway (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3*,” Proc. Natl. Acad. Sci. (U.S.A.) 105(30):10277-10278).

[00270] B7-H3 has also been found to co-stimulate CD4⁺ and CD8⁺ T-cell proliferation. B7-H3 also stimulates IFN- γ production and CD8⁺ lytic activity (Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- γ Production*,” Nature Immunol. 2:269–274; Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily*,” Nature Rev. Immunol. 2:116-126). However, the protein also possibly acts through NFAT (nuclear factor for activated T-cells), NF- κ B (nuclear factor kappa B), and AP-1 (Activator Protein-1) factors to inhibit T-cell activation (Yi. K.H. *et al.* (2009) “*Fine Tuning The Immune Response Through B7-H3 And B7-H4*,” Immunol. Rev. 229:145-151). B7-H3 is also believed to inhibit Th1, Th2, or Th17 *in vivo* (Prasad, D.V. *et al.* (2004) “*Murine B7-H3 Is A Negative Regulator Of T Cells*,” J. Immunol. 173:2500-2506; Fukushima, A. *et al.* (2007) “*B7-H3 Regulates The Development Of Experimental Allergic Conjunctivitis In Mice*,” Immunol. Lett. 113:52-57; Yi. K.H. *et al.* (2009) “*Fine Tuning The Immune Response Through B7-H3 And B7-H4*,” Immunol. Rev. 229:145-151).

[00271] Preferred B7-H3-Binding Molecules possess the VL and/or VH Domains, of humanized anti-human B7-H3 monoclonal antibody “**B7-H3 mAb-B**,” “**B7-H3 mAb-C**,” “**B7-H3 mAb-D**,” or any of the anti-B7-H3 antibodies provided herein; and more preferably possess 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of such anti-B7-H3 monoclonal antibodies.

[00272] Upon humanization, antibody **B7-H3 mAb-B** yielded two variant VH Domains, **B7-H3 mAb-B VH1** and **B7-H3 mAb-B VH2**; and two variant VL Domains **B7-H3 mAb-B VH1 VL1** and **B7-H3 mAb-B VL2**, which may be used in any combination of VH/VL Domains to yield a functional B7-H3 Binding Domain.

[00273] The amino acid sequence of the VH Domain of **B7-H3 mAb-B VH1** is SEQ ID NO:122 (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWMQWVRQA PGQGLEWMGT
IYPGDGDTRY TQKFKGRVTI TADKSTSTAY MELSSLRSED TAVYYCARRG
IPRLWYFDVW GQGTTVTVSS

[00274] The amino acid sequence of the VH Domain of **B7-H3 mAb-B VH2** is SEQ ID NO:123 (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWMQWVRQA PGQGLEWMGT
IYPGGGDTRY TQKFOGRVTI TADKSTSTAY MELSSLRSED TAVYYCARRG
IPRLWYFDVW GQGTTVTVSS

[00275] The amino acid sequence of the VL Domain of **B7-H3 mAb-B VL1** is SEQ ID NO:124 (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY
TSRLHSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
 GTKLEIK

[00276] The amino acid sequence of the VL Domain of **B7-H3 mAb-B VL2** is SEQ ID NO:125 (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS SYLNWYQQKP GKAPKLLIYY
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
 GTKLEIK

[00277] The amino acid sequence of the VH Domain of humanized **B7-H3 mAb-C** is SEQ ID NO:126 (CDR_H residues are shown underlined):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLEWVAT
INSGGSNTYY PDSLKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHD
GGAMDYWGQG TTVTSS

[00278] The amino acid sequence of the VL Domain of humanized **B7-H3 mAb-C** is SEQ ID NO:127 (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASESIY SYLAWYQQKP GKAPKLLVYN
TKTLPEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH HYGTPPWTFG
 QGTRLEIK

[00279] The amino acid sequence of the VH Domain of **B7-H3 mAb-D (SEQ ID NO:128)** is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVAY
ISSGSGTIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARHG
YRYEGFDYWG QGTTVTSS

[00280] The amino acid sequence of the VL Domain of **B7-H3 mAb-D (SEQ ID NO:129)** is shown below (CDR_L residues are shown underlined).

DIQMTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFGSGSGTD FTLTISSLQP EDFAEYFCQQ YNNYPFTFGQ
 GTKLEIK

[00281] Particularly preferred, are B7-H3-Binding Molecules which possess a humanized VH and/or VL Domain including but not limited to “Enoblituzumab” (also known as MGA271; CAS Reg No. 1353485-38-7). Enoblituzumab is an Fc-optimized monoclonal antibody that binds to HER2/neu and mediates enhanced ADCC activity. The amino acid sequences of the complete Heavy and Light Chains of Enoblituzumab are known in the art (see., e.g., WHO Drug Information, 2017, Recommended INN: List 77, 31(1):49). The amino acid sequence of the VH Domain of Enoblituzumab is (SEQ ID NO:130) (CDR_Hs are underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVAY
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCGRGR
ENIYYGSRLD YWGQGTTVTV SS

The amino acid sequence of the VL Domain of Enoblituzumab is (SEQ ID NO:131) (CDR_Ls are underlined):

DIQMTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
 GTKLEIK

[00282] In addition to the above-identified preferred anti-B7-H3 Binding Molecules, the invention contemplates the use of any of the following anti-B7-H3 Binding Molecules: **LUCA1; BLA8; PA20; or SKN2** (see, US Patent Nos. 7,527,969; 8,779,098 and PCT Patent Publication WO 2004/001381); **M30; cM30; M30-H1-L1; M30-H1-L2; M30-H1-L3; M30-H1-L4; M30-H1-L5; M30-H1-L6; M30-H1-L7; M30-H4-L1; M30-H4-L2; M30-H4-L3; and M30-H4-L4** (see, US Patent Publication 2013/0078234 and PCT Patent Publication WO 2012/147713); and **8H9** (see US Patent Nos. 7,666,424; 7,737,258; 7,740,845; 8,148,154; 8,414,892; 8,501,471; 9,062,110; US Patent Publication 2010/0143245 and PCT Patent Publication WO 2008/116219).

2. Exemplary Anti-CEACAM5 and Anti-CEACAM6 Antibodies

[00283] Carcinoembryonic Antigen-Related Cell Adhesion Molecules 5 (CEACAM5) and 6 (CEACAM6) have been found to be associated with various types of cancers including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660), and particularly colorectal, gastrointestinal, pancreatic, non-small cell lung cancer (NSCL), breast, thyroid, stomach, ovarian and uterine carcinomas (Zheng, C. *et al.* (2011) “A Novel Anti-CEACAM5 Monoclonal Antibody, CC4, Suppresses Colorectal Tumor Growth and Enhances NK Cells-Mediated Tumor Immunity,” PLoS One 6(6):e21146, pp. 1-11).

[00284] CEACAM5 has been found to be overexpressed in 90% of gastrointestinal, colorectal and pancreatic cancers, 70% of non-small cell lung cancer cells and 50% of breast cancers (Thompson, J.A. *et al.* (1991) “Carcinoembryonic Antigen Gene Family: Molecular Biology And Clinical Perspectives,” J. Clin. Lab. Anal. 5:344-366). Overexpressed carcinoembryonic antigen-related cellular adhesion molecule 6 (CEACAM6) plays important roles in the invasion and metastasis of a variety of human cancers, including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660; Deng, X. *et al.* (2014) “Expression Profiling Of CEACAM6 Associated With The Tumorigenesis And Progression In Gastric Adenocarcinoma,” Genet. Mol. Res. 13(3):7686-7697; Cameron, S. *et al.* (2012) “Focal Overexpression Of CEACAM6 Contributes To Enhanced Tumourigenesis In Head And Neck Cancer Via Suppression Of Apoptosis,” Mol. Cancer 11:74, pp. 1-11; Chapin, C. *et al.* (2012) “Distribution And Surfactant Association Of Carcinoembryonic Cell Adhesion Molecule 6 In Human Lung,” Amer. J. Physiol. Lung Cell. Mol. Physiol. 302(2):L216-L25; Riley, C.J. *et al.* (2009) “Design And Activity Of A Murine And Humanized Anti-CEACAM6 Single-Chain Variable Fragment In The Treatment Of Pancreatic Cancer,” Cancer Res. 69(5):1933-1940; Lewis-Wambi, J.S. *et al.* (2008) “Overexpression Of CEACAM6 Promotes Migration And Invasion Of Oestrogen-Deprived Breast Cancer Cells,” Eur. J. Cancer 44(12):1770-1779; Blumenthal, R.D. *et al.* (2007) “Expression Patterns Of

CEACAM5 And CEACAM6 In Primary And Metastatic Cancers,” BMC Cancer. 7:2, pp. 1-15). Antibodies that immunospecifically bind CEACAM5 and CEACAM6 are commercially available (Santa Cruz Biotechnology, Inc., Novus Biologicals LLC; Abnova Corporation).

[00285] The amino acid sequence of the VH Domain of the humanized anti- CEACAM5 / ANTI-CEACAM6 antibody **16C3** (EP 2585476) (**SEQ ID NO:132**) is shown below (CDR_H residues are shown underlined):

QVQLQQSGPE VVRPGVSVKI SCKGSGYTFT DYAMHWVKQS HAKSLEWIGL
ISTYSGDTKY NQNFKGKATM TVDKSASTAY MELSSLRSED TAVYYCARGD
YSGSRYWFAY WGQGT_LTVTS S

[00286] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / ANTI-CEACAM6 antibody **16C3** (EP 2585476) (**SEQ ID NO:133**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVIT ITCGASENIY GALNWYQRKP GKSPKLLIWG
ASNLADGMPs RFSGSGSGRQ YTLTISSLQP EDVATYYCQNV VLSSPYTFGG
 GTKLEIK

[00287] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) (**SEQ ID NO:134**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCSSSGFALT DYYMSWVRQA PGKGLEWLGF
IANKANGHTT DYSPSVKGRF TISRDNKNT LFLQMDSLRLP EDTGVYFCAR
DMGIRWNFDV WGQGT_PVTVS S

[00288] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) (**SEQ ID NO:135**) is shown below (CDR_L residues are shown underlined):

DIQLTQSPSS LSASVGDRVIT MTCSASSRVS YIHWYQQKPG KAPKRWIYGT
STLASGVPAR FSGSGSGTDF TFTISSLQPE DIATYYCQQW SYNPPTFGQG
 TKVEIKR

[00289] The present invention specifically includes and encompasses CEACAM5/CEACAM6 Binding Molecules (e.g., CEACAM5/CEACAM6 x CD3 bispecific Binding Molecules) that are capable of binding to CEACAM5 and/or CEACAM6, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-CEACAM5/CEACAM6 monoclonal antibodies **16C3** or **hMN15**.

3. Exemplary Anti-EGFR Antibodies

[00290] Epidermal Growth Factor Receptor (EGFR) is a Cancer Antigen of certain metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer. Exemplary antibodies that bind human EGFR are “**Cetuximab**” and “**Panitumumab**.” Cetuximab is a recombinant human-mouse chimeric epidermal growth factor receptor (EGFR) IgG1 monoclonal antibody (Govindan R. (2004) “*Cetuximab In Advanced Non-Small Cell Lung Cancer*,” Clin. Cancer Res. 10(12 Pt 2):4241s-4244s; Bou-Assaly, W. *et al.* (2010) “*Cetuximab (Erbix)*,” Am. J. Neuroradiol. 31(4):626-627). Panitumumab (Vectibix®, Amgen) is a fully humanized epidermal growth factor receptor (EGFR) IgG2 monoclonal antibody (Foon, K.A. *et al.* (2004) “*Preclinical And Clinical Evaluations Of ABX-EGF, A Fully Human Anti-Epidermal Growth Factor Receptor Antibody*,” Int. J. Radiat. Oncol. Biol. Phys. 58(3):984-990; Yazdi, M.H. *et al.* (2015) “*A Comprehensive Review of Clinical Trials on EGFR Inhibitors Such as Cetuximab and Panitumumab as Monotherapy and in Combination for Treatment of Metastatic Colorectal Cancer*,” Avicenna J. Med. Biotechnol. 7(4):134-144).

[00291] The amino acid sequence of the VH Domain of the chimeric anti-EGFR antibody **Cetuximab (SEQ ID NO:136)** is shown below (CDR_H residues are shown underlined):

QVQLKQSGPG LVQPSQSLSI TCTVSGFSLT NYGVHWVRQS PGKGLEWLGV
IWSGGNTDYN TPFTSRLSIN KDNSKSQVFF KMNSLQSDNT AIYYCARALT
YYDYEFAYWG QGTLVTVSA

[00292] The amino acid sequence of the VL Domain of the chimeric anti-EGFR antibody **Cetuximab (SEQ ID NO:137)** is shown below (CDR_L residues are shown underlined):

DILLTQSPVI LSVSPGERVS FSCRASQSIG TNIHWYQQRT NGSPRLLIKY
ASESISGIPS RFSGSGSGTD FTLSINSVES EDIADYYCQQ NNNWPTTFGA
 GTKLELKR

[00293] The amino acid sequence of the VH Domain of Panitumumab (**SEQ ID NO:138**) is shown below (CDR_H residues are shown underlined):

QVQLQESGPG LVKPSETLSL TCTVSGGSVS SGDYWTWIR QSPGKGLEWI
GHIYYSGNTN YNPSLKSRLT ISIDTSKTQF SLKLSSVTAA DTAIYYCVRD
RVTGAFDIWG QGTMVTVSS

[00294] The amino acid sequence of the VL Domain of Panitumumab (**SEQ ID NO:139**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP GKAPKLLIYD
ASNLETGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQH FDHLPLAFGG
 GTKVEIKR

[00295] The present application specifically includes and encompasses EGFR Binding Molecules (e.g., EGFR x CD3 bispecific Binding Molecules) that are capable of binding to EGFR, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-EGFR monoclonal antibodies Cetuximab or Panitumumab.

4. Exemplary Anti-EphA2 Antibodies

[00296] The receptor tyrosine kinase, Ephrin type-A receptor 2 (**EphA2**) is normally expressed at sites of cell-to-cell contact in adult epithelial tissues, however, recent studies have shown that it is also overexpressed in various types of epithelial carcinomas, with the greatest level of EphA2 expression observed in metastatic lesions. High expression levels of EphA2 have been found in a wide range of cancers and in numerous cancer cell lines, including prostate cancer, breast cancer, non-small cell lung cancer and melanoma (Xu, J. *et al.* (2014) “*High EphA2 Protein Expression In Renal Cell Carcinoma Is Associated With A Poor Disease Outcome*,” *Oncol. Lett.* Aug 2014; 8(2): 687-692; Miao, B. *et al.* (2014) “*EphA2 is a Mediator of Vemurafenib Resistance and a Novel Therapeutic Target in Melanoma*,” *Cancer Discov.* pii: CD-14-0295). EphA2 does not appear to be merely a marker for cancer, but rather appears to be persistently overexpressed and functionally changed in numerous human cancers (Chen, P. *et al.* (2014) “*EphA2 Enhances The Proliferation And Invasion Ability Of LnCap Prostate Cancer Cells*,” *Oncol. Lett.* 8(1):41-46). Exemplary antibodies that bind human EphA2 are “**EphA2 mAb 1**,” “**EphA2 mAb 2**” and “**EphA2 mAb 3**.”

[00297] The amino acid sequence of the VH Domain of **EphA2 mAb 1** (SEQ ID NO:140) is shown below (CDR_H residues are shown underlined):

QVQLKESGPG LVAPSQSLSI TCTVSGFSL **RYSVHWVRQP** PGKGLEWLGM
IWGGGSTDYN SALKSRLSIS KDNSKSQVFL KMNSLQTDDT AMYYCARK**KHG**
NYTMDYWGQ GTSVTVSS

[00298] The amino acid sequence of the VL Domain of **EphA2 mAb 1** (SEQ ID NO:141) is shown below (CDR_L residues are shown underlined):

DIQMTQTTSS LSASLGDRIT ISCR**ASQDIS** **NYLNWYQQKP** DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFC**QQ** **GYTLTYTFGGG**
 TKLEIK

[00299] The amino acid sequence of the VH Domain of **EphA2 mAb 2** (SEQ ID NO:142) is shown below (CDR_H residues are shown underlined):

QIQLVQSGPE LKKPGETVKI SCKASGFTFT NYGMNWVKQA PGKGLKWMGW
INTYIGEPTY ADDFKGRFVF SLETSASTAY LQINNLLKNE MATYFCAREL
GPYYFDYWGQ GTTLTVSS

[00300] The amino acid sequence of the VL Domain of **EphA2 mAb 2** (SEQ ID NO:143) is shown below (CDR_L residues are shown underlined):

DVMTQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
 LLIYKVSNRF SGVPDRFGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
TFGSGTKLEI K

[00301] The amino acid sequence of the VH Domain of **EphA2 mAb 3** (SEQ ID NO:144) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG SVKPGGSLKL SCAASGFTFT DHYMYWVRQT PEKRLEWVAT
ISDGGSFTSY PDSVKGRFTI SRDIAKNNLY LQMSSLKSED TAMYYCTRDE
SDRPFFPYWGQ GTLTVSS

[00302] The amino acid sequence of the VL Domain of **EphA2 mAb 3** (SEQ ID NO:145) is shown below (CDR_L residues are shown underlined):

DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAVAWYQQKP GQSPKLLIFW
ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
 GTKLEIK

[00303] The present application specifically includes and encompasses EphA2 Binding Molecules (*e.g.*, EphA2 x CD3 bispecific Binding Molecules) that are capable of binding to EphA2, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of anti-EphA2 monoclonal antibodies **EphA2 mAb 1**, **EphA2 mAb 2** and **EphA2 mAb 3**.

5. Exemplary Anti-gpA33 Antibodies

[00304] The 43kD transmembrane glycoprotein A33 (**gpA33**) is expressed in >95% of all colorectal carcinomas (Heath, J.K. *et al.* (1997) "The Human A33 Antigen Is A Transmembrane Glycoprotein And A Novel Member Of The Immunoglobulin Superfamily," Proc. Natl. Acad. Sci. (U.S.A.) 94(2):469-474; Ritter, G. *et al.* (1997) "Characterization Of Posttranslational Modifications Of Human A33 Antigen, A Novel Palmitoylated Surface Glycoprotein Of Human Gastrointestinal Epithelium," Biochem. Biophys. Res. Commun. 236(3):682-686; Wong, N.A. *et al.* (2006) "EpCAM and gpA33 Are Markers Of Barrett's

Metaplasia,” J. Clin. Pathol. 59(3):260-263). An exemplary antibody that binds to human gpA33 is “**gpA33 mAb 1**.”

[00305] The amino acid sequence of the VH Domain of **gpA33 mAb 1** (SEQ ID NO:146) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIIY
GNNVYFDVWG QGTTVTIVSS

[00306] The amino acid sequence of the VL Domain of **gpA33 mAb 1** (SEQ ID NO:147) is shown below (CDR_L residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSLEAE DAATYYCQQW SSYPLTFGQG
 TKLEIK

[00307] The present application specifically includes and encompasses gpA33 Binding Molecules (*e.g.*, gpA33x CD3 bispecific Binding Molecules) that are capable of binding to gpA33, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of anti-gpA33 monoclonal antibodies **gpA33 mAb 1**, or of any of the anti-gpA33 monoclonal antibodies provided in WO 2015/026894. The present invention additionally includes and encompasses the exemplary gpA33 x CD3 bispecific Binding Molecules provided in WO 2015/026894.

6. Exemplary Anti-HER2/neu Antibodies

[00308] HER2/neu is a 185 kDa receptor protein that was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. HER2/neu has been extensively investigated because of its role in several human carcinomas (including breast and gastric cancers) and in mammalian development (Hynes *et al.* (1994) Biochim. Biophys. Acta 1198:165-184; Dougall *et al.* (1994) Oncogene 9:2109-2123; Lee *et al.* (1995) Nature 378:394-398). Exemplary antibodies that bind human HER2/neu include “**Margetuximab**,” “**Trastuzumab**” and “**Pertuzumab**.” Margetuximab (also known as MGAH22; CAS Reg No. 1350624-75-7) is an Fc-optimized monoclonal antibody that binds to HER2/neu and mediates enhanced ADCC activity. Trastuzumab (also known as rhuMAB4D5, and marketed as HERCEPTIN®; CAS Reg No 180288-69-1; see, US Patent No. 5,821,337) is the humanized version of antibody 4D5, having IgG1/kappa constant regions. Pertuzumab (also known as rhuMAB2C4, and

marketed as PERJETTM; CAS Reg No 380610-27-5; see for example, WO2001/000245) is a humanized version of antibody 2C4 having IgG1/kappa constant regions.

[00309] The present application specifically includes and encompasses Her2/Neu binding molecule (*e.g.*, Her2/Neu x CD3 bispecific Binding Molecules) that are capable of binding to Her2/Neu, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-Her2/Neu monoclonal antibodies Margetuximab, Trastuzumab or Pertuzumab.

[00310] The amino acid sequence of the VH Domain of Margetuximab is (SEQ ID NO:148) (CDR_H residues are shown underlined):

QVQLQQSGPE LVKPGASLKL SCTASGFNIK DTYIHWVKQR PEQGLEWIGR
IYPTNGYTRY DPKFQDKATI TADTSSNTAY LQVSRLTSED TAVYYCSRWG
GDGFYAMDYW GQGASVTVSS

[00311] The amino acid sequence of the VL Domain of Margetuximab is (SEQ ID NO:149) (CDR_L residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GHSPKLLIYS
ASFRYTGVDPD RFTGSRSGTD FTFTISSVQA EDLAVYYCQQ HYTTPPTFGG
 GTKVEIK

[00312] The amino acid sequences of the complete Heavy and Light Chains of Margetuximab are known in the art (see., *e.g.*, WHO Drug Information, 2014, Recommended INN: List 71, 28(1):93-94).

[00313] The amino acid sequence of the VH Domain of Trastuzumab is (SEQ ID NO:150) (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR
IYPTNGYTRY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCSRWG
GDGFYAMDYW GQGTLVTVSS

[00314] The amino acid sequence of the VL Domain of Trastuzumab is (SEQ ID NO:151) (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVIT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS
ASFLYSGVPS RFSGRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
 GTKVEIK

[00315] The amino acid sequence of the VH Domain of Pertuzumab is (SEQ ID NO:152) (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFT DYTMDWVRQA PGKGLEWVAD
VNPNSGGSIY NQRFKGRFTL SVDRSKNTLY LQMNSLRAED TAVYYCARNL
GPSFYFDYWG QGTLVTVSS

[00316] The amino acid sequence of the VL Domain of Pertuzumab is (SEQ ID NO:153) (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCKASQDVS IGVAWYQQKP GKAPKLLIYS
ASYRYTGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQQ YYIYPYTFGQ
 GTKVEIK

[00317] In addition to the above-identified preferred anti-HER2/neu Binding Molecules, the invention contemplates Her2/Neu Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of any of the following anti-Her-2 Binding Molecules: **1.44.1; 1.140; 1.43; 1.14.1; 1.100.1; 1.96; 1.18.1; 1.20; 1.39; 1.24; and 1.71.3** (US Patent Nos. 8,350,011; 8,858,942; and PCT Patent Publication WO 2008/019290); **F5 and C1** (US Patent Nos. 7,892,554; 8,173,424; 8,974,792; and PCT Patent Publication WO 99/55367); and also the anti-Her-2 Binding Molecules of US Patent Publication US2013017114 and PCT Patent Publication Nos. WO2011/147986 and WO 2012/143524). The present invention additionally includes and encompasses the exemplary Her2/Neu x CD3 bispecific Binding Molecules provided in WO 2012/143524.

7. Exemplary Anti-VEGF Antibodies

[00318] VEGF-A is a chemical signal that stimulates angiogenesis in a variety of diseases, especially in certain metastatic cancers such as metastatic colon cancer, and in certain lung cancers, renal cancers, ovarian cancers, and glioblastoma multiforme of the brain. An exemplary antibody that binds to human VEGF-A is “**Bevacizumab**” (Avastin®). Bevacizumab is a recombinant humanized IgG1 monoclonal antibody (Midgley, R. *et al.* (2005) “*Bevacizumab – Current Status And Future Directions*,” Ann. Oncol. 16(7):999-1004; Hall, R.D. *et al.* (2015) “*Angiogenesis Inhibition As A Therapeutic Strategy In Non-Small Cell Lung Cancer (NSCLC)*,” Transl. Lung Cancer Res. 4(5):515-523; Narita, Y. (2015) “*Bevacizumab For Glioblastoma*,” Ther. Clin. Risk Manag. 11:1759-1765).

[00319] The amino acid sequence of the VH Domain of **Bevacizumab** (SEQ ID NO:154) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTTLVT VSS

[00320] The amino acid sequence of the VL Domain of **Bevacizumab** (SEQ ID NO:155) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF
TSSLHSGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
 GTKVEIKR

[00321] The present application specifically includes and encompasses VEGF Binding Molecules (*e.g.*, VEGF x CD3 bispecific Binding Molecules) that are capable of binding to VEGF, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-VEGF monoclonal antibody Bevacizumab.

8. Exemplary Anti-5T4 Antibodies

[00322] The oncofetal protein, **5T4**, is a tumor-associated protein displayed on the cell membrane of many carcinomas, including kidney, colon, prostate, lung, carcinoma and in acute lymphoblastic leukemia (see, Boghaert, E.R. *et al.* (2008) “*The Oncofetal Protein, 5T4, Is A Suitable Target For Antibody-Guided Anti-Cancer Chemotherapy With Calicheamicin*,” Int. J. Oncol. 32(1):221-234; Eisen, T. *et al.* (2014) “*Naptumomab Estafenatox: Targeted Immunotherapy with a Novel Immunotoxin*,” Curr. Oncol. Rep. 16:370, pp. 1-6). Exemplary antibodies that bind to human 5T4 include “**5T4 mAb 1**” and “**5T4 mAb 2**.”

[00323] The amino acid sequence of the VH Domain of **5T4 mAb 1** (SEQ ID NO:156) is shown below (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SFWMHWVRQA PGQGLEWMGR
IDPNRGGTEY NEKAKSRVTM TADKSTSTAY MELSSLRSED TAVYYCAGGN
PYYPMDYWGQ GTT~~VT~~VSS

[00324] The amino acid sequence of the VL Domain of an exemplary **5T4 mAb 1** (SEQ ID NO:157) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIIYR
ANRLQSGVPS RFGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
 GTKLEIK

[00325] The amino acid sequence of the VH Domain of **5T4 mAb 2** (SEQ ID NO:158) is shown below (CDR residues are shown underlined):

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYWITWVKQR PGQGLEWIGD
IYPGSGRANY NEKFKSKATL TVDTSSSTAY MQLSSLTSED SAVYNCARYG
PLFTTVVDPN SYAMDYWGQG TSVTVSS

[00326] The amino acid sequence of the VL Domain of **5T4 mAb 2** (SEQ ID NO:159) is shown below (CDR residues are shown underlined):

DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK
 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHP
FTFGSGTKLE IK

[00327] The present application specifically includes and encompasses 5T4 Binding Molecules (*e.g.*, 5T4 x CD3 bispecific Binding Molecules) that are capable of binding to 5T4 that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-5T4 monoclonal antibodies **5T4 mAb 1** or **5T4 mAb 2**, or of any of the anti-5T4 antibodies provided in WO 2013/041687 or WO 2015/184203. The present invention additionally includes and encompasses the exemplary 5T4 x CD3 bispecific Binding Molecules provided in WO 2015/184203.

[00328] The present application additionally specifically includes and encompasses 5T4 x CD3 x CD8 trispecific Binding Molecules that are capable of binding to 5T4, to CD3 and to CD8, and particularly such trispecific Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-5T4 monoclonal antibodies **5T4 mAb 1** or **5T4 mAb 2** or of any of the anti-5T4 monoclonal antibodies provided in WO 2015/184203, and/or the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of any of the anti-CD8 monoclonal antibodies provided herein.

9. Exemplary Anti-IL13R α 2 Antibodies

[00329] Interleukin-13 Receptor α 2 (**IL-13R α 2**) is overexpressed in a variety of cancers, including glioblastoma, colorectal cancer, cervical cancer, pancreatic cancer, multiple melanoma, osteosarcoma, leukemia, lymphoma, prostate cancer and lung cancer (PCT Publication No. WO 2008/146911; Brown, C.E. *et al.* (2013) “*Glioma IL13R α 2 Is Associated With Mesenchymal Signature Gene Expression And Poor Patient Prognosis*,”

PLoS One. 18;8(10):e77769; Barderas, R. *et al.* (2012) “*High Expression Of IL-13 Receptor A2 In Colorectal Cancer Is Associated With Invasion, Liver Metastasis, And Poor Prognosis*,” Cancer Res. 72(11):2780-2790; Kasaian, M.T. *et al.* (2011) “*IL-13 Antibodies Influence IL-13 Clearance In Humans By Modulating Scavenger Activity Of IL-13R α 2*,” J. Immunol. 187(1):561-569; Bozinov, O. *et al.* (2010) “*Decreasing Expression Of The Interleukin-13 Receptor IL-13R α 2 In Treated Recurrent Malignant Gliomas*,” Neurol. Med. Chir. (Tokyo) 50(8):617-621; Fujisawa, T. *et al.* (2009) “*A Novel Role Of Interleukin-13 Receptor Alpha2 In Pancreatic Cancer Invasion And Metastasis*,” Cancer Res. 69(22):8678-8685). Antibodies that immunospecifically bind to IL13R α 2 are commercially available and have been described in the art (Abnova Corporation, Biorbyt, LifeSpan BioSciences, United States Biologicals; see also PCT Publication No. WO 2008/146911). Exemplary antibodies that bind to human IL-13R α 2 include “**hu08**” (see, *e.g.*, PCT Publication No. WO 2014/072888).

[00330] The amino acid sequence of the VH Domain of **hu08** (SEQ ID NO:160) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS RNGMSWVRQA PGKGLEWVAT
VSSGGSYIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARQG
TTALATRFED VWGQGTLVTV SS

[00331] The amino acid sequence of the VL Domain of **hu08** (SEQ ID NO:161) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCKASQDVG TAVAWYQQKP GKAPKLLIYS
ASSRSTGVPS RFGSGSGTD FTLTISSLQP EDFATYYCQH HYSAPWTFGG
 GTKVEIK

[00332] The present application specifically includes and encompasses IL13R α 2 Binding Molecules (*e.g.*, IL13R α 2 x CD3 bispecific Binding Molecules) that are capable of binding to IL13R α 2, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti- IL13R α 2 monoclonal antibody **hu08**.

10. Exemplary Anti-CD123 Antibodies

[00333] CD123 (interleukin 3 receptor alpha, IL-3Ra) is a 40 kDa molecule and is part of the interleukin 3 receptor complex (Stomski, F.C. *et al.* (1996) “*Human Interleukin-3 (IL-3) Induces Disulfide-Linked IL-3 Receptor Alpha- And Beta-Chain Heterodimerization, Which Is Required For Receptor Activation But Not High-Affinity Binding*,” Mol. Cell. Biol.

16(6):3035-3046). Interleukin 3 (IL-3) drives early differentiation of multipotent stem cells into cells of the erythroid, myeloid and lymphoid progenitors. CD123 has been reported to be overexpressed on malignant cells in a wide range of hematologic malignancies including acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), and myelodysplastic syndrome (MDS) (Muñoz, L. *et al.* (2001) “*Interleukin-3 Receptor Alpha Chain (CD123) Is Widely Expressed In Hematologic Malignancies*,” *Haematologica* 86(12):1261-1269). Overexpression of CD123 is associated with poorer prognosis in AML (Tettamanti, M.S. *et al.* (2013) “*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric Antigen Receptor*,” *Br. J. Haematol.* 161:389-401).

[00334] An exemplary antibody that binds to human CD123, and that may be employed in the present invention, is “**CD123 mAb 1**” (see, *e.g.*, PCT Patent Publication WO 2015/026892).

[00335] The amino acid sequence of the VH Domain of **CD123 mAb 1** (SEQ ID NO:162) is shown below (CDR_H residues are shown underlined):

EVQLVQSGAE LKKPGASVKV SCKASGYTFT DYYMKWVRQA PGQGLEWIGD
IIPSNGATFY NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCARSH
LLRASWFAYW GQGTLVTVSS

[00336] The amino acid sequence of the VL Domain of **CD123 mAb 1** (SEQ ID NO:163) is shown below (CDR_L residues are shown underlined):

DFVMTQSPDS LAVSLGERVT MSCKSSQSL NSGNQKNYLT WYQQKPGQPP
 KLLIYWASTR ESGVDPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIK

[00337] The present application specifically includes and encompasses CD123 Binding Molecules (*e.g.*, CD123 x CD3 bispecific Binding Molecules) that are capable of binding to CD123, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-CD123 monoclonal antibody **CD123 mAb 1**, and also any of the anti-CD123 antibodies disclosed in US 2017/081424 and WO 2016/036937. The present invention additionally includes and encompasses exemplary CD123 x CD3

bispecific Binding Molecules, including: flotetuzumab (aka MGD007; CAS Registry No. 1664355-28-5), JNJ-63709178 (Johnson & Johnson, also see, WO 2016/036937) and XmAb14045 (Xencor, also see, US 2017/081424).

11. Exemplary Anti-CD19 Antibodies

[00338] CD19 (B lymphocyte surface antigen B4, Genbank accession number M28170) is a component of the B-cell-receptor (BCR) complex, and is a positive regulator of B-Cell signaling that modulates the threshold for B-Cell activation and humoral immunity. CD19 is one of the most ubiquitously expressed antigens in the B-Cell lineage and is expressed on >95% of B-Cell malignancies, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin's Lymphoma (NHL). Notably, CD19 expression is maintained on B-Cell lymphomas that become resistant to anti-CD20 therapy (Davis *et al.* (1999) "Therapy of B-Cell Lymphoma With Anti-CD20 Antibodies Can Result In The Loss Of CD20 Antigen Expression." Clin Cancer Res, 5:611-615, 1999). CD19 has also been suggested as a target to treat autoimmune diseases (Tedder (2009) "CD19: A Promising B-Cell Target For Rheumatoid Arthritis," Nat. Rev. Rheumatol. 5:572-577).

[00339] An exemplary humanized antibody that binds to human CD19, and that may be employed in the present invention, is the anti-CD19 antibody disclosed in WO 2016/048938 (referred to herein as "CD19 mAb 1").

[00340] The amino acid sequence of the VH Domain of CD19 mAb 1 (SEQ ID NO:164) is shown below (CDR_H residues are shown underlined):

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS TSGMGVGVIR QPPGKALEWL
AHIWDDDKR YNPALKSRLT ISKDTSKNQV FLTMTNMDPV DTATYYCARM
ELWSYYFDYW GQGTTVTVSS

[00341] The amino acid sequence of the VL Domain of CD19 mAb 1 (SEQ ID NO:165) is shown below (CDR_L residues are shown underlined):

ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTSSLEAE DAATYYCFQG SVYPFTFGQG
 TKLEIK

[00342] The amino acid sequence of an alternative VL Domain of CD19 mAb 1 (SEQ ID NO:195) is shown below (CDR_L residues are shown underlined):

ENVLTQSPAT LSVTPGEKVT ITCSASSSVS YMHYQQKPG QAPRLLIYDT
SKLASGVPSR FSGSGSGTDH FLTSSLEAE DAATYYCFQG SVYPFTFGQG
 TKLEIK

[00343] The present application specifically includes and encompasses CD19 Binding Molecules (e.g., CD19 x CD3 bispecific Binding Molecules) that are capable of binding to CD19, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_{LS} of the VL Domain and/or 1, 2 or all 3 of the CDR_{HS} of the VH Domain of the anti-CD19 monoclonal antibody **CD19 mAb 1**, or any of the anti-CD19 antibodies disclosed in US Patent US 7,112,324. The present invention specifically includes and encompasses exemplary CD19 x CD3 bispecific Binding Molecules that may be employed in the present invention, including: blinatumomab (BLINCYTO®; amino acid sequence found in WHO Drug Information, 2009, Recommended INN: List 62, 23(3):240-241) and duvortuxizumab (aka MGD011; amino acid sequence found in WHO Drug Information, 2016, Proposed INN: List 116, 30(4):627-629).

B. Exemplary Pathogen-Associated Antigens

[00344] As used herein, the term “**Pathogen Antigen**” denotes an antigen that is characteristically expressed on the surface of a pathogen-infected cell, and that may thus be treated with an Antibody-Based Molecule or an Immunomodulatory Molecule. Examples of Pathogen Antigens include, but are not limited to antigens expressed on the surface of a cell infected with: a Herpes Simplex Virus (e.g., infected cell protein (ICP)47, gD, etc.), a varicella-zoster virus, a Kaposi’s sarcoma-associated herpesvirus, an Epstein-Barr Virus (e.g., LMP-1, LMP-2A, LMP-2B, etc.), a Cytomegalovirus (e.g., UL11, etc.), Human Immunodeficiency Virus (e.g., env proteins gp160, gp120, gp41, etc.), a Human Papillomavirus (e.g., E6, E7, etc.), a human T-cell leukemia virus (e.g., env proteins gp64, gp46, gp21, etc.), Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Vesicular Stomatitis Virus (VSV), *Bacilli*, *Citrobacter*, *Cholera*, *Diphtheria*, *Enterobacter*, *Gonococci*, *Helicobacter pylori*, *Klebsiella*, *Legionella*, *Meningococci*, mycobacteria, *Pseudomonas*, *Pneumococci*, rickettsia bacteria, *Salmonella*, *Serratia*, *Staphylococci*, *Streptococci*, *Tetanus*, *Aspergillus* (fumigatus, niger, etc.), *Blastomyces dermatitidis*, *Candida* (albicans, krusei, glabrata, tropicalis, etc.), *Cryptococcus neoformans*, Genus *Mucorales* (mucor, absidia, rhizopus), *Sporothrix schenkii*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Leptospirosis*, *Borrelia burgdorferi*, helminth parasite (hookworm, tapeworms, flukes, flatworms (e.g. *Schistosomia*), *Giardia lamblia*, *trichinella*, *Dientamoeba Fragilis*, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*). Such antibodies are available

commercially from a wide number of sources, or can be obtained by immunizing mice or other animals (including for the production of monoclonal antibodies) with such antigens.

[00345] Exemplary antibodies, whose VH and VL Domains may be used to construct molecules capable of binding a Pathogen Antigen arrayed on the surface of a pathogen-infected cell are antibodies are provided below, additional antibodies are known in the art.

1. Exemplary Anti-HIV env Antibody

[00346] The env protein of HIV is an exemplary Pathogen-Associated Antigen, and antibodies that bind the env protein of HIV are exemplary of antibodies capable of binding a Pathogen-Associated Antigen.

[00347] The initial step in HIV-1 infection occurs with the binding of cell surface CD4 to trimeric HIV-1 envelope glycoproteins (env), a heterodimer of a transmembrane glycoprotein (gp41) and a surface glycoprotein (gp120). The gp120 and gp41 glycoproteins are initially synthesized as a single gp160 polypeptide that is subsequently cleaved to generate the non-covalently associated gp120/gp41 complex. The ectodomain of env is a heterodimer with mass of approximately 140 kDa, composed of the entire gp120 component, and approximately 20 kDa of gp41 (Harris, A. *et al.* (2011) “*Trimeric HIV-1 Glycoprotein Gp140 Immunogens And Native HIV-1 Envelope Glycoproteins Display The Same Closed And Open Quaternary Molecular Architectures*,” Proc. Natl. Acad. Sci. (U.S.A.) 108(28):11440-11445). Antibodies that that immunospecifically bind to env proteins are commercially available and have been described in the art (see, *e.g.*, GenBank Accession No. AFQ31503; Buchacher, A. *et al.* (1994) “*Generation Of Human Monoclonal Antibodies Against HIV-1 Proteins; Electroporation And Epstein-Barr Virus Transformation For Peripheral Blood Lymphocyte Immortalization*,” AIDS Res. Hum. Retroviruses 10(4):359-369; Shen, R. (2010) “*GP41-Specific Antibody Blocks Cell-Free HIV Type 1 Transcytosis Through Human Rectal Mucosa And Model Colonic Epithelium*,” J. Immunol. 184(7):3648-3655; WO 2012/162068; and WO 2016/054101). Exemplary antibodies that bind to HIV env include “**7B2**” (GenBank Accession No. AFQ31503) and “**A32**” (PCT Publication No. WO 2014/159940). Multiple VH Domains of Antibody A32 have been reported in the art that possess minor changes in framework regions 1 and/or 4 reported (see, *e.g.*, Protein Data Base Accession number PDB: 4YBL_H, US 2015/0239961 and WO 2006/044410). Any of these variant Antibody A32 VH Domains may be employed in accordance with the present invention.

[00348] The amino acid sequence of the VH Domain of **7B2 (SEQ ID NO:166)** is shown below (CDR residues are shown underlined):

QVQLVQSGGG VFKPGGSLRL SCEASGFTFT EYYMTWVRQA PGKGLEWLAY
ISKNGEYSKY SPSSNGRFTI SRDNAKNSVF LQLDRLSADD TAVYYCARAD
GLTYFSELLQ YIFDLWGQGA RVTVSS

[00349] The amino acid sequence of the VL Domain of **7B2 (SEQ ID NO:167)** is shown below (CDR residues are shown underlined):

DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP
 KLLLYWASMR LSGVDPDRFSG SGSGTDFTLT INNLAEDVA IYYCHQYSSH
PPTFGHGTRV EIK

[00350] The amino acid sequence of an exemplary VH Domain of **A32 (SEQ ID NO:168)** is shown below (CDR residues are shown underlined):

QVQLQESGPG LVKPSQTLST SCTVSGGSSS SGAHYWSWIR QYPGKGLEWI
GYIHYSNTY YNPSLKSRIT ISQHTSENQF SLKLNSVTVA DTAVYYCARG
TRLRTLNAF DIWGQGTXTV VSS
 wherein: X is L or M

[00351] The amino acid sequence of such an exemplary VH Domain of **A32 (SEQ ID NO:209)**, wherein X is L, is shown below (CDR residues are shown underlined):

QVQLQESGPG LVKPSQTLST SCTVSGGSSS SGAHYWSWIR QYPGKGLEWI
GYIHYSNTY YNPSLKSRIT ISQHTSENQF SLKLNSVTVA DTAVYYCARG
TRLRTLNAF DIWGQGTTLV VSS

[00352] The amino acid sequence of the VL Domain of **A32 (SEQ ID NO:169)** is shown below (CDR residues are shown underlined):

QSALTQPPSA SGSPGQSVTI SCTTGTSSDVG GYNYVSWYQH HPGKAPKLII
EVNNRPSGV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFF
 FGGGTKLTVL

[00353] The present application specifically includes and encompasses HIV Binding Molecules (*e.g.*, HIV x CD3 bispecific Binding Molecules) that are capable of binding to HIV, and particularly such Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-HIV monoclonal antibodies **7B2**, **A32**, and also any of the anti-HIV antibodies disclosed in WO 2016/054101, WO 2017/011413, WO 2017/011414. The present invention specifically includes and encompasses the exemplary HIV x CD3 bispecific Binding Molecules provided in WO 2014/159940, WO 2015/184203, WO 2017/011413, and WO 2017/011414.

[00354] The present application additionally specifically includes and encompasses HIV x CD3 x CD8 trispecific Binding Molecules that are capable of binding to HIV, to CD3 and to CD8, and particularly such trispecific Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-HIV monoclonal antibodies **7B2** or **A32** or of any of the anti-HIV monoclonal antibodies provided in WO 2015/184203, WO 2016/054101, WO 2017/011413, WO 2017/011414, and/or the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of any of the anti-CD8 monoclonal antibodies provided in WO 2015/184203.

2. Exemplary Anti- RSV glycoprotein F Antibody

[00355] A further illustrative Pathogen-Associated Antigen is RSV glycoprotein F. An exemplary anti-RSV glycoprotein F antibody is palivizumab (see, *e.g.*, Protein Data Bank (PDB) ID No. 2HWZ). Alternative anti-RSV glycoprotein F antibodies include motavizumab (see, *e.g.*, PDB ID No. 3IXT) and a variant of palivizumab that has been engineered to remove a cysteine residue from palivizumab's CDR_{L1}. The amino acid sequence of the VH Domain of the variant of palivizumab (**SEQ ID NO:170**) is shown below (CDR residues are shown underlined):

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMSVGWIR QPPGKALEWL
ADIWDDKKD YNPSLKSRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARS
MITNWFFDVW GAGTTVTVSS

[00356] The amino acid sequence of the VL Domain of the variant of palivizumab (**SEQ ID NO:171**) is shown below (CDR residues are shown underlined):

DIQMTQSPST LSASVGDRVT ITCRASQSVG YMHWYQQKPG KAPKLLIYDT
SKLASGVPSR FSGSGSGTEF TLTISLQPD DFATYYCFQG SGYPFTFGGG
TKLEIK

VII. Exemplary Binding Molecules of the Present Invention

[00357] As discussed below, the present invention is illustrated using several **DA x CD3 Binding Molecules** having different structures including molecules capable of mediating the redirected killing of a tumor cell (*e.g.*, a “**DART-A**”-type diabody or a “**DART-B**”-type diabody or a TRIVALENT-type molecule, as described below).

A. DART-A-Type Diabodies

[00358] **DART-A**-type diabodies are bispecific diabodies capable of binding CD3 and a Disease Antigen (*e.g.*, a Cancer Antigen) that do not comprise an Fc Domain. Provided

herein are illustrative **DART-A**-type diabodies composed of two polypeptide chains having one binding site for CD3 and one binding site for the Cancer Antigen CD123 (see, *e.g.*, **Figure 1**).

[00359] An illustrative **DART-A**-type diabody (designated “**DART-A-WT**”) has a first polypeptide chain having the amino acid sequence of **SEQ ID NO:172**:

```
DFVMTQSPDS LAVSLGERVT MSCSSQSLN NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SSGTDFTLT ISSLQAEDVA VYYCNDYSY
PYTFGQGTKL EIKGGGSGGGGEVQLVESGG GLVQPGGSLR LSCAASGFTF
STYAMNWVRQ APGKGLEWVG RIRSKYNNYA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT LVTVSSGGCG
GGKVAAALKEK VAALKEKVAA LKEKVAALKE
```

[00360] Residues 1-113 of the first polypeptide chain of such illustrative **DART-A**-type diabody correspond to the VL Domain of **CD123 mAb 1 (SEQ ID NO:162)**. Residues 114-121 (double underlined) of the first polypeptide chain of such illustrative **DART-A**-type diabody correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of such illustrative **DART-A**-type diabody correspond to the VH Domain of **CD3 mAb 1 (SEQ ID NO:55)**, wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of such illustrative **DART-A**-type diabody correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of such illustrative **DART-A**-type diabody correspond to the heterodimer-promoting “K-coil” (KVAAALKE-KVAAALKE-KVAAALKE-KVAAALKE; **SEQ ID NO:30**).

[00361] The second polypeptide chain of such illustrative **DART-A**-type diabody **DART-A-WT** has the amino acid sequence of **SEQ ID NO:173**:

```
QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGEV QLVQSGAELK KPGASVKVSC KASGYTFTDY
YMKWVRQAPG QGLEWIGDII PSNGATFYNQ KFKGRVTITV DKSTSTAYME
LSSLRSEDTA VYYCARSHLL RASWFAYWGQ GTLVTVSSGG CGGGEVAAALE
KEVAALKEV AALEKEVAAL EK
```

[00362] Residues 1-110 of the second polypeptide chain of such illustrative **DART-A**-type diabody **DART-A-WT** correspond to the VL Domain of **CD3 mAb 1 (SEQ ID NO:56)**. Residues 111-118 (double underlined) of the second polypeptide chain of such illustrative **DART-A**-type diabody correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-238 of the second polypeptide chain of such illustrative **DART-A**-type diabody

correspond to the VH Domain of **CD123 mAb 1** (SEQ ID NO:163). Residues 239-244 (underlined) of the second polypeptide chain of such illustrative **DART-A**-type diabody correspond to Linker 2 (GGCGGG; SEQ ID NO:17). Residues 245-272 of the second polypeptide chain of such illustrative **DART-A**-type diabody correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; SEQ ID NO:29).

[00363] As will be recognized in view of the instant disclosure, additional **DART-A**-type diabodies having a binding site for an alternative Disease Antigen and/or having the CD3 Binding Domains of a variant anti-CD3 antibody (*i.e.*, a vCD3-Binding Domain) may likewise be constructed (by employing the VL and VH Domains of such antibodies in lieu of the VL and VH Domains of the illustrative **DART-A**-type diabody). Similarly, as provided herein, alternative **DART-A**-type molecules may likewise be constructed incorporating alternative Linkers and/or alternative Heterodimer-Promoting Domains. For example, an illustrative panel of CD123 x CD3 **DART-A**-type diabodies were generated having the same structure as **DART-A-WT** diabody provided above, but comprising the VL and VH Domains of one of the **CD3 mAb 1** variants (**M1-M26**) provided above.

[00364] Each illustrative CD123 x CD3 **DART-A**-type diabody of the panel has a first polypeptide chain having the amino acid sequence of SEQ ID NO: SEQ ID NO:189:

DFVMTQSPDS	LAVSLGERVT	MSCKSSQSLL	NSGNQKNYLT
WYQQKPGQPP	KLLIYWASTR	ESGVPDRFSG	SGSGTDFTLT
ISSLAEDVA	VYYCQNDYSY	PYTFGQGTKL	EIK <u>GGGSGGG</u>
<u>GEVQLVESGG</u>	GLVQPGGSLR	LSCAASGFTF	SX ₁ X ₂ X ₃ MNWVRQ
APGKGLEWVG	RIRSKYNNTA	TYYADSVKX ₄ R	FTISRDDSKN
SLYLQMNSLK	TEDTAVYYCV	RHX ₅ NX ₆ X ₇ NSX ₈ V	X ₉ X ₁₀ FAX ₁₁ WGQGT
LVTVSS <u>GGCG</u>	<u>GGKVAALKEK</u>	VAALKEKVAA	LKEKVAALKE

wherein: X₁ is T, D, or E; X₂ is Y, D or T; X₃ is A or G; X₄ is D or G; X₅ is G, D, E, or K; X₆ is F or I; X₇ is G or I; X₈ is Y, A, G, or Q; X₉ is S or T; X₁₀ is W, F, or Y; and X₁₁ is Y or E.

[00365] Residues 1-113 of the first polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the VL Domain of **CD123 mAb 1** (SEQ ID NO:162). Residues 114-121 (double underlined) of the first polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to Linker 1 (GGGSGGGG; SEQ ID NO:16; double underlined). Residues 122-246 of the first polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the VH Domain of **CD3 mAb 1 M1 - CD3 mAb 1 M22** (SEQ ID NOs: 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92,

94, 96, 98, 100, 102, 104 or 106). Residues 247-252 (single underlined) of the panel of illustrative **DART-A**-type diabodies correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the heterodimer-promoting “K-coil” (KVAAALKE-KVAAALKE-KVAAALKE-KVAAALKE; **SEQ ID NO:30**).

[00366] The second polypeptide chain of such illustrative **DART-A**-type diabody has the amino acid sequence of **SEQ ID NO:190**:

QAVVTQEPSL	TVSPGGTVTL	TCRSSTGAVT	TSNYANWVQQ
KPGQAPRGLI	GX ₁ TN ₂ RAPWT	PARFSGSLLG	GKAALTITGA
QAEDEADYYC	AX ₃ WYSNLWVF	GGGTKLTVLG	<u>GGGSGGGG</u> EV
QLVQSGAELK	KPGASVKVSC	KASGYTFTDY	YMKWVRQAPG
QGLEWIGDII	PSNGATFYNQ	KFKGRVTITV	DKSTSTAYME
LSSLRSEDTA	VYYCARSHLL	RASWFAYWGQ	GTLVTVSS <u>GG</u>
<u>CGGGE</u> VAALE	KEVAALEKEV	AALEKEVAAL	EK

wherein: X₁ is G or D; X₂ is K or G; and X₃ is L, E or Q.

[00367] Residues 1-110 of the second polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the VL Domain of **CD3 mAb 1 M23 – CD3 mAb 1 M26** (**SEQ ID NOs:108, 110, 112, and 114**). Residues 111-118 (double underlined) of the second polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**; double underlined). Residues 119-238 of the second polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the VH Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 239-244 (underlined) of the second polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to Linker 2 (GGCGGG; **SEQ ID NO:17**; single underlined). Residues 245-272 of the second polypeptide chain of the panel of illustrative **DART-A**-type diabodies correspond to the heterodimer-promoting “E-coil” (EVAAALEK-EVAAALEK-EVAAALEK-EVAAALEK; **SEQ ID NO:29**).

[00368] The amino acid sequences and designations of the panel of illustrative **DART-A**-type diabodies comprising the VL and VH of the **CD3 mAb 1** variants are provided in **Table 8** below.

Table 8 Illustrative DART-A-Type Diabodies		
Designation	First Polypeptide Chain SEQ ID NO:	Second Polypeptide Chain SEQ ID NO.
DART-A-M1	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is T; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M2	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is T; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M3	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is I; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M4	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is A; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M5	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is G; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M6	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Q; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M7	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is D; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M8	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is E; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M9	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M10	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is I; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M11	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is F; and X ₁₁ is Y	173
DART-A-M12	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is Y; and X ₁₁ is Y	173
DART-A-M13	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is E	173
DART-A-M14	189 - wherein: X ₁ is D; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173

Table 8 Illustrative DART-A-Type Diabodies		
Designation	First Polypeptide Chain SEQ ID NO:	Second Polypeptide Chain SEQ ID NO.
DART-A-M15	189 - wherein: X ₁ is E; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M16	189 - wherein: X ₁ is T; X ₂ is D; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M17	189 - wherein: X ₁ is T; X ₂ is T; X ₃ is A; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M18	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is G; X ₄ is D; X ₅ is G; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M19	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is I; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M20	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is F; X ₇ is G; X ₈ is G; X ₉ is S; X ₁₀ is W; and X ₁₁ is Y	173
DART-A-M21	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is F; and X ₁₁ is Y	173
DART-A-M22	189 - wherein: X ₁ is T; X ₂ is Y; X ₃ is A; X ₄ is D; X ₅ is K; X ₆ is F; X ₇ is G; X ₈ is Y; X ₉ is S; X ₁₀ is Y; and X ₁₁ is Y	173
DART-A-M23	172	190 - wherein: X ₁ is G; X ₂ is K; and X ₃ is E
DART-A-M24	172	190 - wherein: X ₁ is G; X ₂ is K; and X ₃ is Q
DART-A-M25	172	190 - wherein: X ₁ is D; X ₂ is K; and X ₃ is L
DART-A-M26	172	190 - wherein: X ₁ is G; X ₂ is G; and X ₃ is L

B. DART-B-Type Diabodies

[00369] **DART-B**-type diabodies are bispecific diabodies capable of binding CD3 and a Disease Antigen (*e.g.*, a Cancer or Infectious Disease Antigen) that comprise an Fc Domain. Provided herein are illustrative **DART-B**-type diabodies (**Table 9**) composed of three polypeptide chains and have one binding site for CD3 and one binding site for the Cancer Antigen CD123, 5T4, or CD19 (see, *e.g.*, **Figure 4A**).

Table 9						
DART-B-Type No.	Disease Antigen-Binding Domain	CD3 Binding Domain	Polypeptide Chain			Designation
			First	Second	Third	
			CD123 / CD3 Binding Domains		Fc Domain	
1	CD123 mAb 1	CD3 mAb 1	SEQ ID NO:174	SEQ ID NO:175	SEQ ID NO:176	CD123-WT
2		CD3 mAb 1 M1	SEQ ID NO:177			CD123-M1
3		CD3 mAb 1 M2	SEQ ID NO:178			CD123-M2
4		CD3 mAb 1 M18	SEQ ID NO:179			CD123-M18
5		CD3 mAb 1 M13	SEQ ID NO:198			CD123-M13
6		CD3 mAb 1 M17	SEQ ID NO:199			CD123-M17
7		CD3 mAb 1 M19	SEQ ID NO:200			CD123-M19
8	5T4 mAb 1	CD3 mAb 1	SEQ ID NO:180	SEQ ID NO:181		5T4-WT
9		CD3 mAb 1 M1	SEQ ID NO:182			5T4-M1
10		CD3 mAb 1 M2	SEQ ID NO:183			5T4-M2
11		CD3 mAb 1 M18	SEQ ID NO:184			5T4-M18
12	HIV mAb A32	CD3 mAb 1	SEQ ID NO:185	SEQ ID NO:186		HIV-WT
13		CD3 mAb 1 M18	SEQ ID NO:196			HIV-M18
14	CD19 mAb 1 (alternative VL where indicated)	CD3 mAb 1	SEQ ID NO:191	SEQ ID NO:192		CD19-WT
15		CD3 mAb 1 M18	SEQ ID NO:197			CD19-M18
16		CD3 mAb 1 M18	SEQ ID NO:193	SEQ ID NO:194		CD19.1-M18
17		CD3 mAb 1 M13	SEQ ID NO:201			CD19.1-M13
18		CD3 mAb 1 M17	SEQ ID NO:202			CD19.1-M17
19		CD3 mAb 1 M19	SEQ ID NO:203			CD19.1-M19

1. First Illustrative DART-B-Type Diabody CD123-WT (CD123 x CD3 mAb 1)

[00370] A first illustrative **DART-B**-type diabody (designated “**CD123-WT**”) has a first polypeptide chain having the amino acid sequence of **SEQ ID NO:174**:

DFVMTQSPDS LAVSLGERVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP
 KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
 PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
 STYAMNWVRQ APGKGLEWVG RIRSKYNNA TYYADSVKDR FTISRDDSKN
 SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT LVTVSSGGCG
GGEVAALEKE VAALKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG

PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
 KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
 ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
 QKSLSLSPGK

[00371] Residues 1-113 of the first polypeptide chain of **CD123-WT** correspond to the VL Domain of **CD123 mAb 1 (SEQ ID NO:163)**. Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-WT** correspond to the VH Domain of **CD3 mAb 1 (SEQ ID NO:55)**, wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-WT** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-WT** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-WT** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-WT** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-WT** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00372] The second polypeptide chain of **CD123-WT** has the amino acid sequence of **SEQ ID NO:175**:

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG GGGSGGGGEV QLVQSGAELK KPGASVKVSC KASGYTFTDY
 YMKWVRQAPG QGLEWIGDII PSNGATFYNQ KFKGRVTITV DKSTSTAYME
 LSSLRSEDTA VYYCARSHLL RASWFAYWGQ GTLVTVSSGG CGGGKVAALK
 EKVAALKEKV AALKEKVAAL KE

[00373] Residues 1-110 of the second polypeptide chain of **CD123-WT** correspond to the VL Domain of **CD3 mAb 1 (SEQ ID NO:56)**. Residues 111-118 (double underlined) of the second polypeptide chain of **CD123-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-238 of the second polypeptide chain of **CD123-WT** correspond to the VH Domain of **CD123 mAb 1 (SEQ ID NO:162)**. Residues 239-244 (underlined) of the second polypeptide chain correspond to Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 245-272 of the second polypeptide chain of **CD123-WT** correspond to the heterodimer-promoting “K-coil” (KVAALKE-KVAALKE-KVAALKE-KVAALKE; **SEQ ID NO:30**).

[00374] The third polypeptide chain of **CD123-WT** has the amino acid sequence of **SEQ ID NO:176**:

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
 CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK
 GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLVSKL TVDKSRWQQG
 NVFSCSV MHE ALHNRYTQKS LSLSPGK

[00375] Residues 1-10 of the third polypeptide chain of **CD123-WT** correspond to the Linker **DKTHTCPPCP (SEQ ID NO:40)**. Residues 10-227 of the third polypeptide chain of **CD123-WT** correspond to the IgG1 “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:50**).

[00376] As will be recognized, the third polypeptide chain of **CD123-WT** does not contain any Epitope-Binding Domains and may thus be employed in various **DA x CD3 Binding Molecules** having such **DART-B-type** structure.

2. Second Illustrative DART-B-Type Diabody CD123-M1 (CD123 x CD3 mAb 1 M1)

[00377] A second illustrative **DART-B-type** diabody is similar to the above-described **CD123-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M1** and is designated “**CD123-M1**”. As indicated above, **CD3 mAb 1 M1** is a low affinity variant of **CD3 mAb 1**, (also referred to as “**CD3 mAb 1 Low**”). As also indicated above, the VL Domain of **CD3 mAb 1 M1** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00378] Thus, the second illustrative **DART-B-type** diabody (**CD123-M1**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:177**):

DFVMTQSPDS LAVSLGERVT MSCKSSQSLN NSGNQKNYLT WYQQKPGQPP
 KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLAEDVA VYYCQNDYSY
 PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
 STYAMNWVRQ APGKGLEWVG RIRSKYNNTA TYYADSVKDR FTISRDDSKN
 SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV TWFAFWGQGT LTVSSGGCG
GGEVAALEKE VALEKEVAA LEKEVAALEK GGGDKHTTCP PCPAPEAAGG
 PSVFLFPPKP KDTLMISRTPEVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
 KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
 ENNYKTTTPV LQSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
 QKSLSLSPGK

[00379] Residues 1-113 of the first polypeptide chain of **CD123-M1** correspond to the VL Domain of **CD123 mAb 1 (SEQ ID NO:163)**. Residues 114-121 (double underlined) of

the first polypeptide chain of **CD123-M1** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M1** correspond to the VH Domain of **CD3 mAb 1 M1** (**SEQ ID NO:55**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M1** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M1** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M1** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M1** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M1** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00380] Since the VL Domain of **CD3 mAb 1 M1** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M1** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly, the amino acid sequence of the third polypeptide chain of **CD123-M1** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

3. Third Illustrative DART-B-Type Diabody CD123-M2 (CD123 x CD3 mAb 1 M2)

[00381] A third illustrative **DART-B**-type diabody is similar to the above-described **CD123-M1** diabody, but contains the VH Domain of **CD3 mAb 1 M2** and is designated “**CD123-M2**”. As indicated above, **CD3 mAb 1 M2** has a faster off-rate than **CD3 mAb 1**, and is thus also referred to as “**CD3 mAb 1 Fast**.” As also indicated above, the VL Domain of **CD3 mAb 1 M2** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00382] Thus, the third illustrative **DART-B-type** diabody (**CD123-M2**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:178**):

```
DFVMTQSPDS LAVSLGERVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
STYAMNWVRQ APGKGLEWVG RIRSKYNNYA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHKNFGNSYV TWFAIWGQGT LVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK
```

[00383] Residues 1-113 of the first polypeptide chain of **CD123-M2** correspond to the VL Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-M2** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M2** correspond to the VH Domain of **CD3 mAb 1 M2** (**SEQ ID NO:59**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M2** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M2** correspond to the heterodimer-promoting “E-coil” (EVAALEEK-EVAALEEK-EVAALEEK-EVAALEEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M2** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M2** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M2** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00384] Since the VL Domain of **CD3 mAb 1 M2** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M2** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly, the amino acid sequence of the third polypeptide chain of **CD123-M2** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

4. Fourth Illustrative DART-B-Type Diabody CD123-M18 (CD123 x CD3 mAb 1 M18)

[00385] A fourth illustrative **DART-B-type** diabody is similar to the above-described **CD123-M2** diabody, but contains the VH Domain of **CD3 mAb 1 M18** and is designated

“**CD123-M18**”. As indicated above, the VL Domain of **CD3 mAb 1 M18** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00386] Thus, the fourth illustrative **DART-B**-type diabody (**CD123-M18**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:179**):

```
DFVMTQSPDS LAVSLGERVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
STYGMNWVRQ APGKGLEWVG RIRSKYNNYA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT LVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK
```

[00387] Residues 1-113 of the first polypeptide chain of **CD123-M18** correspond to the VL Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M18** correspond to the VH Domain of **CD3 mAb 1 M18** (**SEQ ID NO:98**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M18** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M18** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M18** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M18** correspond to the Linker DKTHTCPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M18** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00388] Since the VL Domain of **CD3 mAb 1 M18** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M18** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly, the amino acid sequence of the third polypeptide chain of **CD123-M18** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

5. Fifth Illustrative DART-B-Type Diabody CD123-M13 (CD123 x CD3 mAb 1 M13)

[00389] A fifth illustrative **DART-B**-type diabody is similar to the above-described **CD123-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M13** and is designated “**CD123-M13**”. As indicated above, the VL Domain of **CD3 mAb 1 M13** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00390] Thus, the fifth illustrative **DART-B**-type diabody (**CD123-M13**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:198**):

```
DFVMTQSPDS LAVSLGERVT MSCKSSQSLN NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLAEDVA VYYCQNDYSY
PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
STYAMNWVRQ APGKGLEWVG RIRSKYNNA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAEWGQGT LVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK
```

[00391] Residues 1-113 of the first polypeptide chain of **CD123-M13** correspond to the VL Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-M13** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M13** correspond to the VH Domain of **CD3 mAb 1 M13** (**SEQ ID NO:88**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M1** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M13** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M13** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M13** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M13** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00392] Since the VL Domain of **CD3 mAb 1 M13** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M13** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly,

the amino acid sequence of the third polypeptide chain of **CD123-M1** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

6. Sixth Illustrative DART-B-Type Diabody CD123-M17 (CD123 x CD3 mAb 1 M17)

[00393] A sixth illustrative **DART-B**-type diabody is similar to the above-described **CD123-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M17** and is designated “**CD123-M17**”. As indicated above, the VL Domain of **CD3 mAb 1 M17** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00394] Thus, the sixth illustrative **DART-B**-type diabody (**CD123-M17**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:199**):

```
DFVMTQSPDS LAVSLGERVT MSCKSSQSLN NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
STTAMNWVRQ APGKGLEWVG RIRSKYNNYA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHGNFGNSYV SWFAYWGQGT LVTVSSGGCG
GGEVAALEKE VAALEKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
ENNYKTTTPV LDSGGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK
```

[00395] Residues 1-113 of the first polypeptide chain of **CD123-M17** correspond to the VL Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-M17** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M17** correspond to the VH Domain of **CD3 mAb 1 M17** (**SEQ ID NO:96**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M17** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M17** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M17** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M17** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M17** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00396] Since the VL Domain of **CD3 mAb 1 M17** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M17** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly, the amino acid sequence of the third polypeptide chain of **CD123-M17** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

7. Seventh Illustrative DART-B-Type Diabody CD123-M19 (CD123 x CD3 mAb 1 M19)

[00397] A seventh illustrative **DART-B**-type diabody is similar to the above-described **CD123-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M19** and is designated “**CD123-M19**”. As indicated above, the VL Domain of **CD3 mAb 1 M19** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00398] Thus, the seventh illustrative **DART-B**-type diabody (**CD123-M19**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:200**):

```
DFVMTQSPDS LAVSLGERVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP
KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIKGGGSGGG GEVQLVESGG GLVQPGGSLR LSCAASGFTF
STYAMNWVRQ APGKGLEWVG RIRSKYNNTA TYYADSVKDR FTISRDDSKN
SLYLQMNSLK TEDTAVYYCV RHKNIGNSYV SWFAYWGQGT LVTVSSGGCG
GGEVAALEKE VAALKEVAA LEKEVAALEK GGGDKTHTCP PCPAPEAAGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
KAKGQPREPQ VYTLPPSREE MTKNQVSLWC LVKGFYPSDI AVEWESNGQP
ENNYKTTTPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
QKSLSLSPGK
```

[00399] Residues 1-113 of the first polypeptide chain of **CD123-M19** correspond to the VL Domain of **CD123 mAb 1** (**SEQ ID NO:163**). Residues 114-121 (double underlined) of the first polypeptide chain of **CD123-M19** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 122-246 of the first polypeptide chain of **CD123-M19** correspond to the VH Domain of **CD3 mAb 1 M19** (**SEQ ID NO:100**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 247-252 (underlined) of the first polypeptide chain of **CD123-M19** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 253-280 of the first polypeptide chain of **CD123-M19** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 281-283 of the first polypeptide chain of **CD123-M19** correspond to a GGG Linker. Residues 284-293 (underlined) of the first polypeptide chain of **CD123-M1** correspond to the Linker

DKTHTCPPCP (**SEQ ID NO:40**). Residues 294-510 of the first polypeptide chain of **CD123-M19** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00400] Since the VL Domain of **CD3 mAb 1 M19** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD123-M19** is the same as that of the second polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:175**). Similarly, the amino acid sequence of the third polypeptide chain of **CD123-M1** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

8. Eighth Illustrative DART-B-Type Diabody 5T4-WT (5T4 x CD3 mAb 1)

[00401] An eighth illustrative **DART-B**-type diabody is similar to the above-described **CD123-M18** diabody, but comprises a 5T4 Binding Domain in lieu of the CD123 Binding Domain of the **CD123-M18** diabody. Additionally, the eighth illustrative **DART-B**-type diabody contains the VH Domain of **CD3 mAb 1**. This eighth illustrative DART-B-type diabody is designated “**5T4-WT**”.

[00402] Thus, the eighth illustrative **DART-B**-type diabody (**5T4-WT**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:180**):

```
DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISR DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLLVTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPFVLDSGGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK
```

[00403] Residues 1-107 of the first polypeptide chain of **5T4-WT** correspond to the VL Domain of **5T4 mAb 1** (**SEQ ID NO:157**). Residues 108-115 (double underlined) of the first polypeptide chain of **5T4-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 116-240 of the first polypeptide chain of **5T4-WT** correspond to the VH Domain of **CD3 mAb 1** (**SEQ ID NO:55**), wherein Kabat position 65 (double underlined) is glycine (G). Residues 241-246 (underlined) of the first polypeptide chain of **5T4-WT** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 247-274 of the first polypeptide chain of **5T4-WT** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-

EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 275-277 of the first polypeptide chain of **5T4-WT** correspond to a GGG Linker. Residues 278-287 (underlined) of the first polypeptide chain of **5T4-WT** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 288-504 of the first polypeptide chain of **5T4-WT** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00404] The second polypeptide chain of **5T4-WT** has the amino acid sequence (**SEQ ID NO:181**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTSF
WMHWVRQAPG QGLEWMGRID PNRGGTEYNE KAKSRVTMTA DKSTSTAYME
LSSLRSEDTA VYYCAGGNPY YPMDYWGQGT TVTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE

[00405] Residues 1-110 of the second polypeptide chain of **5T4-WT** correspond to the VL Domain of **CD3 mAb 1** (**SEQ ID NO:56**). Residues 111-118 (double underlined) of the second polypeptide chain of **5T4-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-236 of the second polypeptide chain of **5T4-WT** correspond to the VH Domain of **5T4 mAb 1** (**SEQ ID NO:156**). Residues 237-242 (underlined) of the second polypeptide chain of **5T4-WT** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 243-280 of the second polypeptide chain of **5T4-WT** correspond to the heterodimer-promoting “K-coil” (KVAALKE-KVAALKE-KVAALKE-KVAALKE; **SEQ ID NO:30**).

[00406] The third polypeptide chain of **5T4-WT** has the same amino acid sequence as the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

9. Ninth Illustrative DART-B-Type Diabody 5T4-M1 (5T4 x CD3 mAb 1 M1)

[00407] A ninth illustrative **DART-B**-type diabody is similar to the above-described **5T4-WT** diabody, but comprises the VH Domain of **CD3 mAb 1 M1** and is designated “**5T4-M1**.”

[00408] Thus, the ninth illustrative **DART-B**-type diabody (**5T4-M1**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:182**):

DIQMTQSPSS LSASVGDRVIT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ

GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
 WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISR DSKNSLYLQM
 NSLKTEDTAV YYCVRHGNFG NSYVTWFAYW GQGTLVTVSS GGCGGGEVAA
 LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPPAPE AAGGPSVFLF
 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
 EQYNSTYRVV SVLTIVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
 REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
 TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 SPGK

[00409] Residues 1-107 of the first polypeptide chain of **5T4-M1** correspond to the VL Domain of **5T4 mAb 1 (SEQ ID NO:157)**. Residues 108-115 (double underlined) of the first polypeptide chain of **5T4-M1** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 116-240 of the first polypeptide chain of **5T4-M1** correspond to the VH Domain of **CD3 mAb 1 M1 (SEQ ID NO:64)**, wherein Kabat position 65 (double underlined) is aspartate (D). Residues 241-246 (underlined) of the first polypeptide chain of **5T4-M1** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 247-274 of the first polypeptide chain of **5T4-M1** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 275-277 of the first polypeptide chain of **5T4-M1** correspond to a GGG Linker. Residues 278-287 (underlined) of the first polypeptide chain of **5T4-M1** correspond to the Linker DKHTTCPPCP (**SEQ ID NO:40**). Residues 288-504 of the first polypeptide chain of **5T4-M1** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00410] Since the VL Domain of **CD3 mAb 1 M1** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **5T4-M1** is the same as that of the second polypeptide chain of the **5T4-WT** diabody (*i.e.*, **SEQ ID NO:181**). Similarly, the amino acid sequence of the third polypeptide chain of **5T4-M1** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

10. Tenth Illustrative DART-B-Type Diabody 5T4-M2 (5T4 x CD3 mAb 1 M2)

[00411] A tenth illustrative **DART-B**-type diabody is similar to the above-described **5T4-M1** diabody, but comprises the VH Domain of **CD3 mAb 1 M2** and is designated “**5T4-M2**”.

[00412] Thus, the tenth illustrative **DART-B**-type diabody (**5T4-M2**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:183**):

```

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
GTKLEIKGGG SGGGGEVQLV ESGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISR DSKNSLYLQM
NSLKTEDTAV YYCVRHKNFG NSYVTWFAYW GQGTLVTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

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[00413] Residues 1-107 of the first polypeptide chain of **5T4-M2** correspond to the VL Domain of **5T4 mAb 1** (**SEQ ID NO:157**). Residues 108-115 (double underlined) of the first polypeptide chain of **5T4-M2** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 116-240 of the first polypeptide chain of **5T4-M2** correspond to the VH Domain of **CD3 mAb 1 M2** (**SEQ ID NO:66**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 241-246 (underlined) of the first polypeptide chain of **5T4-M2** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 247-274 of the first polypeptide chain of **5T4-M2** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 275-277 of the first polypeptide chain of **5T4-M2** correspond to a GGG Linker. Residues 278-287 (underlined) of the first polypeptide chain of **5T4-M2** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 288-504 of the first polypeptide chain of **5T4-M2** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00414] Since the VL Domain of **CD3 mAb 1 M2** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **5T4-M2** is the same as that of the second polypeptide chain of the **5T4-WT** diabody (*i.e.*, **SEQ ID NO:181**). Similarly, the amino acid sequence of the third polypeptide chain of **5T4-M2** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

11. Eleventh Illustrative DART-B-Type Diabody 5T4-M18 (5T4 x CD3 mAb 1 M18)

[00415] An eleventh illustrative **DART-B**-type diabody is similar to the above-described **5T4-WT** diabody, but comprises the VH Domain of **CD3 mAb 1 M18** and is designated “**5T4-M18**”.

[00416] Thus, the eleventh illustrative **DART-B**-type diabody (**5T4-M18**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:184**):

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
GTKLEIKGGG SGGGGEVQLV ESGGLVQPG GSLRLSCAAS GFTFSTYGMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLLTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTIVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

[00417] Residues 1-107 of the first polypeptide chain of **5T4-M18** correspond to the VL Domain of **5T4 mAb 1** (**SEQ ID NO:157**). Residues 108-115 (double underlined) of the first polypeptide chain of **5T4-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 116-240 of the first polypeptide chain of **5T4-M18** correspond to the VH Domain of **CD3 mAb 1 M18** (**SEQ ID NO:98**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 241-246 (underlined) of the first polypeptide chain of **5T4-M18** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 247-274 of the first polypeptide chain of **5T4-M18** correspond to the heterodimer-promoting “E-coil” (EVAALEK–EVAALEK–EVAALEK–EVAALEK; **SEQ ID NO:29**). Residues 275-277 of the first polypeptide chain of **5T4-M18** correspond to a GGG Linker. Residues 278-287 (underlined) of the first polypeptide chain of **5T4-M18** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 288-504 of the first polypeptide chain of **5T4-M18** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00418] Since the VL Domain of **CD3 mAb 1 M18** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **5T4-M18** is the same as that of the second polypeptide chain of the **5T4-WT** diabody (*i.e.*, **SEQ ID NO:181**). Similarly, the

amino acid sequence of the third polypeptide chain of **5T4-M18** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

12. Twelfth Illustrative DART-B-Type Diabody HIV-WT (HIV x CD3 mAb 1)

[00419] A twelfth illustrative **DART-B**-type diabody is similar to the above-described **CD123-WT** diabody, but comprises the HIV Binding Domain of the anti-HIV antibody A32 in lieu of the CD123 Binding Domain of the **CD123-WT** diabody. This twelfth illustrative DART-B-type diabody is designated “**HIV-WT**”.

[00420] Thus, the twelfth illustrative **DART-B**-type diabody (**HIV-WT**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:185**):

```

QSALTQPPSA SGSPGQSVTI SCTGTSSDVG GYNYVSWYQH HPGKAPKLII
SEVNNRPSGV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFV
FGGGTKLTVL GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFSTY
AMNWVRQAPG KGLEWVGRIK SKYNNYATYY ADSVKGRFTI SRDDSKNSLY
LQMNLSKTED TAVYYCVRHG NFGNSYVSWF AYWGQGTLLV VSSASTKGEV
AACEKEVAAL EKEVAALEKE VAALEKGGGD KTHTCPPCPA PEAAGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
REEQYNSTYR VVSVLTIVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG
QPREPQVYTL PPSREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMEHA LHNHYTQKSL
SLSPGK

```

[00421] Residues 1-110 of the first polypeptide chain of **HIV-WT** correspond to the VL Domain of **A32** (**SEQ ID NO:169**). Residues 111-118 (double underlined) of the first polypeptide chain of **HIV-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-243 of the first polypeptide chain of **HIV-WT** correspond to the VH Domain of **CD3 mAb 1** (**SEQ ID NO:55**), wherein Kabat position 65 (double underlined) is glycine (G). Residues 244-248 (underlined) of the first polypeptide chain of **HIV-WT** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**; single underlined). Residues 249-276 of the first polypeptide chain of **HIV-WT** correspond to the heterodimer-promoting “E-coil” (EVAACEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:31**). Residues 277-279 of the first polypeptide chain of **HIV-WT** correspond to a GGG Linker. Residues 280-289 (underlined) of the first polypeptide chain of **HIV-WT** correspond to the Linker DKHTCPPCP (**SEQ ID NO:40**; single underlined). Residues 290-506 of the first polypeptide chain of **HIV-WT** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00422] The second polypeptide chain of **HIV-WT** has the amino acid sequence (**SEQ ID NO:186**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLQESGPGLV KPSQTLRLSC TVSGGSSSSG
AHYWSWIRQY PGKGLEWIGY IHYSGNTYYN PSLKSRITIS QHTSENQFSL
KLNSVTVADT AVYYCARGTR LRTLRFNAFDI WGQGTLLVTVS SASTKGKVAA
CKEKVAALKE KVAALKEKVA ALKE

[00423] Residues 1-110 of the second polypeptide chain of **HIV-WT** correspond to the VL Domain of **CD3 mAb 1** (**SEQ ID NO:56**). Residues 111-118 (double underlined) of the second polypeptide chain of **HIV-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-241 of the second polypeptide chain of **HIV-WT** correspond to the VH Domain of **A32** (**SEQ ID NO:209** (*i.e.*, **SEQ ID NO:168**, wherein X is L)). Residues 242-246 (underlined) of the second polypeptide chain of **HIV-WT** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**). Residues 247-274 of the second polypeptide chain of **HIV-WT** correspond to the heterodimer-promoting “K-coil” (KVAACKE-KVAALKE-KVAALKE-KVAALKE; **SEQ ID NO:32**).

[00424] The third polypeptide chain of **HIV-WT** has the same amino acid sequence as the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

13. Thirteenth Illustrative DART-B-Type Diabody HIV-M18 (HIV x CD3 mAb 18)

[00425] A thirteenth illustrative **DART-B**-type diabody is similar to the above-described **HIV-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M18**. This illustrative **DART-B**-type diabody is designated “**HIV-M18**”.

[00426] Thus, the thirteenth illustrative **DART-B**-type diabody (**HIV-M18**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:196**):

QSALTQPPSA SGSPGQSVTI SCTGTSSDVG GYNYVSWYQH HPGKAPKLII
SEVNNRPSGV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFV
FGGGTKLTVL GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFSTY
GMNWVRQAPG KGLEWVGRIR SKYNNYATYY ADSVKGRFTI SRDDSKNSLY
LQMNSLKTED TAVYYCVRHG NFGNSYVSWF AYWGQGTLLV VSSASTKGEV
AAACEKEVAAL EKEVAALKE VAALKGGGD KTHTCPPCPA PEAAGGPSVF
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP
REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP IEKTISKAKG
QPREPQVYTL PPSREEMTKN QVSLWCLVKG FYPSDIAVEW ESNGQPENNY
KTTTPVLDSG GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL
SLSPGK

[00427] Residues 1-110 of the first polypeptide chain of **HIV-M18** correspond to the VL Domain of **A32** (**SEQ ID NO:169**). Residues 111-118 (double underlined) of the first polypeptide chain of **HIV-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-243 of the first polypeptide chain of **HIV-M18** correspond to the VH Domain of **CD3 mAb 1 M18** (**SEQ ID NO:55**), wherein Kabat position 65 (double underlined) is glycine (G). Residues 244-248 (underlined) of the first polypeptide chain of **HIV-M18** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**; single underlined). Residues 249-276 of the first polypeptide chain of **HIV-M18** correspond to the heterodimer-promoting “E-coil” (EVAACEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:31**). Residues 277-279 of the first polypeptide chain of **HIV-M18** correspond to a GGG Linker. Residues 280-289 (underlined) of the first polypeptide chain of **HIV-M18** correspond to the Linker DKHTTCPPCP (**SEQ ID NO:40**; single underlined). Residues 290-506 of the first polypeptide chain of **HIV-M18** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00428] Since the VL Domain of **CD3 mAb 1 M18** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **HIV-M18** is the same as that of the second polypeptide chain of the **HIV-WT** diabody (*i.e.*, **SEQ ID NO:186**). Similarly, the amino acid sequence of the third polypeptide chain of **HIV-M18** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

14. Fourteenth Illustrative DART-B-Type Diabody CD19-WT (CD19 x CD3 mAb 1)

[00429] An fourteenth illustrative **DART-B**-type diabody is similar to the above-described **HIV-WT** diabody, but comprises **CD19 mAb 1** in lieu of the **A32** Binding Domain. This fourteenth illustrative **DART-B**-type diabody is designated “**CD19-WT**”.

[00430] Thus, the fourteenth illustrative **DART-B**-type diabody (**CD19-WT**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:191**):

```
ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSLEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSA STKGEVAACE
KEVAALEKEV AALEKEVAAL EKGGGDKTHT CPPCPAPEAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
```

PQVYTLPPSR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTP
 PVLDSGSGFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
 GK

[00431] Residues 1-106 of the first polypeptide chain of **CD19-WT** correspond to the VL Domain of **CD19 mAb 1 (SEQ ID NO:165)**. Residues 107-114 (double underlined) of the first polypeptide chain of **CD19-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19-WT** correspond to the VH Domain of **CD3 mAb 1 (SEQ ID NO:55)**, wherein Kabat position 65 (double underlined) is glycine (G). Residues 240-244 (underlined) of the first polypeptide chain of **CD19-WT** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**; single underlined). Residues 245-272 of the first polypeptide chain of **CD19-WT** correspond to the heterodimer-promoting “E-coil” (EVAACEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:31**). Residues 273-275 of the first polypeptide chain of **CD19-WT** correspond to a GGG Linker (double underlined). Residues 276-285 (single underlined) of the first polypeptide chain of **CD19-WT** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 286-502 of the first polypeptide chain of **CD19-WT** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00432] The second polypeptide chain of **CD19-WT** has the amino acid sequence (**SEQ ID NO:192**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
 GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
 GGGTKLTVLG GGSGGGGQV TLRESGPALV KPTQTLTLTC TFSGFSLSTS
 GMGVGWIRQP PGKALEWLAH IWWDDDKRYN PALKSRLTIS KDTSKNQVFL
 TMTNMDPVDV ATYYCARMEL WSYFYFDYWGQ GTTVTVSSAS TKGKVAACKE
 KVAALKEKVA ALKEKVAALK E

[00433] Residues 1-110 of the second polypeptide chain of **CD19-WT** correspond to the VL Domain of **CD3 mAb 1 (SEQ ID NO:56)**. Residues 111-118 (double underlined) of the second polypeptide chain of **CD19-WT** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-238 of the second polypeptide chain of **CD19-WT** correspond to the VH Domain of **CD19 mAb 1 (SEQ ID NO:164)**. Residues 239-243 (underlined) of the second polypeptide chain of **CD19-WT** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**). Residues 244-271 of the second polypeptide chain of **CD19-WT** correspond to the heterodimer-promoting “K-coil” (KVAACKE-KVAALKE-KVAALKE-KVAALKE; **SEQ ID NO:32**).

[00434] The third polypeptide chain of **CD19-WT** has the same amino acid sequence as the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

**15. Fifteenth Illustrative DART-B-Type Diabody
CD19-M18 (CD19 x CD3 mAb 1 M18)**

[00435] A fifteenth illustrative **DART-B**-type diabody is similar to the above-described **CD19-WT** diabody, but contains the VH Domain of **CD3 mAb 1 M18**. This fifteenth illustrative **DART-B**-type diabody is designated “**CD19-M18**”.

[00436] Thus, the fifteenth illustrative **DART-B**-type diabody (**CD19-M18**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:197**):

```
ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHWYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYGMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSA STKGEVAAACE
KEVAALEKEV AALEKEVAAL EKGGGDKTHT CPPCPAPAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYTLPPSR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTP
PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP
GK
```

[00437] Residues 1-106 of the first polypeptide chain of **CD19-M18** correspond to the VL Domain of **CD19 mAb 1 (SEQ ID NO:165)**. Residues 107-114 (double underlined) of the first polypeptide chain of **CD19-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19-M18** correspond to the VH Domain of **CD3 mAb 1 M18 (SEQ ID NO:98)**, wherein Kabat position 65 (double underlined) is glycine (G). Residues 240-244 (underlined) of the first polypeptide chain of **CD19-M18** correspond to a Linker 2 (ASTKG; **SEQ ID NO:21**; single underlined). Residues 245-272 of the first polypeptide chain of **CD19-M18** correspond to the heterodimer-promoting “E-coil” (EVAACEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:31**). Residues 273-275 of the first polypeptide chain of **CD19-M18** correspond to a GGG Linker. Residues 276-285 (single underlined) of the first polypeptide chain of **CD19-M18** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 286-502 of the first polypeptide chain of **CD19-M18** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00438] Since the VL Domain of **CD3 mAb 1 M18** is the same as that of **CD3 mAb 1**, the amino acid sequence of the second polypeptide chain of **CD19-M18** is the same as that of the second polypeptide chain of the **CD19-WT** diabody (*i.e.*, **SEQ ID NO:192**). Similarly, third polypeptide chain of **CD19-M18** has the same amino acid sequence as the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

16. Sixteenth Illustrative DART-B-Type Diabody CD19.1-M18 (CD19.1 x CD3 mAb 1 M18)

[00439] A sixteenth illustrative **DART-B**-type diabody is similar to the above-described **CD123-M18** diabody, but comprising a CD19 Binding Domain, containing the alternative VL Domain of **CD19 mAb 1**, in lieu of the CD123 Binding Domain of **CD123-M18**. This illustrative **DART-B**-type diabody is designated “**CD19.1-M18**”. As indicated above, the VL Domain of **CD3 mAb 1 M18** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00440] Thus, the sixteenth illustrative **DART-B**-type diabody (**CD19.1-M18**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:193**):

```
ENVLTQSPAT LSVTPGEKVT ITCSASSSVS YMHYQQKPG QAPRLLIYDT
SKLASGVPSR FSGSGSGTDH FLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYGMNW
VRQAPGKGL E WVGRIISKYN NYATYYADSV KDRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

[00441] Residues 1-106 of the first polypeptide chain of **CD19.1-M18** correspond to the alternative VL Domain of **CD19 mAb 1** (**SEQ ID NO:195**). Residues 107-114 (double underlined) of the first polypeptide chain of **CD19.1-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19.1-M18** correspond to the VH Domain of **CD3 mAb 1 M18** (**SEQ ID NO:98**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 240-245 (single underlined) of the first polypeptide chain of **CD19.1-M18** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 246-273 of the first polypeptide chain of **CD19.1-M18** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 274-276 of the first polypeptide chain of **CD19-M18** correspond to a

GGG Linker. Residues 277-286 (single underlined) of the first polypeptide chain of **CD19.1-M18** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 287-503 of the first polypeptide chain of **CD19.1-M18** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00442] The second polypeptide chain of **CD19.1-M18** has the amino acid sequence (**SEQ ID NO:194**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV TLRESGPALV KPTQTLTLTC TFSGFSLSLS
GMGVGWIRQP PGKALEWLAH IWDDDDKRYN PALKSRLTIS KDTSKNQVFL
TMTNMDPVDV ATYYCARMEL WSYFFDYWGQ GTTVTVSSGG CGGGKVAALK
EKVAALKEKV AALKEKVAAL KE

[00443] Residues 1-110 of the second polypeptide chain of **CD19.1-M18** correspond to the VL Domain of **CD3 mAb 1** (**SEQ ID NO:56**). Residues 111-118 (double underlined) of the second polypeptide chain of **CD19.1-M18** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 119-238 of the second polypeptide chain of **CD19.1-M18** correspond to the VH Domain of **CD19 mAb 1** (**SEQ ID NO:164**). Residues 239-244 (single underlined) of the second polypeptide chain of **CD19.1-M18** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 245-272 of the second polypeptide chain of **CD19.1-M18** correspond to the heterodimer-promoting “K-coil” (KVAAALKE-KVAAALKE-KVAAALKE-KVAAALKE; **SEQ ID NO:30**).

[00444] The amino acid sequence of the third polypeptide chain of **CD19.1-M18** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

17. Seventeenth Illustrative DART-B-Type Diabody CD19.1-M13 (CD19.1 x CD3 mAb 1 M13)

[00445] A seventeenth illustrative **DART-B**-type diabody is similar to the above-described **CD19.1-M18** diabody, but contains the VH Domain of **CD3 mAb 1 M13** and is designated “**CD19.1-M13**”. As indicated above, the VL Domain of **CD3 mAb 1 M13** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00446] Thus, the seventeenth illustrative **DART-B**-type diabody (**CD19.1-M13**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:201**):

```
ENVLTQSPAT LSVTPGEKVT ITCSASSSVS YMHWYQQKPG QAPRLLIYDT
SKLASGVPSR FSGSGSGTDH FLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KDRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAEWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALKEEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQFENNYKTT
PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

[00447] Residues 1-106 of the first polypeptide chain of **CD19.1-M13** correspond to the alternative VL Domain of **CD19 mAb 1** (**SEQ ID NO:195**). Residues 107-114 (double underlined) of the first polypeptide chain of **CD19.1-M13** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19.1-M18** correspond to the VH Domain of **CD3 mAb 1 M13** (**SEQ ID NO:88**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 240-245 (single underlined) of the first polypeptide chain of **CD19.1-M13** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 246-273 of the first polypeptide chain of **CD19.1-M13** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 274-276 of the first polypeptide chain of **CD19-M13** correspond to a GGG Linker. Residues 277-286 (single underlined) of the first polypeptide chain of **CD19.1-M13** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 287-503 of the first polypeptide chain of **CD19.1-M13** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00448] Since the VL Domain of **CD3 mAb 1 M13** is the same as that of **CD3 mAb 1** the amino acid sequence of the second polypeptide chain of **CD19.1-M13** is the same as that of the second polypeptide chain of the **CD19.1-M18** diabody (*i.e.*, **SEQ ID NO:194**). The amino acid sequence of the third polypeptide chain of **CD19.1-M13** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

**18. Eighteenth Illustrative DART-B-Type Diabody
CD19.1-M17 (CD19.1 x CD3 mAb 1 M17)**

[00449] An eighteenth illustrative **DART-B**-type diabody is similar to the above-described **CD19.1-M18** diabody, but contains the VH Domain of **CD3 mAb 1 M17** and is designated “**CD19.1-M17**”. As indicated above, the VL Domain of **CD3 mAb 1 M17** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00450] Thus, the eighteenth illustrative **DART-B**-type diabody (**CD19.1-M17**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:202**):

```
ENVLTQSPAT LSVTPGEKVT ITCSASSSVS YMHWYQOKPG QAPRLLIYDT
SKLASGVPSR FSGSGSGTDH FLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTAMNW
VRQAPGKGL WVGRIRSKYN NYATYYADSV KDRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

[00451] Residues 1-106 of the first polypeptide chain of **CD19.1-M17** correspond to the alternative VL Domain of **CD19 mAb 1 (SEQ ID NO:195)**. Residues 107-114 (double underlined) of the first polypeptide chain of **CD19.1-M17** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19.1-M17** correspond to the VH Domain of **CD3 mAb 1 M17 (SEQ ID NO:96)**, wherein Kabat position 65 (double underlined) is aspartate (D). Residues 240-245 (single underlined) of the first polypeptide chain of **CD19.1-M17** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 246-273 of the first polypeptide chain of **CD19.1-M17** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 274-276 of the first polypeptide chain of **CD19-M17** correspond to a GGG Linker. Residues 277-286 (single underlined) of the first polypeptide chain of **CD19.1-M17** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 287-503 of the first polypeptide chain of **CD19.1-M17** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00452] Since the VL Domain of **CD3 mAb 1 M17** is the same as that of **CD3 mAb 1** the amino acid sequence of the second polypeptide chain of **CD19.1-M17** is the same as that of

the second polypeptide chain of the **CD19.1-M18** diabody (*i.e.*, **SEQ ID NO:194**). The amino acid sequence of the third polypeptide chain of **CD19.1-M17** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

19. Nineteenth Illustrative DART-B-Type Diabody CD19.1-M19 (CD19.1 x CD3 mAb 1 M19)

[00453] A nineteenth illustrative **DART-B**-type diabody is similar to the above-described **CD19.1-M18** diabody, but contains the VH Domain of **CD3 mAb 1 M19** and is designated “**CD19.1-M19**”. As indicated above, the VL Domain of **CD3 mAb 1 M19** has the same amino acid sequence as the VL Domain of **CD3 mAb 1**.

[00454] Thus, the nineteenth illustrative **DART-B**-type diabody (**CD19.1-M19**) has a first polypeptide chain that has the amino acid sequence (**SEQ ID NO:203**):

```
ENVLTQSPAT LSVTPGEKVT ITCSASSSVS YMHWYQQKPG QAPRLLIYDT
SKLASGVPSR FSGSGSGTDH FLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KDRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHKNIGN SYVSWFAYWG QGTLVTVSSG GCGGGEVAAL
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLEFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFP SDAVEWESN GQFENNYKTT
PPVLDSGGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

[00455] Residues 1-106 of the first polypeptide chain of **CD19.1-M19** correspond to the alternative VL Domain of **CD19 mAb 1** (**SEQ ID NO:195**). Residues 107-114 (double underlined) of the first polypeptide chain of **CD19.1-M19** correspond to Linker 1 (GGGSGGGG; **SEQ ID NO:16**). Residues 115-239 of the first polypeptide chain of **CD19.1-M19** correspond to the VH Domain of **CD3 mAb 1 M9** (**SEQ ID NO:100**), wherein Kabat position 65 (double underlined) is aspartate (D). Residues 240-245 (single underlined) of the first polypeptide chain of **CD19.1-M19** correspond to a Linker 2 (GGCGGG; **SEQ ID NO:17**). Residues 246-273 of the first polypeptide chain of **CD19.1-M19** correspond to the heterodimer-promoting “E-coil” (EVAALEK-EVAALEK-EVAALEK-EVAALEK; **SEQ ID NO:29**). Residues 274-276 of the first polypeptide chain of **CD19-M19** correspond to a GGG Linker. Residues 277-286 (single underlined) of the first polypeptide chain of **CD19.1-M19** correspond to the Linker DKTHTCPPCP (**SEQ ID NO:40**). Residues 287-503 of the

first polypeptide chain of **CD19.1-M19** correspond to the IgG1 “knob-bearing” CH2-CH3 Domain (**SEQ ID NO:48**).

[00456] Since the VL Domain of **CD3 mAb 1 M19** is the same as that of **CD3 mAb 1** the amino acid sequence of the second polypeptide chain of **CD19.1-M19** is the same as that of the second polypeptide chain of the **CD19.1-M18** diabody (*i.e.*, **SEQ ID NO:194**). The amino acid sequence of the third polypeptide chain of **CD19.1-M13** is the same as that of the third polypeptide chain of the **CD123-WT** diabody (*i.e.*, **SEQ ID NO:176**).

[00457] Additional CD19 x CD3 DART-B-Type Diabodies specifically contemplated are similar to the above-described **CD19-WT** (see, the fourteenth illustrative DART-B-Type Diabody) but will comprise the VH Domain of **CD3 mAb 1 M13**, **M17**, or **M19**. Such diabodies will comprise a first polypeptide chain having one of the following amino acid sequences:

[00458] **SEQ ID NO:204** for such diabody comprising the VH Domain of **CD3 mAb 1 M13**:

```
ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHWYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAEWG QGTLVTVSSA STKGEVAACE
KEVAALEKEV AALEKEVAAL EKGGGDKTHT CPPCPAPEAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYITLPPSR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP
GK
```

[00459] **SEQ ID NO:205** for such diabody comprising the VH Domain of **CD3 mAb 1 M17**:

```
ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHWYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHGNFGN SYVSWFAYWG QGTLVTVSSA STKGEVAACE
KEVAALEKEV AALEKEVAAL EKGGGDKTHT CPPCPAPEAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYITLPPSR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTTP
PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP
GK
```

[00460] SEQ ID NO:206 for such diabody comprising the VH Domain of **CD3 mAb 1 M19**:

```

ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHWYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSEAE DAATYYCFQG SVYPFTFGQG
TKLEIKGGGS GGGGEVQLVE SGGGLVQPGG SLRLSCAASG FTFSTYAMNW
VRQAPGKGLE WVGRIIRSKYN NYATYYADSV KGRFTISRDD SKNSLYLQMN
SLKTEDTAVY YCVRHKNIGN SYVSWFAYWG QGTLVTVSSA STKGEVAAACE
KEVAALEKEV AALEKEVAAL EKGGGDKTHT CPPCPAPEAA GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYTLPPSR EEMTKNQVSL WCLVKGFYPS DIAVEWESNG QPENNYKTP
PVLDSGSGFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNNH YTKSLSLSP
GK

```

[00461] The second polypeptide chains of such diabodies will have the same amino acid sequence of **CD19-WT** (*i.e.*, SEQ ID NO:192) and the third polypeptide chain of such diabodies will have the same amino acid sequence as the third polypeptide chain of **CD123-WT** (*i.e.*, SEQ ID NO:176).

[00462] As will be recognized in view of the instant disclosure, additional **DART-B**-type diabodies having a binding site for an alternative Disease Antigens and/or having the CD3 Binding Domains of alternative variant anti-CD3 antibodies (*i.e.*, vCD3-Binding Domains) may likewise be constructed (by employing the VL and VH Domains of such antibodies). Similarly, as provided herein, alternative **DART-B**-type molecules may likewise be constructed incorporating alternative Linkers and/or alternative Heterodimer-Promoting Domains.

[00463] Additional, exemplary molecules capable of mediating the redirected killing of a cell expressing a Disease Antigen (*e.g.*, a tumor cell) which may be used in the methods of the present invention include bispecific molecules capable of binding: **CD19** and **CD3** (see, *e.g.*, US Patent No. 7,235,641 and WO 2016/048938); **CD123** and **CD3** (see, *e.g.*, Kuo, S.R. *et al.*, (2012) "Engineering a CD123xCD3 Bispecific scFv Immunofusion For The Treatment Of Leukemia And Elimination Of Leukemia Stem Cells," Protein Eng Des Sel. 25:561-9; WO 2015/026892; WO 2016/086189); **gpA33** and **CD3** (*e.g.*, WO 2015/026894); **CEA** and **CD3** (*e.g.*, WO 2013/012414; WO 2017/118675); **B7-H3** and **CD3** (*e.g.*, WO 2017/030926); **HER2** and **CD3** (*e.g.*, WO 2012/143524); **5T4** and **CD3** (*e.g.*, WO 2015/184203 and WO 2013/041687), and other molecules having a CD3 Binding Domain (see, *e.g.*, *etc.*, WO 2013/026835, WO 2013/158856, WO 2014/110601, WO 2016/182751,

WO 2017/053469). As will be recognized in view of the instant disclosure, the vCD3-Binding Domains of the instant invention may be incorporated into such molecules.

C. TRIVALENT-Type Molecules

[00464] **TRIVALENT**-type molecules are trivalent molecules capable of binding up to three different epitopes. In particular, the **TRIVALENT**-type molecules of the instant invention are capable of binding CD3 and a Disease Antigen (*e.g.*, a Cancer or Infectious Disease Antigen) and may further bind an addition antigen such as an additional Disease Antigen (*e.g.*, a Cancer or Infectious Disease Antigen) or an additional antigen expressed on the surface of an effector cell (*e.g.*, CD8), or may bind to a second epitope of CD3 or a second epitope of the Disease Antigen. **TRIVALENT**-type molecules comprise an Fc Domain. Provided herein are illustrative **TRIVALENT**-type diabodies composed of four polypeptide chains and have one binding site for CD3, one binding site for the Cancer Antigen CD123 or for the Cancer Antigen 5T4, and one binding site for CD8 (see, *e.g.*, **Figure 6A**). The illustrative **TRIVALENT**-type molecules of the invention are generated using the first and second polypeptide chains of the **DART-B**-type diabodies provided above in combination with the illustrative third and fourth polypeptide chains provided below, which provide the CD8 Binding Domain. The first and second polypeptide chains form the CD3 and DA Binding Domains while the third and fourth polypeptide chains form the CD8 Binding Domain. The first and third polypeptide chains form an Fc Domain.

[00465] The illustrative **TRIVALENT**-type molecules provide below each incorporate a third polypeptide chain having the amino acid sequence of **SEQ ID NO:187**:

```
QVQLVESGGG VVQPGRSLRL SCAASGFTFS DFGMNWVRQA PGKGLEWVAL
IYYDGSNKFY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKPH
YDGYHFFDS WGQGTSLTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV
KDYFPEPVTV SWNSGALTSG VHTFPAVLQS SGLYSLSSVV TVPSSSLGTQ
TYICNVNHKP SNTKVDKRVE PKSCDKHTC PPCPAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSL SCAVKGFYPSD IAVEWESNGQ PENNYKTTPP
VLDSGDSFFL VSKLTVDKSR WQQGNVFSCS VMHEALHNRY TQKSLSLSPG
K
```

[00466] Residues 1-121 of the third polypeptide chain of such illustrative **TRIVALENT**-type molecules correspond to the VH Domain of the anti-CD8 antibody **TRX2 (SEQ ID NO:120)**. Residues 121-219 of the third polypeptide chain of such illustrative **TRIVALENT**-type molecule correspond to an IgG1 CH1 Domain (**SEQ ID NO:1**).

Residues 220-234 of the third polypeptide chain of such illustrative **TRIVALENT**-type molecule correspond to an IgG1 Hinge Domain (**SEQ ID NO:5**). Residues 235-451 correspond to the IgG1 “hole-bearing” CH2-CH3 Domain (**SEQ ID NO:50**).

[00467] The illustrative **TRIVALENT**-type molecules described below each incorporate a fourth polypeptide chain having the amino acid sequence of **SEQ ID NO:188**:

DIQMTQSPSS LSASVGDRVIT ITCKGSQDIN NYLAWYQQKPGKAPKLLIYN
 TDILHTGVPS RFGSGSGTD FTFTISSLQP EDIATYYCYQ YNNGYTFGQG
 TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYF REAKVQWKVD
 NALQSGNSQE SVTEQDSKDS TYSLSSTLT SKADYEKHKV YACEVTHQGL
 SSPVTKSFNR GEC

[00468] Residues 1-106 of the fourth polypeptide chain of such illustrative **TRIVALENT**-type molecules correspond to the VL Domain of the anti-CD8 antibody **TRX2** (**SEQ ID NO:121**). Residues 107-213 correspond to a CL Kappa Domain (**SEQ ID NO:14**).

[00469] The **SEQ ID NOs.** of the polypeptide chains of Illustrative **TRIVALENT**-type molecules are summarized in **Table 10**.

Table 10 CD123 x CD3 x CD8 TRIDENT Molecules							
TRIDENT- Type No.	Disease Antigen- Binding Domain	CD3 Binding Domain	Polypeptide Chain				Designation
			First	Second	Third	Fourth	
			CD123 / CD3 Binding Domains		CD8 Binding Domains		
1	CD123 mAb 1	CD3 mAb 1	SEQ ID NO:174	SEQ ID NO:175	SEQ ID NO:187	SEQ ID NO:188	T-CD123-WT
2		CD3 mAb 1 M1	SEQ ID NO:177				T-CD123-M1
3		CD3 mAb 1 M2	SEQ ID NO:178				T-CD123-M2
4		CD3 mAb 1 M18	SEQ ID NO:179				T-CD123-M18

1. First Illustrative **TRIVALENT**-Type Molecule T-CD123-WT (CD123 mAb 1 x CD3 mAb 1 x TRX2)

[00470] A first illustrative **TRIVALENT**-type molecule (designated “**T-CD123-WT**”) contains the VH and VL Domains of **CD123 mAb 1**, the VH and VL Domains of **CD3 mAb 1**, and the VH and VL Domains of the anti-CD8 antibody **TRX2**. As indicated above, the amino acid sequence of the first polypeptide chain is the same as that of the above-described **CD123-WT** diabody (**SEQ ID NO:174**). Similarly, the amino acid sequence of the second polypeptide chain is the same as that of the above-described **CD123-WT** diabody (**SEQ ID NO:175**). Also indicated above, the amino acid sequences of the third and fourth

polypeptide chains of all the illustrative **TRIVALENT**-type molecules are **SEQ ID NO:187** and **SEQ ID NO:188**, respectively.

**2. Second Illustrative TRIVALENT-Type Molecule
T-CD123-M1 (CD123 mAb 1 x CD3 mAb 1 M1 x TRX2)**

[00471] A second illustrative **TRIVALENT**-type molecule (designated “**T-CD123-M1**”) contains the VH and VL Domains of **CD123 mAb 1**, the VH and VL Domains of **CD3 mAb 1 M1**, and the VH and VL Domains of **TRX2**. As indicated above, the amino acid sequence of the first polypeptide chain is the same as that of the above-described **CD123-M1** diabody (**SEQ ID NO:177**). Similarly, the amino acid sequence of the second polypeptide chain is the same as that of the above-described **CD123-WT** diabody (**SEQ ID NO:175**). Also as indicated above, the amino acid sequences of the third and fourth polypeptide chains of all the illustrative **TRIVALENT**-type molecules are **SEQ ID NO:187** and **SEQ ID NO:188**, respectively.

**3. Third Illustrative TRIVALENT-Type Molecule
T-CD123-M2 (CD123 mAb 1 x CD3 mAb 1 M2 x TRX2)**

[00472] A third illustrative **TRIVALENT**-type molecule designated **T-CD123-M2** binds contains the VH and VL Domains of **CD123 mAb 1**, the VH and VL Domains of **CD3 mAb 1 M2**, and the VH and VL Domains of **TRX2**. As indicated above, the amino acid sequence of the first polypeptide chain is the same as that of the above-described **CD123-M2** diabody (**SEQ ID NO:178**). Similarly, the amino acid sequence of the second polypeptide chain is the same as that of the above-described **CD123-WT** diabody (**SEQ ID NO:175**). Also indicated above, the amino acid sequences of the third and fourth polypeptide chains of all the illustrative **TRIVALENT**-type molecules are **SEQ ID NO:187** and **SEQ ID NO:188** respectively.

**4. Fourth Illustrative TRIVALENT-Type Molecule
T-CD123-M18 (CD123 mAb 1 x CD3 mAb 1 M18 x TRX2)**

[00473] A fourth illustrative **TRIVALENT**-type molecule designated **T-CD123-M18** binds contains the VH and VL Domains of **CD123 mAb 1**, the VH and VL Domains of **CD3 mAb 1 M18**, and the VH and VL Domains of **TRX2**. As indicated above, the amino acid sequence of the first polypeptide chain is the same as that of the above-described **CD123-M18** diabody (**SEQ ID NO:179**). Similarly, the amino acid sequence of the second

polypeptide chain is the same as that of the above-described **CD123-WT** diabody (**SEQ ID NO:175**). Also indicated above, the amino acid sequences of the third and fourth polypeptide chains of all the illustrative **TRIVALENT**-type molecules are **SEQ ID NO:187** and **SEQ ID NO:188**, respectively.

[00474] As will be recognized in view of the instant disclosure, additional **TRIVALENT**-type diabodies having a binding site for an alternative Disease Antigens and/or having the CD3 Binding Domains of alternative variant anti-CD3 antibodies (*i.e.*, vCD3-Binding Domains) may likewise be constructed (by employing the VL and VH Domains of such antibodies). Similarly, as provided herein, alternative **TRIVALENT**-type molecules may likewise be constructed incorporating alternative Linkers and/or alternative Heterodimer-Promoting Domains.

[00475] Additional, exemplary molecules capable of mediating the redirected killing of a cell expressing a Disease Antigen (*e.g.*, a tumor cell) which may be used in the methods of the present invention include trivalent molecules capable of binding: **B7-H3**, **CD3** and **CD8** (see, *e.g.*, WO 2015/184203); **5T4**, **CD3** and **CD8** (see, *e.g.*, WO 2015/184203); **ROR1**, **CD3** and **CD8** (see, *e.g.*, WO 2015/184203 and WO 2017/106061); **HIV**, **CD3** and **CD8** (see, *e.g.*, WO 2015/184203; WO2017/011413; and WO2017/011414); **gpA33**, **CD3** and **DR5** (see, *e.g.*, WO 2015/184207); **EphA2**, **CD3** and **DR5** (see, *e.g.*, WO 2015/184207); **gpA33**, **CD3** and **EphA2** (see, *e.g.*, WO 2015/184207); and other trivalent molecules (see, *e.g.*, WO 2016/105450; WO 2016/115274; WO 2017/180913). As will be recognized in view of the instant disclosure, the vCD3-Binding Domains of the instant invention may be incorporated into such molecules.

VIII. Methods of Production

[00476] The molecules of the present invention are most preferably produced through the recombinant expression of nucleic acid molecules that encode such polypeptides, as is well-known in the art.

[00477] Polypeptides of the invention may be conveniently prepared using solid-phase peptide synthesis (Merrifield, B. (1986) "*Solid Phase Synthesis*," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A.

(2006) “*Solid-Phase Synthesis In The Twenty-First Century*,” Mini Rev. Med. Chem. 6(1):3-10).

[00478] Antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants (*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) “*Production Of Antibodies And Antibody Fragments In Plants*,” Vaccine 19:2756; Lonberg, N. *et al.* (1995) “*Human Antibodies From Transgenic Mice*,” Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) “*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*,” J. Immunol. Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art, and have been described above. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) “*Making Antibodies By Phage Display Technology*,” Annu. Rev. Immunol. 12:433-455).

[00479] Vectors containing polynucleotides of interest (*e.g.*, polynucleotides encoding the polypeptide chains of the Binding Molecules of the present invention) can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00480] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of expressing a polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells.

[00481] The invention includes polypeptides comprising an amino acid sequence of a binding molecule of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other

degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available.

[00482] The invention includes variants of the disclosed Binding Molecules, including functionally equivalent polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other posttranslational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the Variable Domain. Changes in the Variable Domain can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00483] In one embodiment, a fusion polypeptide is provided that comprises a Light Chain, a Heavy Chain or both a Light and Heavy Chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another

embodiment, the fusion polypeptide contains a VH and a VL Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind CD3, or to both CD3 and to a Disease Antigen, and which contains another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

[00484] The present invention particularly encompasses such Binding Molecules (*e.g.*, antibodies, diabodies, trivalent Binding Molecules, *etc.*) conjugated to a diagnostic or therapeutic moiety. For diagnostic purposes, the Binding Molecules of the invention may be coupled to a detectable substance. Such Binding Molecules are useful for monitoring and/or prognosing the development or progression of a disease as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Examples of detectable substances include various enzymes (*e.g.*, horseradish peroxidase, beta-galactosidase, *etc.*), prosthetic groups (*e.g.*, avidin/biotin), fluorescent materials (*e.g.*, umbelliferone, fluorescein, or phycoerythrin), luminescent materials (*e.g.*, luminol), bioluminescent materials (*e.g.*, luciferase or aequorin), radioactive materials (*e.g.*, carbon-14, manganese-54, strontium-85 or zinc-65), positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the binding molecule or indirectly, through an intermediate (*e.g.*, a Linker) using techniques known in the art.

[00485] For therapeutic purposes, the Binding Molecules of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells such as, for example, *Pseudomonas* exotoxin, Diphtheria toxin, a botulinum toxin A through F, ricin abrin, saporin, and cytotoxic fragments of such agents. A therapeutic agent includes any agent having a therapeutic effect to prophylactically or therapeutically treat a disorder. Such therapeutic agents may be may be chemical therapeutic agents, protein or polypeptide therapeutic agents, and include therapeutic agents that possess a desired biological activity and/or modify a given biological response. Examples of therapeutic agents include alkylating agents, angiogenesis inhibitors, anti-mitotic agents, hormone therapy agents, and antibodies useful for the treatment of cell proliferative disorders. The therapeutic moiety may be coupled or conjugated either directly

to the binding molecule or indirectly, through an intermediate (*e.g.*, a Linker) using techniques known in the art.

IX. Uses of the Binding Molecules of the Present Invention

[00486] As discussed above, molecules capable of binding CD3 and a Disease Antigen are capable of mediating the redirected cell killing of a target cell (*i.e.*, a cancer cell, or a pathogen-infected cell) that expresses such Disease Antigen on its cell surface. Such molecules may be used for therapeutic purposes, for example in subjects with cancer or an infection. Thus, Binding Molecules of the present invention have the ability to treat any disease or condition associated with or characterized by the expression of a Disease Antigen, particularly a Cancer Antigen or a Pathogen-Associated Antigen, on the surface of such target cell. Thus, without limitation, the Binding Molecules of the present invention may be employed in the treatment of cancer, particularly a cancer characterized by the expression of a Cancer Antigen. The Binding Molecules of the present invention may be employed in the treatment of infection, particularly an infection characterized by the expression of a Pathogen-Associated Antigen.

[00487] In particular, the present invention encompasses such methods wherein the molecule capable of binding CD3 comprises an Epitope-Binding Domain of an antibody that is capable of binding CD3 and also comprises an Epitope-Binding Domain capable of binding a Disease Antigen (in particular a Cancer Antigen or a Pathogen-Associated Antigen) on the surface of a target cell so as to mediate the redirected killing of the target cell (for example, by mediating redirected cell killing (*e.g.*, redirected T-cell cytotoxicity)).

[00488] In a specific embodiment, the molecule capable of binding CD3 and the Disease Antigen is a bispecific antibody, or a molecule comprising the Epitope-Binding Domains thereof, (including a bispecific scFv a BiTe, a TandAb).

[00489] In a specific embodiment, the molecule capable of binding CD3 and the Disease Antigen is a bispecific diabody.

[00490] In a specific embodiment, the molecule capable of binding CD3 and the Disease Antigen is a trivalent binding molecule.

[00491] “**Providing a therapy**” or “**treating**” refers to any administration of a composition that is associated with any indicia of beneficial or desired result, including, without

limitation, any clinical result such as decreasing symptoms resulting from the disease, attenuating a symptom of infection (*e.g.*, viral load, fever, pain, sepsis, *etc.*) a shrinking of the size of a tumor (in the cancer context, for example, a tumor of breast, gastric or prostate cancer), a retardation of cancer cell growth, a delaying of the onset, development or progression of metastasis, a decreasing of a symptom resulting from the disease, an increasing of the quality of life of the recipient subject, a decreasing of the dose of other medications being provided to treat a subject's disease, an enhancing of the effect of another medication such as via targeting and/or internalization, a delaying of the progression of the disease, and/or a prolonging of the survival of recipient subject.

[00492] Subjects for treatment include animals, most preferably mammalian species such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00493] Exemplary disorders that may be treated by various embodiments of the present invention include, but are not limited to, proliferative disorders, cell proliferative disorders, and cancer (especially a cancer expressing a Cancer Antigen bound by a molecule capable of mediating redirected cell killing), pathogen-associated diseases (especially a chronic viral infection associated with expression of a Pathogen-Associated Antigen bound by a molecule capable of mediating redirected cell killing). In various embodiments, the invention encompasses methods and compositions for treatment, prevention or management of a disease or disorder in a subject, comprising administering to the subject a therapeutically effective amount the Binding Molecules of the present invention. Such molecules are particularly useful for the prevention, inhibition, reduction of growth, or regression of primary tumors, and metastasis of tumors, and for reducing pathogen load, or eliminating pathogen-infected cells. Although not intending to be bound by a particular mechanism of action, such molecules may mediate effector function against target cells, promote the activation of the immune system against target cells, cross-link cell surface antigens and/or receptors on target cells and enhance apoptosis or negative growth regulatory signaling, or a combination thereof, resulting in clearance and/or reduction in the number of target cells.

[00494] The cancers that may be treated by molecules of the present invention, and by the methods of the present invention, include, but are not limited to: 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.

[00495] In particular, the CD19 x CD3 Binding Molecules, CD19 x CD3 x CD8 Binding Molecules, CD123 x CD3 Binding Molecules and CD123 x CD3 x CD8 Binding Molecules of the present invention may be used in the treatment of a hematological cancer including but not limited to: acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), myelodysplastic syndrome (MDS), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), including Richter's syndrome or Richter's transformation of CLL, hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphoma (NHL), including mantle cell lymphoma (MCL) and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, and Burkitt's lymphoma.

[00496] Pathogen-associated diseases that may be treated by the LAG-3-Binding Molecules of the present invention include chronic viral, bacterial, fungal and parasitic infections. Chronic infections that may be treated by the LAG-3-Binding Molecules of the present invention include Epstein-Barr Virus, Hepatitis A Virus (HAV); Hepatitis B Virus (HBV); Hepatitis C Virus (HCV); herpes viruses (*e.g.* HSV-1, HSV-2, HHV-6, CMV), Human Immunodeficiency Virus (HIV), Vesicular Stomatitis Virus (VSV), *Bacilli*, *Citrobacter*, *Cholera*, *Diphtheria*, *Enterobacter*, *Gonococci*, *Helicobacter pylori*, *Klebsiella*, *Legionella*, *Meningococci*, mycobacteria, *Pseudomonas*, *Pneumonococci*, rickettsia bacteria, *Salmonella*, *Serratia*, *Staphylococci*, *Streptococci*, *Tetanus*, *Aspergillus* (*fumigatus*, *niger*, *etc.*), *Blastomyces dermatitidis*, *Candida* (*albicans*, *krusei*, *glabrata*, *tropicalis*, *etc.*), *Cryptococcus neoformans*, Genus *Mucorales* (*mucor*, *absidia*, *rhizopus*), *Sporothrix schenckii*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Leptospirosis*, *Borrelia burgdorferi*, helminth parasite (hookworm, tapeworms, flukes, flatworms (*e.g.* *Schistosomia*), *Giardia lamblia*, *trichinella*, *Dientamoeba Fragilis*, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*).

X. Pharmaceutical Compositions

[00497] The present invention encompasses compositions comprising a molecule capable of binding CD3 and also capable of binding to a Disease Antigen (*e.g.*, a **DA x CD3 Binding**

Molecule, including, for example, a **DA x CD3 x CD8 Binding Molecule**, a **DA x CD3 x DA Binding Molecule**, *etc.*). The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a molecule capable of binding CD3 and also capable of binding to a Disease Antigen so as to be capable of mediating the redirected killing of a target cell (*e.g.*, a cancer cell, a pathogen-infected cell, *etc.*), or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the Binding Molecules of the present invention and a pharmaceutically acceptable carrier. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects).

[00498] Various formulations of such compositions may be used for administration. In addition to the pharmacologically active agent(s), the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well-known in the art and are relatively inert substances that facilitate administration of a pharmacologically effective substance or which facilitate processing of the active compounds into preparations that can be used pharmaceutically for delivery to the site of action. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers.

[00499] In a specific embodiment, the term “**pharmaceutically acceptable**” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “**carrier**” refers to a diluent, adjuvant (*e.g.*, Freund’s adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette

indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00500] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a binding molecule of the present invention, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00501] The present invention provides kits that can be used in the above methods. A kit can comprise any of the Binding Molecules of the present invention. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers.

XI. Methods of Administration

[00502] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of the pharmaceutical compositions of the present invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00503] Methods of administering a molecule or composition of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular,

intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the Binding Molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00504] The invention also provides that preparations of the Binding Molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the Binding Molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

[00505] The lyophilized preparations of the Binding Molecules of the present invention should be stored at between 2°C and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such Binding Molecules, when provided in liquid form, are supplied in a hermetically sealed container.

[00506] The amount of such preparations of the invention that will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00507] As used herein, an “**effective amount**” of a pharmaceutical composition is an amount sufficient to effect beneficial or desired results including, without limitation, clinical results such as decreasing symptoms resulting from the disease, attenuating a symptom of infection (*e.g.*, viral load, fever, pain, sepsis, *etc.*) or a symptom of cancer (*e.g.*, the proliferation, of cancer cells, tumor presence, tumor metastases, *etc.*), thereby increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication such as via targeting and/or internalization, delaying the progression of the disease, and/ or prolonging survival of individuals. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially, or simultaneously.

[00508] An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient: to kill and/or reduce the proliferation of cancer cells, and/or to eliminate, reduce and/or delay the development of metastasis from a primary site of cancer; or to reduce the proliferation of (or the effect of) an infectious pathogen and to reduce and /or delay the development of the pathogen-mediated disease, either directly or indirectly. In some embodiments, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more chemotherapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art.

[00509] For the Binding Molecules encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. For the Binding Molecules encompassed by the invention, the dosage administered to a patient is typically from about 0.01 µg/kg to about 30 mg/kg or more of the subject’s body weight.

[00510] The dosage and frequency of administration of a binding molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00511] The dosage of a binding molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00512] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00513] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) "New Methods Of Drug Delivery," Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 3 17-327).

[00514] Treatment of a subject with a therapeutically or prophylactically effective amount of a binding molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with a pharmaceutical composition of the invention for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The pharmaceutical compositions of the invention can be administered once a day with such administration occurring once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc.* Alternatively, the pharmaceutical compositions of the invention can be administered twice a day with such administration occurring once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc.* Alternatively, the pharmaceutical compositions of the invention can be administered three times a day with such administration occurring

once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc.* It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

XII. Specific Embodiments of the Invention

[00515] Specific embodiments of the of the invention include Embodiments E1-E27:

E1. A **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_{H1} 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_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (II) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} 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_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (III) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} 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_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} 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_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**.

- E2.** The **DA x CD3 Binding Molecule** of **E1**, 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**.
- E3.** The **DA x CD3 Binding Molecule** of **E1** or **E2**, 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.
- E4.** The **DA x CD3 Binding Molecule** of any one of **E1-E3**, 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.
- E5.** The **DA x CD3 Binding Molecule** of any one of **E1-E4**, wherein said Disease Antigen is a Cancer Antigen.
- E6.** The **DA x CD3 Binding Molecule** of any one of **E1-E4**, wherein said Disease Antigen is a Pathogen-Associated Antigen.

- E7.** The **DA x CD3 Binding Molecule**, of any one of **E4-E6**, wherein said different cell surface molecule of an effector cell is CD2, CD8, CD16, TCR, NKp46, or NKG2D.
- E8.** The **DA x CD3 Binding Molecule** of **E5** or **E7**, wherein said 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^b/Le^y, 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 α 2, Integrin β 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 phosphatase, R24, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T-cell receptor derived peptide, T5A7, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.
- E9.** The **DA x CD3 Binding Molecule** of **E8**, wherein said Disease Antigen is B7-H3, CEACAM5/CEACAM6, EGRF, EphA2, gpA33, HER2/neu, VEGF, 5T4, IL13R α 2, CD123, CD19, or ROR1.
- E10.** The **DA x CD3 Binding Molecule** of **E6** or **E7**, wherein said 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.

E11. The **DA x CD3 Binding Molecule** of any one of **E1-E10**, 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_{DA}); and
- (2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to said epitope of CD3 (VH_{CD3});

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_{CD3}); 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_{DA});

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:

(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.

E12. The **DA x CD3 Binding Molecule** of **E11**, 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.

E13. The **DA x CD3 Binding Molecule** of **E11** or **E12**, wherein the first or second polypeptide chain additionally comprises a Domain 3 comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.

E14. The **DA x CD3 Binding Molecule** of **E13**, wherein said **DA x CD3 Binding Molecule** further comprises a third polypeptide chain comprising a CH2 and CH3 Domain of an immunoglobulin Fc Domain.

E15. The **DA x CD3 Binding Molecule** of any one of **E11-E14**, wherein said **DA x CD3 Binding Molecule** further comprises a CD8-Binding Domain.

E16. The **DA x CD3 Binding Molecule** of any one of **E11-E15**, 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**.

E17. A pharmaceutical composition that comprises the **DA x CD3 Binding Molecule** of any of **E1-E16** and a pharmaceutically acceptable carrier.

E18. A method for the treatment of a disease, comprising administering to a subject in need thereof a therapeutically effective amount of the **DA x CD3 Binding Molecule** of any of **E1-E16** or the pharmaceutical composition of **E17**.

E19. The method of **E18**, wherein said disease is cancer.

- E20.** The method of **E19**, 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.
- E21.** The method of **E18**, wherein said disease is a pathogen-associated disease.
- E22.** The method of **E21**, wherein said 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.
- E23.** The **DA x CD3 Binding Molecule** of any of **E1-E16** or the pharmaceutical composition of **E16** for use in the treatment of a disease.
- E24.** The **DA x CD3 Binding Molecule** or pharmaceutical composition of **E23**, wherein said disease is cancer.
- E25.** The **DA x CD3 Binding Molecule** or pharmaceutical composition of **E24**, 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.
- E26.** The **DA x CD3 Binding Molecule** or pharmaceutical composition of **E23**, wherein said disease is a pathogen-associated disease.
- E27.** The **DA x CD3 Binding Molecule** or pharmaceutical composition of **E26**, wherein said 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.

EXAMPLES

[00516] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

Example 1**Evaluation of CD3 mAb 1 M3 – CD3 mAb 1 M26**

[00517] A CD3 mAb 1 scFv saturation-mutant library at 29 CDR positions was constructed and expressed in *E. coli* (XL-1 Blue). A multi-well format was used to produce soluble scFv. scFv-containing supernatants were captured on anti-His surface and screened for binding to recombinant CD3 (ϵ/γ chain Fos/Jun heterodimer) using an Attana biosensor to identify vCD3-Binding Domains.

[00518] Variants of the CD123 x CD3 **DART-A**-type diabody (designated **DART-A-WT**; amino acid sequences provided above) were generated comprising the VH and VL Domains of the identified scFvs. Thus, a panel of **DART-A**-type diabodies, designated **DART-A-M1 – DART-A-M26**, comprising vCD3-Binding Domains (such vCD3-Binding Domains being respectively designated “**CD3 mAb 1 M1 – CD3 mAb 1 M26**”) were generated. The CD3-binding kinetics of **DART-A-M1 – DART-A-M26** were measured by BIACORE® and compared to **DART-A-WT**. Table 11 summarizes the CD3-binding kinetics of **DART-A-WT** and the **DART-A**-type diabodies comprising the vCD3-Binding Domains of **CD3 mAb 1 M1 – CD3 mAb 1 M26**, ranked by k_a (R denotes k_a , k_d , or K_D ratio of variant **DART-A**-type diabody (comprising a vCD3-Binding Domain) to **DART-A-WT** (comprising the rCD3-Binding Domain of **CD3 mAb 1**)).

Table 11
CD3-Binding Kinetics of CD123 x CD3 DART-A-Type Diabodies
CD3 mAb 1 M1 -- CD3 mAb 1 M26

CD3 mAb1 Variant	Substitution (Relative to CD3 Mab-1)	k_a ($M^{-1}s^{-1}$)	R	k_d (s^{-1})	R	K_D (M)	R
M25	G50D/VL	1.54×10^5	0.3	1.92×10^{-2}	4.7	1.25×10^{-7}	15
M18	A33G/VH	2.87×10^5	0.6	5.88×10^{-2}	14	2.05×10^{-7}	25
M14	T31D/VH	3.08×10^5	0.6	1.43×10^{-2}	3.5	4.66×10^{-8}	5.7
M26	K53G/VL	3.19×10^5	0.6	1.74×10^{-2}	4.3	5.44×10^{-8}	6.7
M13	Y102E/VH	3.21×10^5	0.6	2.20×10^{-2}	5.4	6.86×10^{-8}	8.4
M16	Y32D/VH	3.47×10^5	0.7	1.04×10^{-1}	25	3.01×10^{-7}	37

Table 11 CD3-Binding Kinetics of CD123 x CD3 DART-A-Type Diabodies CD3 mAb 1 M1 -- CD3 mAb 1 M26							
CD3 mAb1 Variant	Substitution (Relative to CD3 Mab-1)	k_a (M⁻¹s⁻¹)	R	k_d (s⁻¹)	R	KD (M)	R
M15	T31E/VH	3.52 × 10 ⁵	0.7	3.75 × 10 ⁻²	9.2	1.07 × 10 ⁻⁷	13
M1	S100dT/VH	4.08 × 10 ⁵	0.8	6.50 × 10 ⁻²	16	1.59 × 10 ⁻⁷	19
M3	G99I/VH	4.49 × 10 ⁵	0.9	8.30 × 10 ⁻³	2	1.85 × 10 ⁻⁸	2.3
M23	L95E/VL	4.59 × 10 ⁵	0.9	1.24 × 10 ⁻²	3	2.69 × 10 ⁻⁸	3.3
CD3 Mab-1	Wild-Type	5.00 × 10⁵	1	4.09 × 10⁻³	1	8.17 × 10⁻⁹	1
M24	L95Q/VL	5.94 × 10 ⁵	1.2	8.61 × 10 ⁻³	2.1	1.45 × 10 ⁻⁸	1.8
M6	Y100bQ/VH	6.99 × 10 ⁵	1.4	2.01 × 10 ⁻²	4.9	2.88 × 10 ⁻⁸	3.5
M10	F98I/VH	7.17 × 10 ⁵	1.4	3.10 × 10 ⁻²	7.6	4.33 × 10 ⁻⁸	5.3
M19	G96K/F98I/VH	7.53 × 10 ⁵	1.5	4.11 × 10 ⁻²	10	5.46 × 10 ⁻⁸	6.7
M4	Y100bA/VH	7.90 × 10 ⁵	1.6	2.03 × 10 ⁻²	5	2.57 × 10 ⁻⁸	3.1
M17	Y32T/VH	8.37 × 10 ⁵	1.7	4.78 × 10 ⁻²	12	5.72 × 10 ⁻⁸	7
M12	W100eY/VH	8.66 × 10 ⁵	1.7	1.11 × 10 ⁻²	2.7	1.28 × 10 ⁻⁸	1.6
M7	G96D/VH	1.02 × 10 ⁶	2	2.29 × 10 ⁻²	5.6	2.25 × 10 ⁻⁸	2.8
M8	G96E/VH	1.13 × 10 ⁶	2.3	7.84 × 10 ⁻³	1.9	6.91 × 10 ⁻⁹	0.8
M5	Y100bG/VH	1.31 × 10 ⁶	2.6	2.80 × 10 ⁻²	6.8	2.14 × 10 ⁻⁸	2.6
M11	W100eF/VH	1.39 × 10 ⁶	2.8	2.67 × 10 ⁻²	6.5	1.92 × 10 ⁻⁸	2.4
M9	G96K/VH	2.15 × 10 ⁶	4.3	7.16 × 10 ⁻³	1.8	3.33 × 10 ⁻⁹	0.4
M22	G96K/W100eY/VH	2.37 × 10 ⁶	4.7	1.56 × 10 ⁻²	3.8	6.60 × 10 ⁻⁹	0.8
M21	G96K/W100eF/VH	2.45 × 10 ⁶	4.9	1.03 × 10 ⁻²	2.5	4.18 × 10 ⁻⁹	0.5
M2	G96K/S100dT/VH	3.07 × 10 ⁶	6.1	3.91 × 10 ⁻²	9.6	1.27 × 10 ⁻⁸	1.6
M20	G96K/Y100bG/VH	3.87 × 10 ⁶	7.7	7.32 × 10 ⁻²	18	1.89 × 10 ⁻⁸	2.3

[00519] The ability of **DART-A-M1 – DART-A-M26** (comprising vCD3-Binding Domains) to mediate T-cell redirected cell killing was measured in CTL assay and compared to **DART-A-WT** (comprising the rCD3-Binding Domain). Briefly, the **DART-A**-type diabodies were incubated with effector Pan-T-cells and MOLM-13 target tumor cells, at an effector:target cell ratio of 5:1 for 18 and 42 hours and the EC₅₀ was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells (e.g., by using the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega) that quantitatively measures LDH release, or similar). A 4420 x CD3 fluorescein-binding **DART-A**-type diabody having the CD3 Binding Domain of **CD3 mAb 1** was employed as a negative control. In addition, the stability of the **DART-A**-type diabodies was evaluated by measuring the T_m using DSF. Representative cytotoxicity curves for **DART-A-WT**;

DART-A-M1; DART-A-M2; DART-A-M15; DART-A-M17; DART-A-M18; DART-A-M19; and DART-A-M20 are presented in **Figure 7A**. **Table 12** summarizes the cytotoxicity (*i.e.*, T-cell redirected killing activity) of **DART-A-M1 – DART-A-M26**, ranked by 18-hour EC₅₀ (R denotes ratio of variant **DART-A**-type diabody (comprising a vCD3-Binding domain) to **DART-A-WT** (comprising the rCD3-Binding Domain of **CD3 mAb 1**); ΔT_m denotes change in T_m as compared to WT (T_m = 63°C). The relationship of the kinetic parameters and cytolytic potency is plotted in **Figures 7B-7D** (**Figure 7B**: affinity vs cytotoxicity (18 hour EC₅₀), **Figure 7C**: association rate vs cytotoxicity (EC₅₀), **Figure 7D**: dissociation rate vs cytotoxicity (EC₅₀)). The **CD3 mAb1** (○), **M18** (■), **M2** (▲) and **M1** (▼) variants are indicated.

Table 12 Cytotoxicity Of Antibodies CD3 mAb 1 M1 – CD3 mAb 1 M26								
CD3 mAb 1 Variant	Substitution (Relative to CD3 Mab-1)	MOLM-13 (E:T=5:1)						R ΔTm (°C)
		EC ₅₀ (ng/mL)	R	max % CytoTox	EC ₅₀ (ng/mL)	R	max % CytoTox	
		18 Hour			42 Hour			
M21	G96K/W100eF/VH	0.026	0.3	36.54	0	0	29.92	-1
M8	G96E/VH	0.069	0.9	38.43	0.005	1.7	29.35	1
M22	G96K/W100eY/VH	0.072	0.9	38.26	0.01	3.5	31.58	-0.5
M9	G96K/VH	0.075	1	38.25	0.001	0.4	30.46	0
CD3 Mab-1	Wild-Type	0.079	1	38.97	0.003	1	29.13	-
M3	G99I/VH	0.108	1.4	39.69	0.006	2.3	28.58	0
M6	Y100bQ/VH	0.162	2.1	41.86	0.011	3.9	29.46	1
M4	Y100bA/VH	0.176	2.2	41.69	0.011	3.9	29.8	1
M11	W100eF/VH	0.223	2.8	48.55	0.016	5.8	32.43	0.5
M5	Y100bG/VH	0.225	2.9	40.75	0.011	3.8	28.41	1
M20	G96K/Y100bG/VH	0.259	3.3	37.48	0.038	13.5	30.89	-0.5
M7	G96D/VH	0.304	3.8	41.38	0.03	10.8	32.54	1
M24	L95Q/VL	0.34	4.3	49.41	0.031	11.1	31.12	0.5
M12	W100eY/VH	0.372	4.7	49.72	0.067	24	31.54	0.5
M23	L95E/VL	0.421	5.3	49.29	0.061	21.6	31.37	-0.5
M10	F98I/VH	0.426	5.4	39.26	0.095	33.7	29.87	0
M26	K53G/VL	0.444	5.6	44.23	0.078	27.9	28.19	0
M14	T31D/VH	0.492	6.2	44.69	0.11	39.3	31.36	1.5
M25	G50D/VL	0.585	7.4	46.56	0.1	35.7	30.41	0

Table 12
Cytotoxicity Of Antibodies
CD3 mAb 1 M1 – CD3 mAb 1 M26

CD3 mAb 1 Variant	Substitution (Relative to CD3 Mab-1)	MOLM-13 (E:T=5:1)						R ΔTm (°C)
		EC ₅₀ (ng/mL)	R	max % CytoTox	EC ₅₀ (ng/mL)	R	max % CytoTox	
		18 Hour			42 Hour			
M19	G96K/F98I/VH	0.68	8.6	41.01	0.11	39.1	32.83	-0.5
M13	Y102E/VH	1.223	15.5	45.08	0.203	72.3	31.48	-0.5
M17	Y32T/VH	1.283	16.2	44.23	0.126	44.9	30.69	-0.5
M15	T31E/VH	4.164	52.7	42.74	0.975	347.2	32.05	1.5
M18	A33G/VH	4.687	59.3	41.3	0.91	324	30.58	0.5
M2	G96K/S100dT/VH	8.113	102.7	32.33	0.693	246.9	32.77	-1
M1	S100dT/VH	19.64	248.6	32.32	2.336	831.9	32.37	0
M16	Y32D/VH	NA	NA	NA	NA	NA	NA	-0.5

[00520] As indicated in **Tables 11-12**, the **DART-A-M1 – DART-A-M26** variants displayed a range of binding kinetics and CTL activity, while retaining their thermal stability. Such **DA x CD3 Binding Molecules**, and their vCD3-Binding Domains, are useful for modulating CD3 binding, redirected T-cell killing activity, and/or T-cell stimulation activity.

Example 2

CTL Activity and Cytokine Release of Representative Variants

[00521] The cytotoxic (CTL) activity and cytokine release profile of a representative set of **DART-A**-type diabodies comprising variant **CD3 mAb 1** VL or VH Domains was assessed by incubating **DART-A-WT**; **DART-A-M2**; **DART-A-M7**; **DART-A-M13**; and **DART-A-M15** in the presence of Pan-T-cells effector cells and MV-4-11 leukemia target tumor cells at an effector:target cell ratio of 5:1 24 hours. The percentage cytotoxicity (*i.e.*, cell killing) and/or EC₅₀ was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells (*e.g.*, by using the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega) that quantitatively measures LDH release, or similar) and is plotted in **Figure 8A**. Cytokines released into the supernatant during the CTL was measured (*e.g.*, using Enzyme-Linked ImmunoSpot (ELISPOT) assay or milliplex cytokine assay). Cytokine release is plotted in **Figures 8B-8E** (**Figure 8B**: INF- γ , **Figure 8C**: TNF- α , **Figure 8D**: IL-6, and **Figure 8E**: IL-2). The EC₅₀ Values for cytotoxicity and cytokine release are provided in **Table 13**. A 4420 x CD3 fluorescein-binding **DART-A**-type

diabody having the CD3 Binding Domain of **CD3 mAb 1** was employed as a negative control (**NegCtrl**).

Table 13					
DART-A-Type	EC₅₀CTL	EC₅₀INF-γ	EC₅₀TNFα	EC₅₀IL-6	EC₅₀IL2
WT	0.029	0.11	0.76	0.13	0.85
M2	0.92	1.7	4.7	10	27
M7	0.052	0.98	6.6	1.1	7.0
M13	0.12	0.83	0.12	1.3	7.5
M15	0.39	3.5	18	7.5	30

[00522] The results from these studies show that **DA x CD3 Binding Molecules** comprising vCD3-Binding Domains displaying having altered affinity retain cytolytic activity and exhibit one or more reduced cytokine responses (max response and/or EC₅₀) as compared to a **DA x CD3 Binding Molecule** comprising the rCD3-Binding Domain.

Example 3 Generation of DART-B-Type Diabodies

[00523] Diabodies possessing the VH Domain of **CD3 mAb 1 M18** were selected for further characterization and comparison to diabodies possessing the VH Domain of **CD3 mAb 1**, **CD3 mAb 1 M1** and **CD3 mAb 1 M2**. Thus, the **DART-B-type** diabodies of **Table 9** were prepared comprising a Disease Antigen (DA) Binding Domain binding the Cancer Antigen CD123, 5T4, or CD19. The amino acid sequences of each chain are provided in detail herein (see First – Nineteenth Illustrative **DART-B-type** Diabodies, *supra*). Briefly, the diabodies were expressed in CHO cells (transient or stably transfected) and purified over a Protein A affinity resin (*e.g.*, MabSelect) followed by HPLC size exclusion chromatography.

Example 4 Ability of DART-B-Type Diabodies to Bind to Disease Antigens

[00524] The ability of such diabodies to bind to their respective Disease Antigen (*i.e.*, CD123 or 5T4) on the surface of MOLM-13 leukemia (CD123) or A-498 kidney carcinoma (5T4) target cancer cells was assessed using FACS. Briefly, cells were incubated with the diabody molecules (in FACS buffer containing 10% human AB serum) in microtiter plates. The cells were washed and incubated with biotin-conjugated mouse anti-EK-coil antibody that recognizes the E-coil/K-coil (EK) Heterodimer-Promoting Domain of the diabodies mixed with streptavidin-phycoerythrin. Representative data of such assays are shown in

Figures 9A-9B. The data shows that the representative diabodies were capable of binding to their respective Disease Antigens.

Example 5

Ability of DART-B-Type Diabodies to Bind to CD4⁺ and CD8⁺ T-cells

[00525] The ability of the CD123-binding diabodies: **CD123-WT**, **CD123-M1**, **CD123-M2** and **CD123-M18** to bind to CD4⁺ and CD8⁺ T-cells was also assessed using FACS. A 4420 x CD3 fluorescein-binding **DART-A**-type diabody having the CD3 Binding Domain of **CD3 mAb 1** was employed as a control for CD3 binding ("**4420-CD3**"). Briefly, CD4⁺ and CD8⁺ T-cells were incubated with the diabody molecules (in FACS buffer containing 10% human AB serum) in microtiter plates. The cells were washed and incubated with a labeled anti-human Fc secondary antibody. The cells were then washed and resuspended with FACS buffer, and analyzed by flow cytometry. Representative data of such assays are shown in **Figure 10A** (binding to CD8⁺ T-cells) and **Figure 10B** (binding to CD4⁺ T-cells). **T-CD123-M1** and **T-CD123-M18** exhibit reduced binding to CD3 expressing CD4⁺ and CD8⁺ T-cells.

[00526] The ability of the CD123-binding diabodies: **CD123-WT**, **CD123-M1**, **CD123-M2** and **CD123-M18** to bind to human CD3 and CD123 was also evaluated using BIAcore®. Briefly, diabodies at concentrations of 62.5-1000 nM were passed over soluble human CD3 that had been immobilized to a surface (normalized; 1:1 Binding Fit). The high analyte concentrations (62.5-1000 nM) were used in order to allow evaluation of parameters for weak CD3 interactions, however, high concentrations of analyte can be associated with a contribution of non-specific binding. In separate studies diabodies at concentrations of 62.5-100 nM were passed over soluble CD123 that had been His-tagged and captured to an anti-PentaHis surface (normalized; 1:1 Binding Fit). **Table 14** presents the calculated **k_a**, **k_d** and **KD** from these studies.

Table 14						
CD3 Variant	Binding to human CD3			Binding to human CD123		
	k_a (x10⁴)	k_d (x10⁻³)	KD (nM)	k_a (x10⁵)	k_d (x10⁻⁴)	KD (nM)
CD123-WT	9.1	6.1	67	2.7	5.3	2.0
CD123-M1	9.5	80	842	3.2	3.9	1.2
CD123-M2	3.8	41	108	4.2	4.5	1.1
CD123-M18	8.6	51	593	2.4	5.4	2.3

[00527] The ability of the 5T4-binding diabodies: **5T4-WT**, **5T4-M1**, **5T4-M2** and **5T4-M18** to bind to CD3 was also evaluated using BIAcore®, as described above. **Table 15A** presents the calculated k_a , k_d and **KD**.

Table 15A			
CD3 Variant	Binding to human CD3		
	k_a ($\times 10^5$) ($M^{-1}s^{-1}$)	k_d ($\times 10^{-3}$) (s^{-1})	KD (nM)
5T4-WT	1.5	5.4	36
5T4-M1	0.95	31	326
5T4-M2	3.5	41	118
5T4-M18	0.75	34	453

[00528] In additional studies the ability of CD123-binding diabodies: **CD123-WT**, **CD123-M13**, **CD123-M17** and **CD123-M19** to bind to human CD3 and cynomolgus CD3 was also evaluated using BIAcore®. Briefly, diabodies at concentrations of 6.25-400 nM were passed over immobilized human CD3 or cyno CD3 (1:1 Langmuir Binding Fit). **Table 15B** presents the calculated k_a , k_d and **KD**.

Table 15B						
CD3 Variant	Binding to human CD3			Binding to cyno CD3		
	k_a ($\times 10^4$)	k_d ($\times 10^{-3}$)	KD (nM)	k_a ($\times 10^4$)	k_d ($\times 10^{-3}$)	KD (nM)
CD123-WT	9.3	4.8	52	11	4.3	39
CD123-M13	5.9	27	458	5	29	580
CD123-M17	19	55	290	17	56	329
CD123-M19	22	48	218	22	50	227

Example 6

Ability of Exemplary DART-B-Type Diabodies to Mediate Redirected Cell Killing

[00529] Exemplary DART-B-type diabodies were evaluated for their ability to mediate redirected cell killing. Where indicated, **HIV-WT** or **HIV-M18** (described above) are used here as a negative control as they do not bind a Cancer Antigen. It will be understood that the **HIV-WT** and **HIV-M18** diabodies will bind cells expressing the epitope bound by the A32 antibody (HIV env) on their cell surface (e.g., HIV infected cells), see for example: WO 2014/1599401 and WO 2016/054101, and are capable of mediating redirected cell killing of such cells.

[00530] The results of representative studies of redirected cell killing mediated by exemplary CD123 x CD3 **DART B**-type diabody constructs are presented in **Figures 11A-11Q**, **Figures 12A-12E**, and **Figures 26A-26E**. The results of representative studies of

redirected cell killing mediated by exemplary 5T4 x CD3 **DART B**-type diabody constructs are presented in **Figures 13A-13Q**. The results of representative studies of redirected cell killing mediated by exemplary CD19 x CD3 **DART B**-type diabody constructs are presented in **Figures 14A-14J**. These assays were performed essentially as described above using the indicated effector and target cells, effector:target cell ratios, and incubation times described below (also see, **Figures 11A, 12A, 13A and 14A**). Where indicated, the release of IFN- γ , TNF- α , IL-6, and IL-2 cytokines was determined at the end of the CTL assay using standard commercial reagents.

[00531] **Figures 11A-11Q** show the results of representative studies of redirected cell killing mediated by CD123 x CD3 **DART B**-type diabody constructs (possessing Fc Domains) **CD123-WT**, **CD123-M2** and **CD123-M18** using Pan-T effector cells and MOLM-13 acute monocytic leukemia (AML) target cells (E:T= 5:1, 24 h). Percent cytotoxicity is plotted in **Figure 11A**. Cytokine responses and cytotoxicity are plotted in **Figures 11B-11Q** (**Figures 11B-11E**: IFN-gamma; **Figures 11F-11I**: TNF-alpha; **Figures 11J-11M**: IL-6; **Figures 11N-11Q**: IL-2). **Figures 11B, 11F, 11J and 11N**: **CD123-WT**; **Figures 11C, 11G, 11K and 11O**: **CD123-M2**; **Figures 11D, 11H, 11L and 11P**: **CD123-M18**; **Figures 11E, 11I, 11M and 11Q**: **HIV-WT** (Negative Control). Similar cytotoxicity was observed against another AML cell line, MV-4-11.

[00532] **Figure 11A** shows that CD123 x CD3 Binding Molecules comprising different **CD3 mAb 1** variants exhibited markedly differing abilities to mediate cytotoxicity particularly as measured by comparing EC₅₀, but reaching a similar maximum cytotoxicity. In addition, these molecules exhibited differing abilities to mediate cytokine responses. For example, as seen in **Figures 11B-11Q**, although **CD123-M18** exhibited levels of maximal cytotoxicity that were similar to those exhibited by **CD123-WT**, the levels of cytokines IFN- α , TNF- α and IL-6 released by treatment with **CD123-M18** were approximately 50% of the levels released by treatment with **CD123-WT**, and the level of IL-2 observed with **CD123-M18** was significantly less than the IL-2 level observed with **CD123-WT**. Thus, **CD123-M18** was found to be able to provide a therapeutic value that was comparable to that of **CD123-WT**, but with less attending side effects than **CD123-WT**.

[00533] **Figures 12A-12E** show the results of representative studies of redirected cell killing mediated by CD123 x CD3 **DART B**-type diabody constructs (possessing Fc Domains) using PBMC effector cells and MOLM-13 AML target cells. Percent cytotoxicity

is plotted in **Figure 12A** (E:T= 15:1, 24 h). Cytokine responses (measured in a milliplex cytokine assay) are plotted in **Figures 12B-12E** (**Figure 12B**: IFN-gamma; **Figure 12C**: TNF-alpha; **Figure 12D**: IL-6; **Figure 12E**: IL-2).

[00534] The results again demonstrate that **CD123-WT** and **CD123-M18** exhibit similar levels of maximal cytotoxicity, but **CD123-M18** exhibited markedly reduced cytokine responses. Additionally, the EC₅₀ values of **CD123-M18** for release of IFN- γ , TNF- α or IL-6 were substantially more those of **CD123-WT** indicating that treatment with **CD123-M18** resulted in significantly less cytokine release as compared to treatment with **CD123-WT**.

[00535] **Figures 26A-26D** and **Figures 27A-27D** show the results of studies of redirected cell killing mediated by CD123 x CD3 **DART B-type** diabody constructs (possessing Fc Domains) **CD123-WT**, **CD123-M1**, **CD123-M13**, **CD123-M17**, **CD123-M18** and **CD123-M19** using Pan-T effector cells and MOLM-13 acute monocytic leukemia (AML) target cells (E:T= 5:1, 48 to 96 hours). As noted below, **DART-A-WT** was included as a comparator in some studies. **Figures 26A-26D** show the results of a representative study performed for 48 hours. In **Figure 26A** cytotoxicity is plotted, as a function of %LDH released. Cytokine responses and cytotoxicity are plotted in **Figures 26B-26E** (**Figure 26B**: IFN-gamma; **Figure 26C**: TNF-alpha; **Figure 26D**: IL-6; **Figure 26E**: IL-2). **Figures 27A-27D** summarize the results from 4-7 such studies performed for 48 and 96 hours that included **DART-A-WT**. **Figures 27A-27C** provide comparative plots of the cytotoxicity (CTL) activity at 48 and 96 hours from four such studies (**Figure 27A**: CTL activity EC₅₀ values in pM; **Figure 27B**: CTL activity as a multiple of the EC₅₀ value of **CD123-WT**, **Figure 27C**: CTL activity E_{max} as a percent of **CD123-WT**). **Figure 27D** plots the Therapeutic Index for CTL Activity against the cytokine IL-2.

[00536] **Figure 26A** shows that while CD123 x CD3 Binding Molecules comprising different **CD3 mAb 1** variants exhibited markedly different cytotoxicity curves with **CD123-M13**, **CD123-M17**, **CD123-M18** and **CD123-M19**, they are able to reach a similar maximum cytotoxicity. As was seen above, each of the variants mediated lower cytokine responses. For example, as seen in **Figures 26B-26D**, although **CD123-M13**, **CD123-M17**, **CD123-M18** and **CD123-M19** exhibited levels of maximal cytotoxicity that were similar to those exhibited by **CD123-WT**, the levels of cytokines IFN- α , TNF- α , IL-6 and IL-2 released were significantly less than the level observed with **CD123-WT** treatment. Thus,

each of the diabody molecules comprising the **CD3 mAb 1** variants **M13**, **M17**, **M18** and **M19** were found to be able to provide a therapeutic value that was comparable to that of diabody constructs comprising wild-type **CD3 mAb 1**, but with less attending side effects.

[00537] The results shown in **Figure 27A-27C** further demonstrate that **CD123-M13**, **CD123-M17**, **CD123-M18** and **CD123-M19** exhibit marked different cytotoxicity EC_{50} values but reach a maximum CTL activity that is comparable to **CD123-WT** and **DART-A-WT**. Using IL-2 as a representative cytokine, a Therapeutic Index (TI) was determined as follows:

$$TI = E_{\max} (CTL) : E_{\max} (cytokine)$$

[00538] The calculated TI values normalized to the values for **CD123-WT** are plotted in **Figure 27D** and further demonstrate that **DA x CD3 Binding Molecules** comprising the **CD3 mAb 1** variants **M13**, **M17**, **M18** and **M19** exhibit an enhanced TI over those comprising wild-type **CD3 mAb 1**.

[00539] **Figures 13A-13Q** show the results of representative studies of redirected cell killing mediated by 5T4 x CD3 **DART B**-type diabody constructs (possessing Fc Domains,) **5T4-WT**, **5T4-M1**, **5T4-M2**, and **5T4-M18**, using Pan-T effector cells and A498 renal cell carcinoma target cells (E:T= 5:1, 24 h). Percent cytotoxicity is plotted in **Figure 13A**. Cytokine responses and cytotoxicity are plotted in **Figures 13B-13Q** (**Figures 13B-13E**: IFN-gamma; **Figures 13F-13I**: TNF-alpha; **Figures 13J-13M**: IL-6; **Figures 13N-13Q**: IL-2). **Figures 13B**, **13F**, **13J** and **13N**: **5T4-WT**; **Figures 13C**, **13G**, **13K** and **13O**: **5T4-M2**; **Figures 13D**, **13H**, **13L** and **13P**: **5T4-M18**; **Figures 13E**, **13I**, **13M** and **13Q**: **HIV-WT** (Negative Control). Cytotoxicity was also observed against JIMT-1 breast carcinoma cells. These results demonstrate that **5T4-WT** and **5T4-M18** exhibit similar levels of maximal cytotoxicity, but markedly different cytokine responses, with **5T4-M18** exhibiting significantly reduced levels of cytokine release as compared to **5T4-WT**.

[00540] **Figures 14A-14J** show the results of representative studies of redirected cell killing mediated by CD19 x CD3 **DART B**-type diabody constructs (possessing Fc Domains), **CD19-WT**, and **CD19.1-M18**, using Pan-T, or PBMC effector cells and Raji lymphoblastoid target cells (E:T= 30:1 for PBMCs and 10:1 for Pan-T-cells, 24-48 h). Percent cytotoxicity (48 hrs) is plotted in **Figures 14A** (PBMCs) and **Figure 14F** (Pan-T-cells). Cytokine responses at 48 hours using PBMCs are plotted in **Figures 14B-14E**

(PBMCs) and **Figures 14G-14J** (Pan T-cells) (**Figures 14B** and **14G**: IFN-gamma; **Figures 14C** and **14H**: TNF-alpha; **Figures 14D** and **14I**: IL-6; **Figures 14E** and **14J**: IL-2; **HIV-M18** (Negative control)). **CD19.1-M18** exhibited similar cytotoxicity and reduced cytokine release against Daudi target cells. These results demonstrate that **CD19-WT** and **CD19.1-M18** exhibit similar levels of maximal cytotoxicity, but markedly different cytokine responses with **CD19.1-M18** exhibiting significantly reduced levels of cytokine release as compared to **CD19-WT**.

[00541] The results of the above studies confirm that constructs comprising the **CD3 mAb 1 M18** variant exhibited higher cytotoxic (CTL) activity in CTL assays than those comprising the M1 and M2 variants. The CTL studies also indicate that constructs comprising the M18 variant exhibited lower cytokine responses as compared to WT, and similar to or only slightly above those exhibited by the less active M2 variant. Thus, the M18 variant appears to have a larger window for CTL active vs cytokine release.

Example 7

Ability of Exemplary DART-B-Type Diabodies to Mediate T-Cell Activation

[00542] The ability to mediate T-cell activation measured by evaluating the ability of the diabodies to affect expression of CD25 and CD69, which are markers of T-cell activation, on CD4⁺ and CD8⁺ T-cell populations. The T-cell populations were obtained from CTL assays, which were performed essentially as described above. Where indicated, CD4⁺ and CD8⁺ T lymphocyte populations were assessed for up-regulation of the activation markers CD69 and CD25 by flow cytometry at the end of the CTL assay.

[00543] Representative data for CD123 x CD3 **DART-B**-type diabody constructs is shown in **Figures 15A-15E**. Cytotoxicity is plotted in **Figure 15A**. Activation of CD4⁺ T-cells as determined by measuring CD25 is plotted in **Figure 15B**. Activation of CD4⁺ T-cells as determined by measuring CD69 is plotted in **Figure 15C**. Activation of CD8⁺ T-cells as determined by measuring CD25 is plotted in **Figure 15D**. Activation of CD8⁺ T-cells as determined by measuring CD69 is plotted in **Figure 15E**.

[00544] Representative data for 5T4 x CD3 **DART-B**-type diabody constructs is shown in **Figures 16A-16E**. Cytotoxicity is plotted in **Figure 16A**. Activation of CD4⁺ T-cells as determined by measuring CD25 is plotted in **Figure 16B**. Activation of CD4⁺ T-cells as determined by measuring CD69 is plotted in **Figure 16C**. Activation of CD8⁺ T-cells as

determined by measuring CD25 is plotted in **Figure 16D**. Activation of CD8⁺ T-cells as determined by measuring CD69 is plotted in **Figure 16E**.

[00545] The results of these studies show that constructs comprising the **CD3-M18** variant exhibited enhanced T-cell activation activity relative to constructs comprising the M1 and M2 variants.

Example 8 ***in vivo* Activity of Exemplary DART-B-Type Diabodies in Murine Models**

[00546] The *in vivo* activity of the CD123 x CD3 **DART-B**-type diabodies **CD123-WT** and **CD123-M18** were evaluated in a co-mix KG1A cell AML model (E:T = 1:5). Briefly, NOD/SCID mice (6 per group) were injected with KG1A (AML) cells co-mixed with activated human CD4⁺ or CD8⁺ T-cells (E:T = 1:5) on Day 0. Vehicle control, **CD123-WT** (50 µg/kg), or **CD123-M18** (5 µg/kg or 50 µg/kg) were subsequently administered. Tumor volume was monitored over the course of the study.

[00547] The results of this study are provided in **Figure 17A** (CD4⁺ T-cells) and **Figure 17B** (CD8⁺ T-cells). The results show that constructs comprising the M18 variant exhibited anti-tumor activity comparable to that of constructs comprising the WT CD3 Binding Domains.

[00548] A further study to evaluate the *in vivo* activity of the CD123 x CD3 **DART-B**-type diabodies was performed using a reconstituted tumor model in which 5 x 10⁶ KG1A (AML) cells were subcutaneously (SC) injected into MHC1^{-/-} mice (5 female per group) on Day 0. On Day 9 1 x 10⁷ PBMC cell were injected intraperitoneal (IP). Vehicle control, **CD123-WT**, **CD123-M2** or **CD123-M18** (each at 0.5, 5, 50, or 500 µg/kg) were administered intravenously (IV) twice a week (2QW) starting on Day 15. Tumor volume was monitored over the course of the study.

[00549] The results of this study are provided in **Figures 18A-18D**. The results show that **CD123-M2** exhibited no activity in this model (**Figure 18B**). In contrast, **CD123-M18** (**Figure 18C**) exhibited anti-tumor activity comparable to that of **CD123-WT** (**Figure 18A**) particularly at 50 µg/kg and 500 µg/kg doses (**Figure 18D**).

[00550] Another study to evaluate the *in vivo* activity of the CD123 x CD3 **DART-B**-type diabodies was performed using a PBMC engraftment model in which 5 x 10⁶ MV4-11

(leukemia) cells were injected SC and 1×10^7 PBMC cell were injected retro-orbitally (RO) into MHCII^{-/-} mice (6 males per group) on Day 0. Vehicle control, **CD123-WT** (at 0.5, 5, 50, or 500 µg/kg), **CD123-M18** or **CD123-M2** (each at 5, 50, 500 or 1000 µg/kg) were administered intravenously (IV) twice a week (2QW) starting on Day 14. Tumor volume was monitored over the course of the study.

[00551] The results of this study are provided in **Figures 19A-19D**. The results show that **CD123-WT** exhibited anti-tumor activity at 0.5 µg/kg and above (**Figure 19A**). **CD123-M18** exhibited anti-tumor activity at 50 µg/kg and above (**Figure 19B**). In contrast, **CD123-M2** only exhibited anti-tumor activity at 1000 µg/kg (**Figure 19C**). As shown in **Figure 19D**, CD123-M18 anti-tumor activity is comparable to that of **CD123-WT** at 500 µg/kg, while CD123-M2 exhibited little or no anti-tumor activity at this concentration.

[00552] The *in vivo* activity of the 5T4 x CD3 **DART-B**-type diabodies, **5T4-WT**, **5T4-M1** and **5T4-M18**, were evaluated in a in PBMC engraftment model in which 5×10^6 SKOV3 (ovarian carcinoma) cells were injected SC and 1×10^7 PBMC cell were injected RO into MHCII^{-/-} mice (8 females per group) on Day 0. Vehicle control, **5T4-WT** (10, 50, 100, or 500 µg/kg), **5T4-M18** (10, 50, 100, or 500 µg/kg), or **5T4-M2** (500 µg/kg) were administered intravenously (IV) twice a week (2QW) starting on Day 7. Tumor volume was monitored over the course of the study.

[00553] The results of this study are provided in **Figures 20A-20B**. The results show that **5T4-WT** exhibited potent anti-tumor activity at all doses tested (**Figure 20A**). **5T4-M18** exhibited dose dependent anti-tumor activity that was comparable to **5T4-WT** at 500 µg/kg, while **5T4-M2** exhibited significantly lower activity at 500 µg/kg (**Figure 20B**).

[00554] The *in vivo* cytokine release profile induced by CD123 x CD3 **DART-B**-type diabodies was examined in a PBMC co-mix model. Briefly, 5×10^6 KG1A (AML) and 1×10^7 PBMC cells were mixed and incubated overnight, the next day the mixed cells were injected SC into NSG mice (6 males per group) and a single dose of vehicle control, **CD123-WT**, **CD123-M18** or **CD123-M2** (each at 50, or 500 µg/kg) was administered intravenously (IV). Six hours post administration serum cytokine levels were evaluated. The cytokine release profiles are plotted in **Figures 20A-20D** (**Figure 21A**: IFN-γ; **Figure 21B**: TNF-α; **Figure 21C**: IL-6; and **Figure 21D**: IL-2). The results of these studies show that treatment

with **DA x CD3 Binding Molecules** comprising the variant VL and VH Domains of **CD3 mAb 1 M2**, and **CD3 mAb 1 M18** exhibit lower levels of cytokine release

[00555] Two further studies to evaluate the *in vivo* activity of the CD123 x CD3 **DART-B**-type diabodies were performed using a reconstituted tumor model in which PBMC reconstituted (8×10^6 PBMC injected retro-orbitally on Day 0) NSG/MHCl^{-/-} mice (7-8 mice per group), were subcutaneously (SC) injected with 5×10^6 KG1A (AML) cells on day 7. In one study, **CD123-WT** (0.5 mg/kg), **CD123-M18** and **CD123-M13** (at 0.005, 0.05, 0.5 and 1 mg/kg) or vehicle were administered intravenously (IV) twice a week (2QW) starting on Day 28. In the other study **CD123-WT** (0.05 mg/kg), **CD123-M18** and **CD123-M17** (at 0.005, 0.05, 0.5 and 1 mg/kg) or vehicle were administered intravenously (IV) twice a week (2QW) starting on Day 28. Tumor volume was monitored over the course of the study.

[00556] The results of these studies are provided in **Figures 28A-28B** (treatment with **CD123-WT**, **CD123-M18** and **CD123-M13**) and **Figures 29A-29B** (treatment with **CD123-WT**, **CD123-M18** and **CD123-M17**). The results show that **CD123-M18** anti-tumor activity was similar to that of **CD123-WT** at doses of 0.5 mg/kg and above (**Figures 28A** and **29A**) and that **CD123-M13** and **CD123-M17** exhibited anti-tumor activity similar to that of **CD123-WT** starting at just 0.05 mg/kg, a 10 fold lower dose than for **CD123-M18** (**Figures 28B** and **29B**).

[00557] The *in vivo* cytokine release profiles induced by CD123 x CD3 **DART-B**-type diabodies **CD123-WT**, **CD123-M13**, **CD123-M17** and **CD123-M18** were examined in a PBMC co-mix model. Briefly, 5×10^6 KG1A (AML) and 1×10^7 PBMC cells were mixed and incubated overnight, the next day the mixed cells were injected SC into NSG mice (7-8 per group) and a single dose of **CD123-WT** (0.5 mg/kg), **CD123-M13**, **CD123-M17** and **CD123-M18** (at 0.05, 0.5 and 1 mg/kg), or vehicle was administered intravenously (IV). Six hours post administration serum cytokine levels were evaluated. In one study animals were treated with **CD123-WT**, **CD123-M18** and **CD123-M13**, and in a separate study, animals were treated with **CD123-WT**, **CD123-M18** and **CD123-M17**. The IL-2 cytokine release profiles are plotted in **Figures 30A-30B** (**Figure 30A**: **CD123-WT**, **CD123-M18** and **CD123-M13**); **Figure 30B**: **CD123-WT**, **CD123-M18** and **CD123-M17**) and show that treatment with **DA x CD3 Binding Molecules** comprising the variant CD3 mAb 1 VL and

VH Domains results in reduced levels of cytokine release as compared to the **DA x CD3 Binding Molecule** comprising the wild type VL and VH domains.

[00558] The results of these animal studies show that administration of the **DA x CD3 Binding Molecules** comprising the variant VL and VH Domains of **CD3 mAb 1**, particularly the VL and VH Domains of **CD3 mAb 1 M2**, **CD3 mAb 1 M13**, **CD3 mAb M17**, and **CD3 mAb 1 M18** (*i.e.*, vCD3-Binding Domains) results in reduced levels of cytokine release as compared to **DA x CD3 Binding Molecules** comprising the VL and VH Domains of **CD3 mAb 1** (*i.e.*, rCD3-Binding Domain). In particular, the results of these studies demonstrate that **DA x CD3 Binding Molecules** comprising the variant VL and VH Domains of **CD3 mAb 1 M13**, **CD3 mAb M17**, and **CD3 mAb 1 M18** exhibit lower levels of cytokine release while retaining anti-tumor activity *in vivo*.

Example 9 Generation of TRIVALENT-Type Molecules

[00559] The VH and VL Domains of **CD3 mAb 1**, **CD3 mAb 1 M1**, **CD3 mAb 1 M2**, or **CD3 mAb 1 M18** were used to generate **TRIVALENT**-type molecules comprising a Disease Antigen (DA) Binding Domain binding the Cancer Antigen CD123 and a Binding Domain binding the effector cell antigen CD8 ("**DA x CD3 x CD8 TRIVALENT**-type molecule"). **Table 10** summarizes the CD3 Binding Domains and SEQ ID NOs. for each polypeptide chain. The amino acid sequences of each chain are provided in detail herein (see First - Fourth Illustrative **TRIVALENT**-type molecules, *supra*).

Example 10 Characterization of DA x CD3 x CD8 TRIVALENT-Type Molecules

[00560] The ability of **T-CD123-WT**, **T-CD123-M1**, **T-CD123-M2** and **T-CD123-M18** to bind CD123 expressed on MOLM-13 cells was evaluated essentially as described above. In addition, the ability of these molecules to bind CD4⁺ T-cells and CD8⁺ T-cells was evaluated essentially as described above. The **DART-B-type diabody CD123-WT** is included in these studies for comparison. Representative data from these studies is provided in **Figure 22A** (binding to MOLM-13 cells), **Figure 22B** (binding to CD4⁺ T-cells) and **Figure 22C** (binding to CD8⁺ T-cells). All of the tested molecules exhibit comparable binding to CD123 expressing MOLM-13 cells and CD8 expressing CD8⁺ T-cells. **T-CD123-M1** and **T-CD123-M18** exhibit significantly reduced binding to CD3 expressing

CD4⁺ T-cells as measured by MFI (geo mean) . Binding to CD8⁺ T-cells is mediated by both the CD3- and CD8-Binding Domains present in the **TRIVALENT**-type molecules.

[00561] The ability of **T-CD123-WT**, **T-CD123-M1**, **T-CD123-M2** and **T-CD123-M18** to mediate redirected cell killing was evaluated. Briefly, the **TRIVALENT**-type molecules were incubated in the presence of Pan-T-cell or purified CD4⁺ or CD8⁺ T-cell effector cells and MOLM-13 target tumor cells at an effector:target cell ratio of 1:1 for 48 hours. The cytotoxicity was determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells (*e.g.*, using the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega) that quantitatively measures LDH release, or similar). Cytokine response was examined in the Pan-T-cell samples. The **DART-B-type diabody CD123-WT** is included in these studies for comparison. Percent cytotoxicity is plotted in in **Figure 23A-23C** (**Figure 23A**: Pan-T-cells; **Figure 23B**: CD4⁺ T-cells; and **Figure 23C**: CD8⁺ T-cells). Cytokine responses are plotted in **Figures 23D-23G** (**Figure 23D**: IFN-gamma; **Figure 23E**: TNF-alpha; **Figure 23F**: IL-6; **Figure 23G**: IL-2).

Example 11 Toxicology Studies

[00562] The safety and cytokine release profiles of representative CD123 x CD3 Binding Molecules was assessed in a dosing study in cynomolgus monkeys. In this study the potential toxicity and cytokine release profiles of **CD123-M18** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M18**) and **CD123-WT** (comprising the rCD3-Binding Domain of **CD3 mAb 1**), when administered by repeated intravenous infusions was evaluated. Cell killing activity is not readily accessed in this model. The study design is presented in **Table 16**.

Table 16				
Group No.	Test Material	Dose Level (mg/kg)	Dosing Days	No. of Animals (male)
1	Control	0	0	2
2	CD123-WT	0.003	0, 7	3
3	CD123-M18	10	0, 7	3
4	CD123-M18	20	0, 7	2

[00563] No mortality, body weight loss or other adverse observations were observed in the **CD123-M18** treatment groups (10 and 20 mg/kg). In addition, no significant hematology or clinical chemistry changes were observed for in these groups. In contrast, the **CD123-WT** molecule (0.003 mg/kg) was not well tolerated. Cytokine release syndrome and

mortality (1/3) was observed in this group. The serum cytokine levels (Days 0-9) are plotted in **Figures 24A-24E** (**Figure 24A**: IFN- γ , **Figure 24B**: TNF- α , **Figure 24C**: IL-6, **Figure 24D**: IL-2, and **Figure 24E**: IL-15). In addition, T-cell proliferation was examined by FACS using expression of Ki67 as a marker of proliferating cells. **Figure 24F** plots CD4⁺ T-cell expansion, and **Figure 24G** plots CD8⁺ T-cells expansion as a percent of CD4⁺ or CD8⁺ cells positive for Ki67 (Days 0-14). **Figures 24H-24I** present plots of several hematology and clinical chemistry markers of significance for the animals in the treatment groups (**Figure 24H**: Platelet counts; **Figure 24I**: C-reactive protein; **Figure 24J**: Urea Nitrogen). The results of this study show that **DA x CD3 Binding Molecules** comprising the vCD3-Binding Domain **CD3 mAb 1 M18** are well tolerated in cynomolgus monkeys and exhibit minimal, transient increases in release of TNF- α , IFN- γ , IL-2 and IL-6 even at doses exceeding projected therapeutic levels, and exhibit smaller changes in multiple clinical chemistry markers. In addition, **DA x CD3 Binding Molecules** comprising the vCD3-Binding Domain **CD3 mAb 1 M18** were seen to preferentially stimulate proliferation of CD8⁺ T-cells.

[00564] A further toxicology study was performed with **CD123-M13** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M13**) dosed at 1 mg/kg and 10 mg/kg, **CD123-M17** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M17**) dosed at 1 mg/kg and 10 mg/kg, and **CD123-M19** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M19**) dosed at 10 mg/kg. In these studies, **CD123-M13** was observed to exhibited higher cytokine release than **CD123-M17** or **CD123-M19**, particularly in the 10 mg/kg group. Some mortality was observed in this study, particularly in the **CD123-M13** high dose group and transient hematological and clinical chemistry changes were observed. **Table 17** provides a summary of observed mortality from this study and previous toxicology studies with **CD123-WT** and **CD123-M19**.

Table 17				
CD3 Variant	Dose	# Dosed	# Died	
CD123-WT	10 µg/kg	1	1	Euthanasia on Day 3
	3 → 3 µg/kg (Day 1 → 8)	3	1	Euthanasia on Day 8
	3 → 10 µg/kg (Day 1 → 8)	2	1	Euthanasia on Day 8
	3 → 30 µg/kg (Day 1 → 8)	2	2	Euthanasia on Day 8 or 9
CD123-M13	1 mg/kg	2	1	Euthanasia on Day 3
	10 mg/kg	2	2	Death or euthanasia on Day 3
CD123-M17	1 mg/kg	2	0	No mortality
	10 mg/kg	5	1	Euthanasia on Day 4
CD123-M18	No mortality at 10 (n=3) or 20 mg/kg (n=2)			
CD123-M19	No mortality at 10 mg/kg (n=2) (higher doses not tested)			

[00565] As shown in **Table 17** mortality is observed in animals treated with as little as 3 µg/kg (0.003 mg/kg) **CD123-M13** (comprising the rCD3-Binding Domain of **CD3 mAb 1**) while **CD123 x CD3 Binding Molecules** comprising the vCD3-Binding Domain of **CD3 mAb 1** **M13**, **M17**, **M18**, and **M19** are tolerable at much higher doses and exhibit reduced cytokine release profiles as compared to **CD123-M13** comprising the rCD3-Binding Domain of **CD3 mAb 1**. The tolerated dose ranking from these studies is **CD123-M18** ≥ **CD123-M19** > **CD123-M17** > **CD123-M13** > **CD123-WT**. These findings track with the Therapeutic Index evaluation provided above.

Example 12

Ability of Exemplary CD123 x CD3 Molecules to Mediate AML Blast Depletion

[00566] Exemplary CD123 x CD3 diabodies were evaluated for their ability to mediate AML blast cell depletion from peripheral blood samples from an AML patient. Briefly, peripheral blood cells from an AML patient were incubated in supplemented medium in the presence of increasing concentrations of **DART-A-WT**, **CD123-WT**, **CD123-M1** and **CD123-M18**. Cellularity (CD34⁺ blasts, CD3⁺ and CD8⁺ T-cells) were analyzed by flow cytometry at time 0 and on day 6 and is plotted as a percent of untreated control or as fold increase of baseline. Cytokine levels were analyzed by cytokine-bead array (BD) on supernatants harvested on day 4 of incubation. The results of this study are presented in **Figures 25A-25G**. As shown in **Figure 25A**, **CD123-M18** was able to mediate depletion AML blast cells to the same extent as **DART-A-WT** and **CD123-WT**. However, **CD123-M18** exhibited significantly reduced expansion of T-cell population (**Figure 25B**: CD4⁺ T-cells; **Figure 25C**: CD8⁺ T-cells). Furthermore, **CD123-M18** exhibited significantly lower

levels of cytokine release (**Figure 25D**: IFN- γ ; **Figure 25E**: TNF- α ; **Figure 25F**: IL-6; and **Figure 25G**: IL-2).

[00567] These results further demonstrate that **DA x CD3 Binding Molecules** comprising the vCD3-Binding Domains of **CD3 mAb 1 M18** retained maximum killing potential with slightly reduced potency, but commensurably greater reduction in target-induced cytokine release *in vitro* and *in vivo*. Incorporating such vCD3-Binding Domains into **DA x CD3 Binding Molecules** may expand the therapeutic index in redirected T-cell killing applications.

Example 13

Ability of Exemplary CD19 x CD3 Molecules to Mediate Autologous B-cell Depletion

[00568] In one set of studies, the exemplary CD19 x CD3 diabodies **CD19-WT** (a positive control comprising the rCD3-Binding Domain of **CD3 mAb 1**) and **CD19.1-M18** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M18**), were evaluated for their ability to mediate autologous B-cell depletion *in vitro* and *in vivo*. For the *in vitro* studies PMBCs from human and cynomolgus monkey were utilized. Briefly, PMBCs isolated from human or cynomolgus monkey were incubated in supplemented medium in the presence of increasing concentrations of **CD19-WT** (a positive control) or **CD19.1-M18** or the negative control **HIV-M18**. B-cell levels were analyzed by flow cytometry (using CD20 as a B-cell marker) at 48 hours post incubation. Cytokine levels in the supernatants from the human samples were analyzed by cytokine-bead array (BD). The results of this study are presented in **Figures 31A-31F**. As shown in **Figures 31A-31B**, **CD19.1-M18** was able to deplete autologous B-cells from both human and cynomolgus monkey PMBCs to the same extent as **CD19-WT**. Furthermore, **CD19.1-M18** exhibited significantly lower levels of cytokine release (**Figure 31C**: IFN- γ ; **Figure 31D**: TNF- α ; **Figure 31E**: IL-6; and **Figure 31F**: IL-2).

[00569] The ability of the positive control, **CD19-WT** and **CD19.1-M18** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M18**), to mediate autologous B-cell depletion *in vivo* was assessed in a dosing study in cynomolgus monkeys. The study design is presented in **Table 18**.

Table 18			
Group No.	Test Material	Dose Level (mg/kg)	No. of Animals (male)
1	CD19-WT	0.1	2
2	CD19.1-M18	1	2
3	CD19.1-M18	10	2
4	CD19.1-M18	30	2

[00570] The CD19 x CD3 diabodies were administered by a single 2-hr intravenous infusion on Day 0. Peripheral blood samples were taken predose and periodically postdose. B-cell levels in peripheral blood samples were analyzed by flow cytometry (using CD20 as a B-cell marker). Representative data from 1 of 2 monkeys treated in groups 1-3 are shown in **Figures 32A-32D** (**Figure 32A**: predose Day 0; **Figure 32B**: Day 1; **Figure 32C**: Day 8; **Figure 32D**: Day 15; B-cell populations are indicated with an oval). In addition, inguinal lymph nodes collected predose, at Day 7, and at Day 15 were stained for B-cells (using CD20 as a B-cell marker). Representative immunohistochemistry images from pre-dose and Day7 samples from 1 of 2 monkeys treated in groups 1, 3 and 4 are shown in **Figure 33A-33C** (**Figure 33A**: group 1; **Figure 33B**: group 3; **Figure 33C**: group 4; predose on the left and Day 7 on the right; stained B-cells appear dark). The results of this *in vivo* study show that B-cells were efficiently depleted in peripheral blood within one day and that the depletion persisted for up to 15 days after administration (see **Figures 32A-32D**) of a single dose of exemplary CD19 x CD3 diabody **CD19.1-M18**. Similarly, B-cells were efficiently depleted in lymph nodes within 7 days (the earliest time point examined after administration) demonstrating that **CD19.1-M18** at doses of as little as 1 mg/kg is capable of mediating autologous B-cell depletion via T-cell redirected killing *in vivo* to a similar degree as the **CD19-WT** positive control.

[00571] The ability of additional CD19 x CD3 Binding Molecules to mediate autologous B-cell depletion was assessed in cynomolgus monkeys. In this study the activity of **CD19-WT** (a positive control comprising the rCD3-Binding Domain of **CD3 mAb 1**); **CD19.1-M13** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M13**); and **CD19.1-M17** (comprising the vCD3-Binding Domain of **CD3 mAb 1 M17**) were evaluated for their ability to mediate autologous B-cell depletion when administered by repeated intravenous infusions. The study design is presented in **Table 19**.

Table 19				
Group No.	Test Material	Dose Level (mg/kg)	Dosing Days	No. of Animals
1	CD19-WT	0.1	1, 8	2M
2	CD19.1-M13	1	1, 8	3M
3	CD19.1-M17	1	1, 8	3M

[00572] No mortality, body weight loss or other significant adverse observations were observed, cold limbs were observed after dosing in one animal in group 3 on Day 1 and in one animal in group 2 on Day 8, both resolved by the next day. B-cell levels in peripheral blood samples (taken predose and periodically postdose) were analyzed by flow cytometry (using CD20 as a B-cell marker). In addition, tissue samples (spleen, bone marrow and lymph nodes (LN)) were evaluated by immunohistochemistry for CD20 staining. Flow cytometry data from groups 1-3 are shown in **Figure 34** and tissue staining data from each animal (and an untreated negative control animal) are summarized in **Table 20**. Serum cytokine levels were evaluated over the course of the study (predose and periodically postdose). Serum levels of TNF- α , IFN- γ , IL-2, IL-6, and IL-15 for each treatment group are plotted in **Figures 35A-35E**, respectively. In addition, T-cell populations in peripheral blood were examined by FACS using expression of Ki67 as a marker of proliferating cells. **Figure 36A** plots CD4⁺ T-cell expansion, and **Figure 36B** plots CD8⁺ T-cells expansion as a percent of CD4⁺ or CD8⁺ cells positive for Ki67 (taken predose and periodically postdose).

Table 20: CD20 Tissue Staining			
	Neg Control[‡]	CD19-WT	
Animal Number	5001	1001	1002
Spleen	X	2+	1+
Bone marrow	2+	0	0
LN, axillary	3+	1+	1+
LN, mandibular	3+	1+	1+
LN, mesenteric	3+	1+	1+
LN, inguinal	3+	1+	1+
	CD19.1-M13		
Animal Number	2001	2002	2003
Spleen	1+	2+	1+
Bone marrow	0	0	0
LN, axillary	1+	2+	1+
LN, mandibular	1+	1+	1+
LN, mesenteric	0	1+	1+
LN, inguinal	1+	1+	1+

Table 20: CD20 Tissue Staining			
	CD19.1-M17		
Animal Number	3001	3002	3003
Spleen	1+	1+	1+
Bone marrow	0	1+	1+
LN, axillary	1+	2+	2+
LN, mandibular	1+	2+	2+
LN, mesenteric	1+	1+	2+
LN, inguinal	1+	2+	2+

‡ one animal in negative control group

X – Not examined

0 – No staining observed

1+ – Weak staining observed

2+ – Moderate staining observed

3+ – Strong staining observed

[00573] The results of this study show that CD19 x CD3 Binding Molecules comprising the vCD3-Binding Domain **CD3 mAb 1 M13** or **CD3 mAb 1 M17** were active with animals treated with 1 mg/kg **CD19.1-M13** exhibiting autologous B-cell depletion to a similar degree as the **CD19-WT** positive control. Animals treated with 1 mg/kg **CD19.1-M17** also exhibited autologous B-cell depletion, but to a slightly lesser extent than the positive control. It is expected that **CD19.1-M17** would achieve comparable depletion as higher dosages. The variants mediated much lower increases in the release of INF- γ , TNF- α , IL-2, and IL-6 and slight reductions in the release of IL-15. In addition, binding molecules comprising **CD3 mAb 1 M13** and **CD3 mAb 1 M17** were seen to stimulate proliferation of T-cells, with molecules comprising **CD3 mAb 1 M13** exhibiting higher levels of proliferation. In addition, both molecules exhibited preferential stimulation of proliferation of CD8⁺ T-cells.

[00574] Together the studies provided in the above examples show that **DA x CD3 Binding Molecules** comprising the vCD3-Binding Domain of **CD3 mAb 1** (e.g., **M13**, **M17**, **M18**, **M19**), exhibit a range of binding affinities, a range of cytotoxicity EC₅₀ values but all reach a maximum CTL activity that is comparable to molecules comprising the rCD3-Binding Domain of **CD3 mAb 1**, thus exhibit an enhanced Therapeutic Index. These studies further show that such molecules are tolerated and active at mediating T-cell redirected cell killing, and at stimulating T-cell activation and proliferation *in vivo*.

[00575] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will

be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

WHAT IS CLAIMED IS:

Claim 1. A **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_{H1} 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_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (II) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} 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_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;

- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} Domain comprising the amino acid sequence of **SEQ ID NO:62**; or
- (III) (A) a CDR_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} Domain comprising the amino acid sequence of **SEQ ID NO:61**; and
- (F) a CDR_{L3} 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_{H1} Domain comprising the amino acid sequence of **SEQ ID NO:57**;
- (B) a CDR_{H2} Domain comprising the amino acid sequence of **SEQ ID NO:58**;
- (C) a CDR_{H3} Domain comprising the amino acid sequence of **SEQ ID NO:59**;
- (D) a CDR_{L1} Domain comprising the amino acid sequence of **SEQ ID NO:60**;
- (E) a CDR_{L2} 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_{L3} 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 the Cancer Antigens: 19-9, 4.2, ADAM-9, AH6, ALCAM, B1, B7-H3, BAGE, beta-catenin, blood group ALe^b/Le^y, 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 α 2, Integrin β 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, R24, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T-cell receptor derived peptide, T5A7, TAG-72, TL5, TNF-receptor, TNF- γ receptor, TRA-1-85, a Transferrin Receptor, 5T4, TSTA, VEGF, a VEGF Receptor, VEP8, VEP9, VIM-D5, and Y hapten, Le^y.
- 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 α 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 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.

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_{DA}); and
- (2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to said epitope of CD3 (VH_{CD3});

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_{CD3}); 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_{DA});

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:

- (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.

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 of claims 1-16 and a pharmaceutically acceptable carrier.
- Claim 18. A method for the treatment of a disease, comprising administering to a subject in need thereof a therapeutically effective amount of the **DA x CD3 Binding Molecule** of any of claims 1-16 or the pharmaceutical composition of claim 17.
- Claim 16. The method of claim 18, wherein said disease is cancer.
- Claim 20. The method 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 method of claim 18, wherein said disease is a pathogen-associated disease.
- Claim 22. The method of claim 21, wherein said 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.
- Claim 23. The **DA x CD3 Binding Molecule** of any of claims 1-16 or the pharmaceutical composition of claim 16 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 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.