(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 December 2017 (14.12.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/214092 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 35/00* (2006.01)
C07K 16/28 (2006.01) *A61P 31/00* (2006.01)

(21) International Application Number:
PCT/US2017/036075

(22) International Filing Date:
06 June 2017 (06.06.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/346,854 07 June 2016 (07.06.2016) US
62/432,299 09 December 2016 (09.12.2016) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,

(54) Title: COMBINATION THERAPY

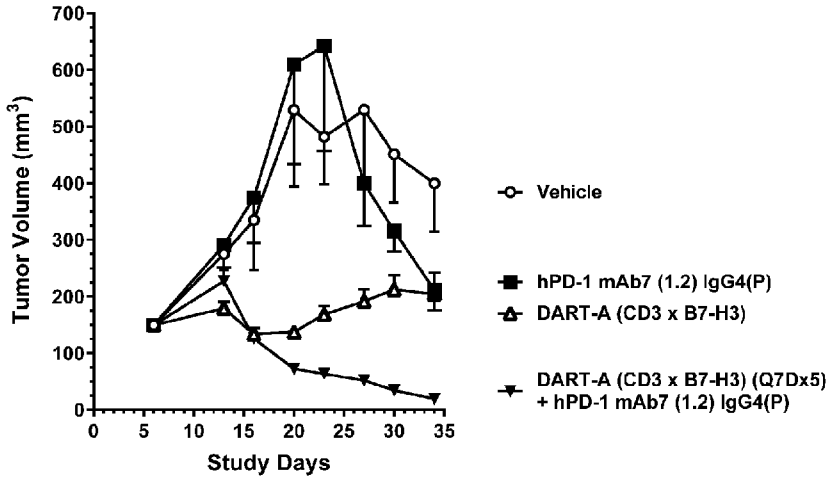

Figure 8A

(57) Abstract: The present invention is directed to a combination therapy for the treatment of cancer and pathogen-associated diseases, that comprises the administration of: (I) a molecule (e.g., a diabody, an scFv, an antibody, a TandAb, etc.) capable of binding PD-I or a natural ligand of PD-I, and (2) a molecule (e.g., a diabody, a BiTe, a bispecific antibody, a CAR, etc.) capable of mediating the redirected killing of a target cell (e.g., a cancer cell or a pathogeninfected cell, etc.) expressing a Disease Antigen. The invention particularly concerns the embodiment in which the molecule capable of mediating the redirected killing of the target cell is a bispecific binding molecule that comprises a first epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and a second epitope-binding site that is capable of immunospecifically binding an epitope of such target cells.

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[Continued on next page]

PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

TITLE OF THE INVENTION

Combination Therapy

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Applications Serial Nos. 62/346,854 (filed on June 7, 2016; pending) and 62/432,299 (filed on December 9, 2016; pending), each of which applications is herein incorporated by reference in its 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_0142PCT_ST25.txt, created on May 31, 2017, and having a size of 225,335 bytes), which file is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention is directed to a combination therapy for the treatment of cancer and pathogen-associated diseases, that comprises the administration of: (1) a molecule (*e.g.*, a diabody, an scFv, an antibody, a TandAb, *etc.*) capable of binding PD-1 or a natural ligand of PD-1, and (2) a molecule (*e.g.*, a diabody, a BiTe, a bispecific antibody, a CAR, *etc.*) 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. The invention particularly concerns the embodiment in which the molecule capable of mediating the redirected killing of the target cell is a bispecific binding molecule that comprises a first epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and a second epitope-binding site that is capable of immunospecifically binding an epitope of such target cells (*i.e.*, a Disease Antigen such as a Cancer Antigen or a Pathogen-Associated Antigen). 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 CD3γ chain, a CD3δ chain, and two CD3ε 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 CD3ε: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 CD3ε 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 "$CD4^+$"). $CD4^+$ 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 histocompability 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 histocompability 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 histocompability 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. CD2

[0016] **CD2** is a cell adhesion molecule found on the surface of T-cells and natural killer (NK) cells. CD2 enhances NK cell cytotoxicity, possibly as a promoter of NK cell nanotube formation (Mace, E.M. *et al.* (2014) "*Cell Biological Steps and Checkpoints in Accessing NK Cell Cytotoxicity*," Immunol. Cell. Biol. 92(3):245-255; Comerci, C.J. *et al.* (2012) "*CD2 Promotes Human Natural Killer Cell Membrane Nanotube Formation*," PLoS One 7(10):e47664:1-12).

C. The T Cell Receptor ("TCR")

[0017] 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).

[0018] 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).

D. The Fc Receptors: CD16, CD32 and CD64

[0019] As discussed in detail below, natural IgG antibodies are composed of four polypeptide chains: two identical "light" chains and two identical "heavy" chains. The Heavy Chains contain C-terminal "CH2" and "CH3" domains, and the association of the two Heavy Chains creates an "**Fc Domain**" that is capable of ligating (binding) to receptors (singularly referred to as an "**Fc gamma receptor**" "**FcγR**," and collectively as "**FcγRs**") found on the surfaces of multiple types of immune system cells (*e.g.*, B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). Such receptors have an "**extracellular**" portion (which is thus capable of ligating to an Fc Domain), a "**transmembrane**" portion (which extends through the cellular membrane), and a "**cytoplasmic**" portion (positioned inside the cell). Multiple types of FcγRs have been identified: **CD16A (FcγRIIIA)**, **CD16B (FcγRIIIB)**, **CD32A (FcγRIIA)**, **CD32B (FcγRIIB)**, and **CD64 (FcγRI)**. Such binding results in the transduction of activating or inhibitory signals to the immune system.

[0020] **CD16** is a generic name for the activating Fc receptors, **FcγRIIIA (CD16A)** and **FcγRIIIB (CD16B)**. CD16 is expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that bind aggregated but not monomeric human IgG (Peltz, G.A. *et al.* (1989) "*Human Fc Gamma RIII: Cloning, Expression, And Identification Of The Chromosomal Locus Of Two Fc Receptors For IgG*," Proc. Natl. Acad. Sci. (U.S.A.) 86(3):1013-1017; Bachanova, V. *et al.* (2014) "*NK Cells In Therapy Of Cancer*," Crit. Rev. Oncog. 19(1-2):133-141; Miller, J.S. (2013) "*Therapeutic Applications: Natural Killer Cells In The Clinic*," Hematology Am. Soc. Hematol. Educ. Program. 2013:247-253; Youinou, P. *et al.* (2002) "*Pathogenic Effects Of Anti-Fc Gamma Receptor IIIB (CD16) On Polymorphonuclear Neutrophils In Non-Organ-Specific Autoimmune Diseases*," Autoimmun Rev. 1(1-2):13-19; Peipp, M. *et al.* (2002) "*Bispecific Antibodies Targeting Cancer Cells*," Biochem. Soc. Trans. 30(4):507-511). These receptors bind the Fc portion of IgG antibodies, thereby triggering the release of cytokines. If such antibodies are bound to a Disease Antigen that is expressed on the surface of a cell (*e.g.*, a cancer cell, pathogen-infected cell, *etc.*), then such release mediates the killing of the targeted cell. Since such killing is antibody-dependent, it is termed **antibody-dependent cell-mediated cytotoxicity (ADCC)**.

[0021] **CD32A (FcγRIIA)** (Brandsma, A.M. (2015) "*Fc Receptor Inside-Out Signaling And Possible Impact On Antibody Therapy*," Immunol Rev. 268(1):74-87; van Sorge, N.M. *et al.* (2003) "*FcgammaR Polymorphisms: Implications For Function, Disease Susceptibility And Immunotherapy*," Tissue Antigens 61(3):189-202; Selvaraj, P. *et al.* (2004) "*Functional Regulation Of Human Neutrophil Fc Gamma Receptors*," Immunol. Res. 29(1-3):219-230) and **CD64 (FcγRI)** (Lu, S. *et al.* (2015) "*Structural Mechanism Of High Affinity FcγRI recognition Of Immunoglobulin G*," Immunol. Rev. 268(1):192-200; Swisher, J.F. *et al.* (2015) "T*he Many Faces Of FcγRI: Implications For Therapeutic Antibody Function*," Immunol. Rev. 268(1):160-174; Thepen, T. *et al.* (2009) "*Fcgamma Receptor 1 (CD64), A Target Beyond Cancer*," Curr. Pharm. Des. 15(23):2712-2718; Rouard, H. *et al.* (1997) "*Fc Receptors As Targets For Immunotherapy*," Int. Rev. Immunol. 16(1-2):147-185) are activating Fc receptors that are expressed on macrophages, neutrophils, eosinophils and dendritic cells (and for **CD32A**, also on platelets and Langerhan cells). In contrast, **CD32B (FcγRIIB)** is an inhibiting Fc receptor on B lymphocytes (macrophages, neutrophils, and eosinophils) (Stopforth, R.J. *et al.* (2016) "*Regulation of Monoclonal Antibody Immunotherapy by FcγRIIB*," J. Clin. Immunol. [2016 Feb 27 Epub],

pp. 1-7; Bruhns, P. *et al.* (2009) "*Specificity And Affinity Of Human Fcgamma Receptors And Their Polymorphic Variants For Human IgG Subclasses*," Blood. 113(16):3716-3725; White, A.L. *et al.* (2014) "FcγRIIB *As A Key Determinant Of Agonistic Antibody Efficacy*," Curr. Top. Microbiol. Immunol. 382:355-372; Selvaraj, P. *et al.* (2004) "*Functional Regulation Of Human Neutrophil Fc Gamma Receptors*," Immunol. Res. 29(1-3):219-230).

[0022] The ability of the different FcγRs to mediate diametrically opposing functions reflects their structural differences, and in particular whether the FcγR possesses an immunoreceptor tyrosine-based activation motif ("**ITAM**") or an immunoreceptor tyrosine-based inhibitory motif ("**ITIM**"). The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγRs include FcγRI, FcγRIIA, FcγRIIIA, and activate the immune system when bound to Fc Domains (*e.g.*, aggregated Fc Domains present in an immune complex). FcγRIIB is the only currently known natural ITIM-containing FcγR; it acts to dampen or inhibit the immune system when bound to aggregated Fc Domains.

E. The NKG2D Receptor

[0023] The Natural Killer Group 2D ("**NKG2D**") receptor is expressed on all human (and other mammalian) Natural Killer cells (Bauer, S. *et al.* (1999) "*Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA*," Science 285(5428):727-729; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29) as well as on all $CD8^+$ T cells (Groh, V. *et al.* (2001) "*Costimulation Of CD8αβ T Cells By NKG2D Via Engagement By MIC Induced On Virus-Infected Cells*," Nat. Immunol. 2(3):255-260; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29). NKG2D ligands are completely absent, or are present only at low levels, on the surfaces of normal cells, but they are overexpressed by infected, transformed, senescent or stressed cells. Such binding ligands, and particularly those which are not expressed on normal cells, include the histocompatibility 60 (H60) molecule, the product of the retinoic acid early inducible gene-1 (RAE-1), and the murine UL16-binding protein-like transcript 1 (MULT1) (Raulet D.H. (2003) "*Roles Of The NKG2D Immunoreceptor And Its Ligands*," Nature Rev. Immunol. 3:781-790; Coudert, J.D. *et al.* (2005) "*Altered NKG2D Function In NK Cells Induced By Chronic Exposure To Altered NKG2D Ligand-Expressing Tumor Cells*," Blood 106:1711-1717).

III. Interacting Molecules of Immune System Cells

[0024] Interactions involving several different kinds of Antigen-Presenting Cell molecules and T Cell molecules affect the required second interaction of the immune response immune response.

A. CD80/CD86 and CD28/CTLA-4

[0025] Binding between the B7.1 (**CD80**) and B7.2 (**CD86**) ligands of Antigen-Presenting Cells and the **CD28** and **CTLA-4** receptors of $CD4^+$ T lymphocytes is of particular importance to the required second interaction of the immune response (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; 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). Binding of B7.1 or of B7.2 to CD28 stimulates T-cell activation; binding of B7.1 or B7.2 to CTLA-4 inhibits such activation (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; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548). CD28 is constitutively expressed on the surface of T-cells (Gross, J., *et al.* (1992) "*Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse*," J. Immunol. 149:380–388), whereas CTLA-4 expression is rapidly upregulated following T-cell activation (Linsley, P. *et al.* (1996) "*Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement*," Immunity 4:535–543). Since CTLA-4 is the higher affinity receptor (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Topalian, S.L. *et al.* (2015) "*Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy*," Cancer Cell 27:450-461), binding first initiates T-cell proliferation (via CD28) and then inhibits it (via nascent expression of CTLA-4), thereby dampening the effect when proliferation is no longer needed.

B. PD-1 and B7-H1 / B7-DC

[0026] Programmed Death-1 ("**PD-1**," also known as "CD279") is type I membrane protein member of the extended CD28/CTLA-4 family of T-cell regulators that broadly negatively regulates immune responses (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell*

Death," EMBO J. 11:3887-3895; United States Patent Application Publications No. 2007/0202100; 2008/0311117; 2009/00110667; United States Patents No. 6,808,710; 7,101,550; 7,488,802; 7,635,757; 7,722,868; PCT Publication No. WO 01/14557).

[0027] Although PD-1 and CTLA-4 both provide inhibitory immune signals, the signals provided by PD-1 are mounted later in the course of the disease, and can profoundly diminish the immune response by limiting the initial production ("burst") of disease-responsive T-cells. As such PD-1 can partially convert a potentially effective T-cell response into one of tolerance (Topalian, S.L. *et al.* (2015) "*Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy*," Cancer Cell 27:450-461).

[0028] The receptor-ligand interactions of the PD-1 system appear to be even more complex than those of the CD28/CTLA-4 system. PD-1 is expressed on the cell surface of activated T-cells, B-cells, and monocytes (Agata, Y. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772; Yamazaki, T. *et al.* (2002) "*Expression Of Programmed Death 1 Ligands By Murine T-Cells And APC*," J. Immunol. 169:5538-5545) and at low levels in natural killer (NK) T-cells (Nishimura, H. *et al.* (2000) "*Facilitation Of Beta Selection And Modification Of Positive Selection In The Thymus Of PD-1-Deficient Mice*," J. Exp. Med. 191:891-898; Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298).

[0029] The extracellular region of PD-1 consists of a single immunoglobulin (Ig)V domain with 23% identity to the equivalent domain in CTLA-4 (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). The extracellular IgV domain is followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Blank, C. *et al.* (2006) "*Contribution Of The PD-L1/PD-1 Pathway To T-Cell Exhaustion: An Update On Implications For Chronic Infections And Tumor Evasion Cancer*," Immunol. Immunother. 56(5):739-745).

[0030] PD-1 mediates its inhibition of the immune system by binding **B7-H1** and **B7-DC** (also known as PD-L1 and PD-L2) (Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; United States Patents Nos. 6,803,192; 7,794,710; United States Patent Application Publication Nos. 2005/0059051; 2009/0055944; 2009/0274666; 2009/0313687; PCT Publication Nos. WO 01/39722; WO 02/086083).

[0031] B7-H1 and B7-DC are broadly expressed on the surfaces of many types of human and murine tissues, such as heart, placenta, muscle, fetal liver, spleen, lymph nodes, and thymus as well as murine liver, lung, kidney, islets cells of the pancreas and small intestine (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). In humans, B7-H1 protein expression has been found in human endothelial cells (Chen, Y. *et al.* (2005) "*Expression of B7-H1 in Inflammatory Renal Tubular Epithelial Cells*," Nephron. Exp. Nephrol. 102:e81-e92; de Haij, S. *et al.* (2005) "*Renal Tubular Epithelial Cells Modulate T-Cell Responses Via ICOS-L And B7-H1*" Kidney Int. 68:2091-2102; Mazanet, M.M. *et al.* (2002) "*B7-H1 Is Expressed By Human Endothelial Cells And Suppresses T-Cell Cytokine Synthesis*," J. Immunol. 169:3581-3588), myocardium (Brown, J.A. *et al.* (2003) "*Blockade Of Programmed Death-1 Ligands On Dendritic Cells Enhances T-Cell Activation And Cytokine Production*," J. Immunol. 170:1257-1266), syncyciotrophoblasts (Petroff, M.G. *et al.* (2002) "*B7 Family Molecules: Novel Immunomodulators At The Maternal-Fetal Interface*," Placenta 23:S95-S101). The molecules are also expressed by resident macrophages of some tissues, by macrophages that have been activated with interferon (IFN)-γ or tumor necrosis factor (TNF)-α (Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T-Cell Activation*," Nat. Immunol 2:261-268), and in tumors (Dong, H. (2003) "*B7-H1 Pathway And Its Role In The Evasion Of Tumor Immunity*," J. Mol. Med. 81:281-287).

[0032] The interaction between B7-H1 and PD-1 has been found to provide a crucial negative costimulatory signal to T and B-cells (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298) and functions as a cell death inducer (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Subudhi, S.K. *et al.* (2005) "*The Balance Of Immune Responses: Costimulation Verse Coinhibition*," J. Molec. Med. 83:193-202). More specifically,

interaction between low concentrations of the PD-1 receptor and the B7-H1 ligand has been found to result in the transmission of an inhibitory signal that strongly inhibits the proliferation of antigen-specific $CD8^+$ T-cells; at higher concentrations, the interactions with PD-1 do not inhibit T-cell proliferation but markedly reduce the production of multiple cytokines (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). T-cell proliferation and cytokine production by both resting and previously activated CD4 and CD8 T-cells, and even naïve T-cells from umbilical-cord blood, have been found to be inhibited by soluble B7-H1-Fc fusion proteins (Freeman, G.J. *et al.* (2000) "*Engagement Of The PD-1 Immunoinhibitory Receptor By A Novel B7 Family Member Leads To Negative Regulation Of Lymphocyte Activation*," J. Exp. Med. 192:1-9; Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T-Cell Activation*," Nature Immunol. 2:261-268; Carter, L. *et al.* (2002) "*PD-1:PD-L Inhibitory Pathway Affects Both CD4(+) and CD8(+) T-cells And Is Overcome By IL-2*," Eur. J. Immunol. 32(3):634-643; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126).

[0033] The role of B7-H1 and PD-1 in inhibiting T-cell activation and proliferation has suggested that these biomolecules might serve as therapeutic targets for treatments of inflammation and cancer. Thus, the use of anti-PD-1 antibodies to treat infections and tumors and to up-modulate an adaptive immune response has been proposed (see, United States Patent Application Publication Nos. 2010/0040614; 2010/0028330; 2004/0241745; 2008/0311117; 2009/0217401; United States Patents Nos. 7,521,051; 7,563,869; 7,595,048; PCT Publications Nos. WO 2004/056875; WO 2008/083174). Antibodies capable of specifically binding PD-1 have been reported by Agata, T. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772; and Berger, R. *et al.* (2008) "*Phase I Safety And Pharmacokinetic Study Of CT-011, A Humanized Antibody Interacting With PD-1, In Patients With Advanced Hematologic Malignancies*," Clin. Cancer Res. 14(10):3044-3051 (see, also, United States Patents No. 8,008,449 and 8,552,154; US Patent Publication Nos. 2007/0166281; 2012/0114648; 2012/0114649; 2013/0017199; 2013/0230514 and 2014/0044738; and PCT Patent Publication Nos. WO 2003/099196; WO 2004/004771; WO 2004/056875; WO 2004/072286; WO 2006/121168; WO 2007/005874; WO 2008/083174; WO 2009/014708; WO 2009/073533; WO 2012/135408, WO 2012/145549; and WO 2013/014668).

[0034] Despite such 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 is directed to this and other goals.

SUMMARY OF THE INVENTION

[0035] The present invention is directed to a combination therapy for the treatment of cancer and pathogen-associated diseases, that comprises the administration of: (1) a molecule (*e.g.*, a diabody, an scFv, an antibody, a TandAb, *etc.*) capable of binding PD-1 or a natural ligand of PD-1, and (2) a molecule (*e.g.*, a diabody, a BiTe, a bispecific antibody, a CAR, *etc.*) 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. The invention particularly concerns the embodiment in which the molecule capable of mediating the redirected killing of the target cell is a bispecific binding molecule that comprises a first epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and a second epitope-binding site that is capable of immunospecifically binding an epitope of such target cells (*i.e.*, a Disease Antigen such as a Cancer Antigen or a Pathogen-Associated Antigen). The present invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[0036] In detail, the invention provides a method for the treatment of cancer or a pathogen-associated disease, comprising administering to a subject in need thereof a therapeutically effective amount of:

(1) a molecule capable of binding PD-1 or a natural ligand of PD-1, and

(2) a molecule capable of mediating the redirected killing of a target cell, wherein the target cell is:

  (a) a cancer cell that expresses a Cancer Antigen; or

  (b) a pathogen-infected cell that expresses a Pathogen-Associated Antigen.

[0037] The invention particularly concerns the embodiment of such method wherein the molecule capable of binding PD-1 or a natural ligand of PD-1 is capable of inhibiting binding between PD-1 and a natural ligand of PD-1.

[0038] The invention further concerns the embodiment of such method, wherein the method comprises administration of two binding molecules that cumulatively comprise three epitope-binding domains, the two binding molecules being:

(A) a binding molecule that comprises an epitope-binding domain of an antibody that is capable of binding PD-1, or an epitope-binding domain of an antibody that is capable of binding a natural ligand of PD-1; and

(B) a binding molecule that comprises:

(1) an epitope-binding domain of an antibody that is capable of binding a cell surface molecule of the effector cell; and

(2) an epitope-binding domain of an antibody that that is capable of binding the Cancer Antigen or the Pathogen Antigen of the target cell;

wherein the epitope-binding domain of the binding molecule (A) is capable of binding PD-1 or a natural ligand of PD-1, and the epitope-binding domains (1) and (2) of the binding molecule (B) are capable of mediating the redirected killing of the target cell.

[0039] The invention further concerns the embodiment of such method, wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a diabody, scFv, antibody or TandAb, and the binding molecule (B) comprises a bispecific diabody, a CAR, a BiTe, or bispecific antibody.

[0040] The invention further concerns the embodiment of such methods wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to PD-1.

[0041] The invention further concerns the embodiment of such methods wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to a natural ligand of PD-1.

[0042] The invention further concerns the embodiment of such methods wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding PD-1, wherein such epitope-binding domains:

(a) compete for binding the same epitope of PD-1; or

(b) do not compete for binding the same epitope of PD-1.

[0043] The invention further concerns the embodiment of such methods wherein the PD-1-epitope-binding domains are capable of simultaneous binding to the same PD-1 molecule.

[0044] The invention further concerns the embodiment of such methods wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding the natural ligand of PD-1, wherein such epitope-binding domains:

(a) compete for binding to the same epitope of such natural ligand of PD-1; or

(b) do not compete for binding to the same epitope of such natural ligand of PD-1.

[0045] The invention further concerns the embodiment of such methods wherein the PD-1 ligand-epitope-binding domains are capable of simultaneous binding the same molecule of the natural ligand of PD-1.

[0046] The invention further concerns the embodiment of such methods wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding an epitope of a molecule that is not PD-1 or a natural ligand of PD-1.

[0047] The invention further concerns the embodiment of such methods wherein in the second epitope-binding domain binds an epitope of CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA.

[0048] The invention further concerns the embodiment of such methods wherein the binding molecule capable of mediating the redirected killing of the target cell comprises a third epitope-binding domain capable of binding a cell surface molecule of the effector cell.

[0049] The invention further concerns the embodiment of such methods wherein the third epitope-binding-domain of the binding molecule capable of mediating the redirected killing of the target cell is capable of binding a different cell surface molecule of the effector cell, such that the binding molecule capable of mediating the redirected killing is capable of binding two different cell surface molecules of the effector cell.

[0050] The invention further concerns the embodiment of such methods wherein the binding molecule capable of mediating the redirected killing of the target cell comprises a third epitope-binding domain capable of binding to a Cancer Antigen or a Pathogen-Associated Antigen of the target cell.

[0051] The invention further concerns the embodiment of such methods wherein the third epitope-binding-domain of the binding molecule capable of mediating the redirected killing of the target cell is capable of binding a different Cancer Antigen or a different Pathogen Antigen of the target cell, such that the binding molecule capable of mediating the redirected killing is capable of binding to two different Cancer Antigens or two different Pathogen Antigens of the target cell.

[0052] The invention further concerns the embodiment of such methods wherein the cell surface molecule of the effector cell is selected from the group consisting of: CD2, CD3, CD8, CD16, TCR, and NKG2D.

[0053] The invention further concerns the embodiment of such methods wherein the Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, 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, $D_1$56-22, DR5, E_1 series, EGFR, an Ephrin receptor, 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, IL13Rα2, Integrin β6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6,L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R_{24}, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_5A_7, 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.

[0054] The invention further concerns the embodiment of such methods wherein the method comprises the administration of the pharmaceutical composition, and 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, *etc.*), 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.

[0055] The invention further provides a pharmaceutical composition that comprises:

(A) therapeutically effective amounts of:

(1) a molecule capable of binding PD-1 or a natural ligand of PD-1, and

(2) a molecule capable of mediating the redirected killing of a target cell expressing a Cancer Antigen or a Pathogen Antigen; and

(B) a pharmaceutically acceptable carrier.

[0056] The invention further concerns the embodiment of such pharmaceutical composition wherein the pharmaceutical composition comprises two binding molecules that cumulatively comprise three epitope-binding domains, the two binding molecules being:

(A) a binding molecule that comprises an epitope-binding domain of an antibody that is capable of binding PD-1, or an epitope-binding domain of an antibody that is capable of binding a natural ligand of PD-1; and

(B) a binding molecule that comprises:

(1) an epitope-binding domain of an antibody that is capable of binding a cell surface molecule of the effector cell; and

(2) an epitope-binding domain of an antibody that that is capable of binding a Cancer Antigen or a Pathogen-Associated Antigen of the target cell;

wherein the epitope-binding domain of the binding molecule (A) is capable of binding PD-1 or a natural ligand of PD-1, and the epitope-binding domains (1) and (2) of the binding molecule (B) are capable of mediating the redirected killing of the target cell.

[0057] The invention further concerns the embodiment of such pharmaceutical compositions wherein the binding molecule (A) comprises a diabody, scFv, antibody, or TandAb, and the binding molecule (B) comprises a diabody, a CAR, a BiTe, or bispecific antibody.

[0058] The invention further concerns the embodiment of such pharmaceutical compositions wherein the molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to PD-1.

[0059] The invention further concerns the embodiment of such pharmaceutical compositions wherein the molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to a natural ligand of PD-1.

[0060] The invention further concerns the embodiment of such pharmaceutical compositions wherein the molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding PD-1, wherein such PD-1-epitope-binding domains:

(a) compete for binding to the same epitope of PD-1; or

(b) do not compete for binding the same epitope of PD-1.

[0061] The invention further concerns the embodiment of such pharmaceutical compositions wherein the PD-1-epitope-binding domains are capable of simultaneous binding the same PD-1 molecule.

[0062] The invention further concerns the embodiment of such pharmaceutical compositions wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding the natural ligand of PD-1, wherein such epitope-binding domains:

(a) compete for binding to the same epitope of such natural ligand of PD-1; or

(b) do not compete for binding to the same epitope of such natural ligand of PD-1.

[0063] The invention further concerns the embodiment of such pharmaceutical compositions wherein the PD-1 ligand-epitope-binding domains are capable of simultaneous binding the same molecule of the natural ligand of PD-1.

[0064] The invention further concerns the embodiment of such pharmaceutical compositions wherein the binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding an epitope of a molecule that is not PD-1 or a natural ligand of PD-1.

[0065] The invention further concerns the embodiment of such pharmaceutical compositions wherein the second epitope-binding domain binds an epitope of CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA.

[0066] The invention further concerns the embodiment of such pharmaceutical compositions wherein the molecule capable of mediating the redirected killing of the target cell comprises a third epitope-binding domain, wherein such three epitope-binding domains are capable of simultaneous binding, and wherein the third epitope-binding site is capable of binding an epitope of a cell surface molecule of the effector cell.

[0067] The invention further concerns the embodiment of such pharmaceutical compositions wherein the third epitope-binding-domain of the binding molecule capable of mediating the redirected killing of the target cell is capable of binding a different cell surface molecule of the effector cell, such that the binding molecule capable of mediating the redirected killing is capable of binding two different cell surface molecules of the effector cell.

[0068] The invention further concerns the embodiment of such pharmaceutical compositions wherein the binding molecule capable of mediating the redirected killing of the target cell comprises a third epitope-binding domain capable of binding to a Cancer Antigen or a Pathogen-Associated Antigen of the target cell.

[0069] The invention further concerns the embodiment of such pharmaceutical compositions wherein the third epitope-binding-domain of the binding molecule capable of mediating the redirected killing of the target cell is capable of binding a different Cancer Antigen or a different Pathogen-Associated Antigen of the target cell, such that the binding molecule capable of mediating the redirected killing is capable of binding to two different Cancer Antigens or two different Pathogen-Associated Antigens of the target cell.

[0070] The invention further concerns the embodiment of such pharmaceutical compositions wherein the cell surface molecule of the effector cell is selected from the group consisting of: CD2, CD3, CD8, CD16, TCR, and NKG2D.

[0071] The invention further concerns the embodiment of such pharmaceutical compositions wherein the Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, 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, $D_1$56-22, DR5, E_1 series, EGFR, an Ephrin receptor, 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, IL13Rα2, Integrin β6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6,L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R_{24}, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_5A_7, 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.

[0072] The invention further concerns the embodiment of such pharmaceutical compositions wherein the Pathogen-Associated Antigen is selected from the group consisting of the Pathogen 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, *etc.*), 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.

[0073] The invention further provides a kit comprising any of the above-described pharmaceutical compositions, wherein the binding molecules thereof are compartmentalized in one or more containers.

BRIEF DESCRIPTION OF THE DRAWINGS:

[0074] **Figure 1** provides a schematic of a representative covalently bonded diabody 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 and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0075] **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.

[0076] **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.

[0077] **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.

[0078] **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.

[0079] **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.

[0080] **Figure 7** shows the result of providing $MHCI^{-/-}$ mice that had received 5 x 10^6 LOX-IMVI human metastatic melanoma cancer cells (ID) and 10^6 human PBMC (IP) with the humanized anti-human PD-1 antibody, **hPD-1 mAb7 (1.2) IgG4(P)**, the CD3 x B7-H3 bispecific diabody, **DART-A**, with both **hPD-1 mAb7 (1.2) IgG4(P)** and **DART-A**, or with vehicle alone (control).

[0081] **Figures 8A-8B** show the result of providing $MHCI^{-/-}$ mice that had received 5 x 10^6 Detroit562 human metastatic pharyngeal carcinoma cancer cells (ID) and 10^6 human PBMC (IP) with the humanized anti-human PD-1 antibody, **hPD-1 mAb7 (1.2) IgG4(P)**, the CD3 x B7-H3 bispecific diabody, **DART-A**, with both **hPD-1 mAb7 (1.2) IgG4(P)** and **DART-A**, or with vehicle alone (control). **Figure 8A** shows the results for Vehicle Control, **hPD-1 mAb7 (1.2) IgG4(P)** (Q7Dx5), **DART-A** (Q7Dx5), and **hPD-1 mAb7 (1.2) IgG4(P)** + **DART-A** (Q7Dx5). **Figure 8B** shows the results for Vehicle Control, **hPD-1 mAb7 (1.2) IgG4(P)** (Q7Dx5), **DART-A** (Q7Dx5), **hPD-1 mAb7 (1.2) IgG4(P)** + **DART-A** (Q7Dx5) and **hPD-1 mAb7 (1.2) IgG4(P)** + **DART-A** (Q14Dx3).

[0082] **Figure 9** shows the results of a study on the effect of the administration of the combination therapy of the present invention. The results show an enhancement of the immune response of recipient animals as determined by an increase in the concentration of their $CD3^+$ cells.

[0083] **Figures 10A-10B** show the results of a study on the effect of the combination therapy of the present invention on T-cell signaling in a luciferase reporter assay. MDA-MB-231 tumor target cells expressing PD-1 and B7-H3 were mixed with MNFAT-luc2/PD-1 Jurkat T-cells at an effector:target cell ratio of 1:1 (**Figure 10A**) or 3:1 (**Figure 10B**) and cultured alone or with a fixed concentration (12.5 nM) of the PD-1 binding molecules **hPD-1 mAb7 (1.2) IgG4(P)**, **DART-1,** or control antibody (hIgG), in the presence of increasing concentations of **DART-A**. These results show an enhancement in signaling activity in the presence of both molecules as determined by increased luminescence.

[0084] **Figures 11A-11B** show that administration of the combination therapy of the present invention reduces tumor recurrence in the presensence of anergic T-cells. NOG mice that had received 5 x 10^6 A375 INFγ treated melanoma cells and 5 x 10^6 activated or anergic human T-cells with vehicle alone, 0.5 mg/kg **DART-2** (Q7Dx4), 0.5 mg/kg **DART-B** (QDx1), or both 0.5 mg/kg **DART-2** (Q7Dx4) and 0.5 mg/kg **DART-B** (QDx1). **Figure 11A** shows the results for mice that received activated T-cells and **Figure 11B** shows the results for mice that received anergic T-cells.

[0085] **Figures 12A-12H** demonstrate the unexpected benefit of the combined therapy of a molecule capable of binding PD-1 and a molecule capable of mediating the redirected killing of a target cell relative to administration of either molecule alone. Tumor

volume caused by A375 melanoma cells was measured as a function of time and is plotted in **Figures 12A-12H**. **Figure 12A** shows the results for Groups 1, 2, 5 and 6 through day 50; **Figures 12B-12H** show the spider plots, through day 80, for the individual animals in Group 2 (**Figure 12B**), Group 5 (**Figure 12C**), Group 6 (**Figure 12D**), Group 3 (**Figure 12E**), Group 7 (**Figure 12F**), Group 4 (**Figure 12G**), and Group 8 (**Figure 12H**).

DETAILED DESCRIPTION OF THE INVENTION

[0086] The present invention is directed to a combination therapy for the treatment of cancer and pathogen-associated diseases, that comprises the administration of: (1) a molecule (*e.g.*, a diabody, an scFv, an antibody, a TandAb, *etc.*) capable of binding PD-1 or a natural ligand of PD-1, and (2) a molecule (*e.g.*, a diabody, a BiTe, a bispecific antibody, a CAR, *etc.*) 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. The invention particularly concerns the embodiment in which the molecule capable of mediating the redirected killing of the target cell is a bispecific binding molecule that comprises a first epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and a second epitope-binding site that is capable of immunospecifically binding an epitope of such target cells (*i.e.*, a Disease Antigen such as a Cancer Antigen or a Pathogen-Associated Antigen). The present invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[0087] The binding domains of the molecules of the present invention bind epitopes 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 to binding means "immunospecific" binding. Two molecules are said to be capable of binding one another in

a "**physiospecific**" manner, if such binding exhibits the specificity with which receptors bind their respective ligands.

[0088] As indicated above, the therapeutic molecules of the present invention particularly include bispecific binding molecules that comprises an epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and also an epitope-binding site that is capable of immunospecifically binding an epitope of a target cell that expresses a Disease Antigen. As used herein, the term "**Disease Antigen**" 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

[0089] The binding molecules of the present invention may be antibodies. "**Antibodies**" are immunoglobulin molecules capable of specific binding a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Domain of the immunoglobulin molecule. 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 fragments 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 site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) 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**"). An epitope-containing molecule may have immunogenic activity, such that it elicits an antibody production response in an animal; such molecules are termed "**antigens**."

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.

[0090] 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 fragments, *etc.*), single-chain (scFv) binding molecules and 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 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. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0091] 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.

[0092] 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).

A. Characteristics of Antibody Variable Domains

[0093] The Variable Domains of an IgG molecule consist of the complementarity determining regions ("**CDR**"), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments ("**FR**"), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the VL and VH Domains have the structure **n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c**. 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 fragment**" denotes a fragment of a molecule capable of immunospecifically binding an epitope. An epitope-binding fragment 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. Preferably, however, an epitope-binding fragment will contain all 6 of the CDR Domains of such antibody. An epitope-binding fragment 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 Fab_2 fragment, *etc.*). Unless specifically noted, the order of domains of the protein molecules described herein is in the "**N-terminal to C-terminal**" direction.

[0094] The invention also particularly encompasses epitope-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 site 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 portion 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. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415

[0095] The epitope-binding site 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; Verhoeyen, 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.

[0096] A number of humanized antibody molecules comprising an epitope-binding site 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. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

B. Characteristics of Antibody Constant Regions

[0097] Throughout the present specification, the numbering of the residues in the constant region of an IgG Heavy Chain 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. 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 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, and 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.

1. Constant Regions of the Heavy Chain: Fc Domains

[0098] 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.

[0099] 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

[00100] 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

[00101] 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:3**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[00102] 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:4**): EPKSCDKTHTCPPCP.

[00103] 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:5**): ERKCCVECPPCP.

[00104] 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:6**): 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:7**): ESKYGPPCPPCP.

[00105] The CH2 and CH3 Domains of the two Heavy Chains of an antibody interact to form an "**Fc Domain**," which is a domain 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.

[00106] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:8**):

```
231       240         250         260         270         280
APELLGGPSV FLFPPKPKDT 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 YKTTPPVLDS 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.

[00107] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (**SEQ ID NO:9**):

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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 YKTTPPMLDS 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.

[00108] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (**SEQ ID NO:10**):

```
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 YNTTPPMLDS 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.

[00109] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (**SEQ ID NO:11**):

```
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 YKTTPPVLDS 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.

[00110] 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 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.

2. Constant Regions of the Light Chain

[00111] As indicated above, each Light Chain of an antibody contains a Variable Domain ("**VL**") and a Constant Domain ("**CL**").

[00112] 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:12**):

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

[00113] 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:13**):

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

II. Chimeric Antigen Receptors

[00114] The binding molecules of the present invention that are capable of mediating the redirected killing of a target cell (*i.e.*, a cancer cell, a pathogen-infected cell, *etc.*) may alternatively be monospecific single-chain molecules such Chimeric Antigen Receptors ("**CARs**") incorporating a single chain variable fragment (**scFv**) capable of binding a

Cancer Antigen or a **Pathogen-Associated Antigen**. As indicated above, scFvs are made by linking Light and Heavy Chain Variable Domains together via a short linking peptide. First-generation CARs typically had the intracellular domain from the CD3 ζ- chain, which is the primary transmitter of signals from endogenous TCRs. Second-generation CARs possessed additional intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 41BB, ICOS, *etc.*) to the cytoplasmic tail of the CAR in order to provide additional signals to the T-cell. Third-generation CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, in order to further augment potency (Tettamanti, 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; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62.

[00115] The intracellular domain of the CARs of the present invention is preferably selected from the intracellular domain of any of: 41BB-CD3ζ, b2c-CD3ζ, CD28, CD28-4-1BB-CD3ζ, CD28-CD3ζ, CD28-FcεRIγ, CD28mut-CD3ζ, CD28-OX40-CD3ζ, CD28-OX40-CD3ζ, CD3ζ, CD4-CD3ζ, CD4-FcεRIγ, CD8-CD3ζ, FcεRIγ, FcεRIγCAIX, Heregulin-CD3ζ, IL-13-CD3ζ, or Ly49H-CD3ζ (Tettamanti, 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; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

III. Bispecific Antibodies and Multispecific Diabodies

[00116] 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).

[00117] 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 binding sites) for the same epitope and/or antigen.

[00118] 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 fragment (*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 fragments (*e.g.*, two Fab fragments or scFvs). Alternative formats use linker peptides to fuse an epitope-binding fragment (*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 Publications 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 Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. PCT Publications 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 Publications 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 Publications 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).

[00119] 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 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).

[00120] The design of a diabody is based on the structure of the single-chain Variable Domain fragment (**scFv**), in which Light and Heavy Chain Variable Domains are linked to one another using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge

approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[00121] The provision of bispecific binding molecules (*e.g.*, non-monospecific diabodies) provides a significant advantage over antibodies, including but not limited to, a "trans" binding capability sufficient to co-ligate and/or co-localize different cells that express different epitopes and/or a "cis" binding capability sufficient to co-ligate and/or co-localize different molecules expressed by the same cell. Bispecific binding molecules (*e.g.*, non-monospecific diabodies) thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221-1225).

[00122] The ability to produce bispecific diabodies 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), bispecific (or tri- or multispecific) diabodies 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, 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.

[00123] However, the advantages of the above-described bispecific diabodies come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; 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; 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).

[00124] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, 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).

[00125] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART® (Dual-Affinity Re-Targeting)** diabodies; see, *e.g.*, 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; and 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 Fcgamma 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). 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.

[00126] 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.

[00127] 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 Publications No: 2011-0206672; European Patent Publication No. EP 2371866, and; PCT Publications 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.

[00128] The present invention provides bispecific 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. Such bispecific binding molecules are capable of binding a "**first epitope**" and a "**second epitope**," such epitopes not being identical to one another. Such bispecific molecules comprise "**VL1**" / "**VH1**" domains that are capable of binding the first epitope, and "**VL2**" / "**VH2**" domains that are capable of binding the second epitope. The notation "**VL1**" and "**VH1**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "first" epitope of such bispecific molecules. Similarly, the notation "**VL2**" and "**VH2**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind the "second" epitope of such bispecific molecules. 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 domains of the polypeptide chains of the binding molecules of the present invention. In one embodiment, one of such epitopes is an epitope of a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell and the other epitope is an epitope of a Disease Antigen (*e.g.*, a Cancer Antigen or a Pathogen-Associated Antigen). In certain embodiments, a bispecific molecule comprises more than two epitope-binding sites. The instant invention particular encompasses bispecific diabodies, BiTEs, antibodies, and TandAbs produced using any of the methods provided herein.

A. Diabodies Lacking Fc Domains

[00129] In one embodiment, the diabodies of the invention are bispecific and will comprise 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. 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 $VL_{Epitope\ 1}$ or $VL_{Epitope\ 2}$), a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding the second epitope (if such first polypeptide chain contains $VL_{Epitope\ 1}$) or a VH Domain of a monoclonal antibody capable of binding the first epitope (if such first polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figure 1**).

[00130] 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.*, $VL_{Epitope\ 1}$ or $VL_{Epitope\ 2}$, 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.*, $VH_{Epitope\ 1}$ or $VH_{Epitope\ 2}$, 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 (**Figure 1**). 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.

[00131] 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 site 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 site 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 $VL_{Epitope\ 1}/VH_{Epitope\ 1}$ and $VL_{Epitope\ 2}/VH_{Epitope\ 2}$).

[00132] 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:14**): GGGSGGGG.

[00133] 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:15**). Alternatively, Linker 2 does not comprise a cysteine (*e.g.*,

GGG, GGGS (**SEQ ID NO:16**), LGGGSG (**SEQ ID NO:17**), GGGSGGGSGGG (**SEQ ID NO:18**), ASTKG (**SEQ ID NO:19**), LEPKSS (**SEQ ID NO:20**), APSSS (**SEQ ID NO:21**), *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.

[00134] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:22**) or VEPKSC (**SEQ ID NO:23**) or AEPKSC (**SEQ ID NO:24**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:25**) or FNRGEC (**SEQ ID NO:26**) on the other polypeptide chain (US2007/0004909).

[00135] 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:27**: 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:28**: 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:29**), and a modified K-coil having the amino acid sequence KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:30**).

[00136] 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 Module*s," 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:31**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00137] As disclosed in WO 2012/162068 (herein incorporated by reference), "**deimmunized**" variants of **SEQ ID NO:31** 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 YKNLI**D**$_{66}$NAK**S**$_{70}$ **A**$_{71}$EGVKALIDE ILAALP (**SEQ ID NO:32**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKN**A**$_{64}$**A**$_{65}$NNAKT VEGVKALI**A**$_{79}$E ILAALP (**SEQ ID NO:33**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**S**$_{66}$NAK**S**$_{70}$ VEGVKALI**A**$_{79}$E ILAALP (**SEQ ID NO:34**),

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:16**: GGGS.

B. Diabodies Comprising Fc Domains

[00138] One embodiment of the present invention relates to multispecific diabodies (*e.g.*, bispecific, trispecific, tetraspecific, *etc.*) capable of simultaneously binding a first and to a second epitope (*i.e.*, a different epitope of the same antigen molecule or an epitope of a molecule that is a different antigen) that comprise an Fc Domain. 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.

[00139] 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 a first epitope and to a second epitope. Incorporating an IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain bispecific Fc Region-containing diabody to form (**Figure 2**).

[00140] 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:22**), VEPKSC (**SEQ ID NO:23**), or AEPKSC (**SEQ ID NO:24**), 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:25**) or FNRGEC (**SEQ ID NO:26**). 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:27**: EVAALEK-EVAALEK-EVAALEK-EVAALEK or **SEQ ID NO:29:** EVAACEK-EVAALEK-EVAALEK-EVAALEK-EVAALEK); and the "K-coil" domains (**SEQ ID NO:28**: KVAALKE-KVAALKE-KVAALKE-KVAALKE or **SEQ ID NO:30:** KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00141] 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). Linkers that may be employed in the bispecific Fc Domain-containing diabody molecules of the present invention include: GGGS (**SEQ ID NO:16**), LGGGSG (**SEQ ID NO:17**), GGGSGGGSGGG (**SEQ ID NO:18**), ASTKG (**SEQ ID NO:19**), LEPKSS (**SEQ ID NO:20**), APSSS (**SEQ ID NO:21**), APSSSPME (**SEQ ID NO:35**), VEPKSADKTHTCPPCP (**SEQ ID NO:36**), LEPKSADKTHTCPPCP (**SEQ ID NO:37**), DKTHTCPPCP (**SEQ ID NO:38**), GGC, and GGG. LEPKSS (**SEQ ID NO:20**) may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or LEPKSS (**SEQ ID NO:20**) may be immediately followed by DKTHTCPPCP (**SEQ ID NO:38**) to form the alternate linkers: GGGDKTHTCPPCP (**SEQ ID NO:39**); and LEPKSSDKTHTCPPCP (**SEQ ID NO:40**). 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:4**) from IgG1, ERKCCVECPPCP (**SEQ ID NO:5**) from IgG2, ESKYGPPCPSCP (**SEQ ID NO:6**) from IgG4, and ESKYGPPCPPCP (**SEQ ID NO:7**) an IgG4 Hinge variant comprising a stabilizing S228P substitution (as numbered by the EU index as set forth in Kabat) to reduce strand exchange.

[00142] 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	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Tetraspecific	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH4-HPD-CH2-CH3-COOH
	4th Chain	NH_2-VL4-VH3-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00143] 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.

[00144] 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 site that is capable of binding either the first or second epitope, as well as a VL2/VH2 epitope-binding site 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 Region-containing diabodies may have either of two orientations (**Table 2**):

Table 2

First Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00145] 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 site immunospecific for either the first or second epitope, as well as a VL2/VH2 epitope-binding site that is capable of binding the other of such epitopes.

[00146] 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.

[00147] 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 site 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.

[00148] 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)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH2-HPD-COOH
Bispecific (3x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL1-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH1-HPD-COOH
Trispecific (2x1x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH3-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL3-VH2-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00149] 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 site 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 site immunospecific for the second molecule, and one epitope-binding site immunospecific for the third epitope.

[00150] 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: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (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:8**), IgG2 (**SEQ ID NO:9**), IgG3 (**SEQ ID NO:10**), and IgG4 (**SEQ ID NO:11**) are presented above.

[00151] 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 FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*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.

[00152] 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.*, FcγR(s)), more preferably such variant Fc Domain have altered binding FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (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.*).

[00153] 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 Fcgamma 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:8** 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			

Table 4 Variations of Preferred Activating Fc Domains†	
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
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	

[00154] Exemplary variants of human IgG1 Fc Domains with reduced binding CD32B and/or increased binding CD16A contain F243L, R292P, Y300L, V305I or P296L substitutions. 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 P296L substitution.

[00155] 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:8**). In a specific embodiment, the Fc Domain-containing binding molecules of the present invention comprise an IgG Fc Domain that exhibits reduced 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. 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:8**)) is utilized. In a specific embodiment, the Fc Domain-containing binding molecules of the present invention comprise an IgG2 Fc Domain (**SEQ ID NO:9**) or an IgG4 Fc Domain (**SEQ ID NO:11**). 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:7**). 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.

[00156] 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:41**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

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

[00157] 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.

[00158] 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, 256, 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. 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. Patents No. 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.

[00159] 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.

[00160] 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 FcγR binding; and
(B) one or more mutations which extend serum half-life.

[00161] 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 first and third polypeptide chains are desired to not be identical), it is desirable to reduce or prevent homodimerization from occurring between the CH2-CH3 Domains of 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).

[00162] 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 the hole-bearing third 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 of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the hole-bearing third 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 first polypeptide chain. In an alternative embodiment, the

hole-bearing third polypeptide chain may incorporate amino acid substitutions at positions 434 and 435 (N434A/N435K).

[00163] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Domain-containing molecule of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:42**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**W**CLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

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

[00164] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Domain-containing molecule of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Domain-containing molecule having three, four, or five polypeptide chains) will have the "**hole-bearing**" sequence (**SEQ ID NO:43**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL TVDKSRWQQG NVFSCSVMHE
ALHN**R**YTQKS LSLSPG**X**

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

[00165] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:42,** and **SEQ ID NO:43** 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), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (**SEQ ID NO:8**). 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 FγR 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.

[00166] It is preferred that the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:42**. However, as will be recognized, a "hole-

bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:43** could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:42**) 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).

[00167] 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.

IV. Trivalent Binding Molecules Containing Fc Domains

[00168] 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 site 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 site, 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 site. Thus, as used herein Fab- Type, and scFv-Type Binding Domains are distinct from Diabody-Type Binding Domains.

[00169] 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.

[00170] 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.

[00171] 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 site 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:14**): 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.

[00172] 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 site. One preferred intervening spacer peptide for this purpose has the sequence: GGGGSGGGGSGGGGS (**SEQ ID NO:44**).

[00173] 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.

[00174] 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 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Four Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Three Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[00175] As provided above, such trivalent binding molecules may comprise three, four, five, or more polypeptide chains.

V. Embodiments of the Invention

[00176] As stated above, the present invention is directed to a combination therapy for the treatment of cancer that comprises the administration of:

(1) a molecule capable of binding PD-1 or a natural ligand of PD-1; and

(2) a molecule (*e.g.*, a diabody, a BiTe, a bispecific antibody, *etc.*) capable of mediating the redirected killing of a target cell.

The present invention is also directed to pharmaceutical compositions that comprise such molecule(s).

[00177] As used herein, the term "**administration**" relates to the provision of such molecules at a relative dosage and in temporal proximity so as to provide a recipient with both binding of PD-1 or a natural ligand of PD-1, and the redirected killing of the target cell (e.g., a cancer cell or a pathogen-infected cell).

[00178] With regard to the molecule capable of binding PD-1 or a natural ligand of PD-1, the invention particularly concerns the embodiment in which such molecule possesses the ability to immunospecifically bind an epitope of PD-1 so as to inhibit (*i.e.*, block or interfere with) the inhibitory activity of PD-1. For example, such a molecule may bind PD-1 thereby inhibit cell signaling and/or inhibit binding between PD-1 and a natural ligand of PD-1. Alternatively, such molecule may bind a natural ligand of PD-1 (*e.g.*, B7-H1 or B7-DC) so as to inhibit (*i.e.*, block or interfere with) the inhibitory activity of such natural ligand. For example, such a molecule may bind a natural ligand of PD-1 to thereby inhibit cell signaling and/or binding between such ligand and PD-1. In one embodiment, such molecules will be monospecific so as to possess the ability to bind only a single epitope (*e.g.*, an epitope of PD-1 or an epitope of a natural ligand of PD-1). Alternatively, such molecules may be multispecific, *i.e.*, capable of binding two, or more than two, epitopes of PD-1 (*e.g.*, 2, 3, 4, or more than 4 epitopes of PD-1), or capable of binding two, or more than two (*e.g.*, 2, 3, 4, or more than 4) epitopes of one or more natural ligand(s) of PD-1, or be capable of binding at least one epitope of PD-1 and at least one epitope of a natural ligand of PD-1. Alternatively, such multispecific molecules are capable of binding at least one epitope of PD-1 and binding at least one epitope of a different molecule that is not PD-1, or capable of binding at least one epitope of a natural ligand of PD-1 and at least one epitope of a different molecule that is not a natural ligand of PD-1. Preferably, the epitope of the different molecule is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA, and particularly CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA, see for example PCT Publications Nos. WO 2015/200119 and WO 2011/159877). Thus, for example, such molecule may bind:

(1) a single epitope of PD-1;

(2) two or more epitopes of PD-1;

(3) a single epitope of a natural ligand of PD-1;

(4) two or more epitopes of the same natural ligand of PD-1;

(5) an epitope of a first natural ligand of PD-1 and an epitope of a second natural ligand of PD-1;

(6) two or more epitopes of a first natural ligand of PD-1 and one or more epitopes of a second natural ligand of PD-1;

(7) one or more epitopes of PD-1 and one or more epitopes of a natural ligand of PD-1;

(8) one or more epitopes of PD-1 and one or more epitopes of a different molecule; or

(9) one or more epitopes of natural ligand of PD-1 and one or more epitopes of a different molecule.

[00179] With regard to the molecules of the present invention that are capable of mediating the redirected killing of a target cell (e.g., a cancer cell or a pathogen-infected cell), the invention particularly concerns the embodiment in which such molecule comprises a first epitope-binding site capable of immunospecifically binding an epitope of a cell surface molecule of an effector cell and a second epitope-binding site that is capable of immunospecifically binding an epitope of a Disease Antigen that is arrayed on the surface of such target cell. In one embodiment, such molecules possess the ability to bind only a single epitope of a cell surface molecule of an effector cell and only to a single epitope of a Disease Antigen that is arrayed on the surface of the target cell. Alternatively, with respect to either or both binding specificities such molecules may be capable of binding one, two, or more than two, epitopes of cell surface molecule(s) of the effector cell, and be capable of binding one, two, or more than two epitopes of Disease Antigen(s). Thus, for example, such molecule may bind:

(1) only a single epitope of a cell surface molecule of an effector cell and a single epitope of a Disease Antigen that is arrayed on the surface of the target cell;

(2) only a single epitope of such cell surface molecule of such effector cell and two, or more than two, epitopes of such Disease Antigen;

(3) only a single epitope of such cell surface molecule of such effector cell and one, two, or more than two, epitopes of such Disease Antigen and one, two, or more than two, epitopes of a different Disease Antigen;

(4) two, or more than two epitopes of such cell surface molecule of such effector cell and a single epitope of a Disease Antigen that is arrayed on the surface of the target cell;

(5) two, or more than two epitopes of such cell surface molecule of such effector cell and two, or more than two, epitopes of such Disease Antigen;

(6) two, or more than two epitopes of such cell surface molecule of such effector cell and one, two, or more than two, epitopes of such Disease Antigen and one, two, or more than two, epitopes of such different Disease Antigen;

(7) one, two, or more than two epitopes of such cell surface molecule of such effector cell and one, two, or more than two, epitopes 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) and a single epitope of a Disease Antigen that is arrayed on the surface of the target cell;

(8) one, two, or more than two epitopes of such cell surface molecule of such effector cell and one, two, or more than two, epitopes 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) and two, or more than two, epitopes of such Disease Antigen; or

(9) one, two, or more than two epitopes of such cell surface molecule of such effector cell and one, two, or more than two, epitopes 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) and one, two, or more than two, epitopes of such Disease Antigen and one, two, or more than two, epitopes of such different Disease Antigen.

[00180] As an example, the invention contemplates a binding molecule that comprises a first epitope-binding site capable of immunospecifically binding an epitope of CD3 (as the cell surface molecule of an effector cell); a second epitope-binding site 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 site capable of immunospecifically binding an epitope of CD8 (as the different cell surface molecule of an effector cell).

[00181] **Table 6A** illustrates possible combination binding specificities of exemplary molecules of the invention capable of binding PD-1 or a natural ligand of PD-1. **Table 6B** illustrates possible combination binding specificities of exemplary multispecific molecules of the invention capable of binding PD-1 or a natural ligand of PD-1 and a molecule other than PD-1 or a natural ligand of PD-1. **Table 7** illustrates possible combination binding specificities of exemplary molecules of the invention capable of mediating the redirected killing of a target cell.

Table 6A

Number of Epitopes Recognized by Exemplary Molecule of the Invention Capable of Binding PD-1 or a Natural Ligand of PD-1

PD-1	PD-1 Ligand	
	1st PD-1 Ligand	2nd PD-1 Ligand
1	0	0
1	1	
1		1
1	2	
1		2
1	>2	
1		>2
2	0	0
2	1	
2		1
2	2	
2		2
2	>2	
2		>2
>2	0	0
>2	1	
>2		1
>2	2	
>2		2
>2	>2	
>2		>2
0	1	0
0	1	1
0	2	0
0	2	1
0	2	2
0	>2	1
0	>2	2

Table 6A		
Number of Epitopes Recognized by Exemplary Molecule of the Invention Capable of Binding PD-1 or a Natural Ligand of PD-1		
PD-1	PD-1 Ligand	
	1st PD-1 Ligand	2nd PD-1 Ligand
1	0	0
0	>2	>2

Table 6B				
Number of Epitopes Recognized by Exemplary Molecule of the Invention Capable of Binding PD-1 or a Natural Ligand of PD-1, and a Different Molecule				
PD-1	Different Molecule		Ligand of PD-1	Different Molecule
1	1		1	1
1	2		1	2
1	>2		1	>2
2	1		2	1
2	2		2	2
2	>2		2	>2
>2	1		>2	1
>2	2		>2	2
>2	>2		>2	>2

Table 7			
Number of Epitopes Recognized by Exemplary Molecules of the Invention Capable of Mediating the Redirected Killing of a Target Cell			
Cell Surface Molecule of an Effector Cell		Disease Antigen	
1st Surface Molecule	2nd Surface Molecule	1st Disease Antigen	2nd Disease Antigen
1	0	1	0
1	0	1	1
1	0	2	0
1	0	2	1
1	0	2	2
1	0	>2	0
1	0	>2	1
1	0	>2	2
2	0	1	0
2	0	1	1
2	0	2	0
2	0	2	1
2	0	2	2
2	0	>2	0
2	0	>2	1
2	0	>2	2

Table 7

Number of Epitopes Recognized by Exemplary Molecules of the Invention Capable of Mediating the Redirected Killing of a Target Cell

Cell Surface Molecule of an Effector Cell		Disease Antigen	
1st Surface Molecule	2nd Surface Molecule	1st Disease Antigen	2nd Disease Antigen
>2	0	1	0
>2	0	1	1
>2	0	2	0
>2	0	2	1
>2	0	2	2
>2	0	>2	0
>2	0	>2	1
>2	0	>2	2
1	1	1	0
1	1	1	1
1	1	2	0
1	1	2	1
1	1	2	2
1	1	>2	0
1	1	>2	1
1	1	>2	2
2	1	1	0
2	1	1	1
2	1	2	0
2	1	2	1
2	1	2	2
2	1	>2	0
2	1	>2	1
2	1	>2	2
2	2	1	0
2	2	1	1
2	2	2	0
2	2	2	1
2	2	2	2
2	2	>2	0
2	2	>2	1
2	2	>2	2
>2	1	1	0
>2	1	1	1
>2	1	2	0
>2	1	2	1
>2	1	2	2
>2	1	>2	0
>2	1	>2	1
>2	1	>2	2

Table 7			
Number of Epitopes Recognized by Exemplary Molecules of the Invention Capable of Mediating the Redirected Killing of a Target Cell			
Cell Surface Molecule of an Effector Cell		Disease Antigen	
1st Surface Molecule	2nd Surface Molecule	1st Disease Antigen	2nd Disease Antigen
>2	2	1	0
>2	2	1	1
>2	2	2	0
>2	2	2	1
>2	2	2	2
>2	2	>2	0
>2	2	>2	1
>2	2	>2	2
>2	>2	1	0
>2	>2	1	1
>2	>2	2	0
>2	>2	2	1
>2	>2	2	2
>2	>2	>2	0
>2	>2	>2	1
>2	>2	>2	2

[00182] No limitation is placed on the nature 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 that is capable of inhibiting binding PD-1 or a natural ligand of PD-1 from such binding and does not prevent the molecule that is capable of mediating the redirected killing of a target cell from mediating such redirected killing.

A. Exemplary Molecules Capable Of Binding PD-1 or A Natural Ligand Of PD-1

1. Binding Molecules Immunospecific For PD-1

[00183] Antibodies that are immunospecific for PD-1 are known and may be employed or adapted to serve as a molecule (*e.g.*, a diabody, an scFv, an antibody, a CAR, a TandAb, *etc.*) capable of binding PD-1 or a natural ligand of PD-1 in accordance with the present invention (see, *e.g.*, United States Patent Applications No. 62/198,867; 62/239,559; 62/255,140 United States Patents No. 8,008,449; 8,552,154; PCT Patent Publications WO 2012/135408; WO 2012/145549; and WO 2013/014668). Preferred molecules capable of binding PD-1 or a natural ligand of PD-1 will exhibit the ability to bind a continuous or

discontinuous (*e.g.*, conformational) portion (**epitope**) of human PD-1 (CD279) and will preferably also exhibit the ability to bind PD-1 molecules of one or more non-human species, in particular, primate species (and especially a primate species, such as cynomolgus monkey). Additional desired antibodies may be made by isolating antibody-secreting hybridomas elicited using PD-1 or a peptide fragment thereof. A representative human PD-1 polypeptide (NCBI Sequence NP_005009.2; including a 20 amino acid residue signal sequence, shown underlined) and the 268 amino acid residue mature protein) has the amino acid sequence (**SEQ ID NO:45**):

MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA
TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTQL
PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE
VPTAHPSPSP RPAGQFQTLV VGVVGGLLGS LVLLVWVLAV ICSRAARGTI
GARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPVP CVPEQTEYAT
IVFPSGMGTS SPARRGSADG PRSAQPLRPE DGHCSWPL

[00184] Preferred PD-1-binding molecules that may be used to bind PD-1 are characterized by any (one or more) of the following criteria:

(1) specifically binds human PD-1 as endogenously expressed on the surface of a stimulated human T-cell;

(2) specifically binds human PD-1 with an equilibrium binding constant (K_D) of 40 nM or less;

(3) specifically binds human PD-1 with an equilibrium binding constant (K_D) of 5 nM or less;

(4) specifically binds human PD-1 with an on rate (k_a) of 1.5×10^4 $M^{-1}min^{-1}$ or more;

(5) specifically binds human PD-1 with an on rate (k_a) of 90.0×10^4 $M^{-1}min^{-1}$ or more;

(6) specifically binds human PD-1 with an off rate (k_d) of 7×10^{-4} min^{-1} or less;

(7) specifically binds human PD-1 with an off rate (k_d) of 2×10^{-4} min^{-1} or less;

(8) specifically binds non-human primate PD-1 (*e.g.*, PD-1 of cynomolgus monkey);

(9) inhibits (*i.e.*, blocks or interferes with) the binding/the inhibitory activity) of PD-1 ligand (PD-L1/PD-L2) to PD-1;

(10) stimulates an immune response; and/or

(11) synergizes with an anti-human LAG-3 antibody to stimulate an antigen-specific T-cell response.

[00185] The preferred anti-human PD-1-binding molecules of the present invention that may be used to bind PD-1 possess humanized VH and/or VL Domains of murine anti-human PD-1 monoclonal antibodies "**PD-1 mAb 1**," "**PD-1 mAb 2**," "**PD-1 mAb 3**," "**PD-1 mAb 4**," "**PD-1 mAb 5**," "**PD-1 mAb** 6," "**PD-1 mAb** 7," "**PD-1 mAb 8**," "**PD-1 mAb 9**," "**PD-1 mAb 10**," "**PD-1 mAb 11**," "**PD-1 mAb 12**," "**PD-1 mAb 13**," "**PD-1 mAb 14**," or "**PD-1 mAb 15**," and more preferably possess 1, 2 or all 3 of the CDR_{HS} of the VH Domain and/or 1, 2 or all 3 of the CDR_{LS} of the VL Domain of such antibodies. The invention particularly relates to such PD-1-binding molecules comprising a PD-1 binding domain that possess:

(A) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 1**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 1**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 1** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 1**;

(4) the VH Domain of **hPD-1 mAb 1 VH1**;

(5) the VL Domain of **hPD-1 mAb 1 VL1**;

(6) the VH and VL Domains of **hPD-1 mAb 1**;

(B) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 2**;

(2) the three CDR_{LS} of the VL Domain of the **PD-1 mAb 2**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 2** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 2**;

(4) the VH Domain of **hPD-1 mAb 2 VH1**;

(5) the VL Domain of **hPD-1 mAb 2 VL1**;

(6) the VH and VL Domains of **hPD-1 mAb 2**;

(C) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 3**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 3**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 3** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 3**;

(D) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 4**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 4**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 4** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 4**;

(E) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 5**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 5**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 5** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 5**;

(F) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 6**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 6**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 6** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 6**;

(G) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 7**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 7**, or **hPD-1 mAb 7 VL2**, or **hPD-1 mAb 7 VL3**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 7** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 7**, or **hPD-1 mAb 7 VL2**, **hPD-1 mAb 7 VL3**;

(4) the VH Domain of **hPD-1 mAb 7 VH1**, or **hPD-1 mAb 7 VH2**;

(5) the VL Domain of **hPD-1 mAb 7 VL1**, or **hPD-1 mAb 7 VL2**, or **hPD-1 mAb 7 VL 3**;

(6) the VH and VL Domains of the **hPD-1 mAb 7(1.1)**, or **hPD-1 mAb 7(1.2)**, or **hPD-1 mAb 7(1.3)**, or **hPD-1 mAb 7(2.1)**, or **hPD-1 mAb 7(2.2)**, or **hPD-1 mAb 7(2.3)**;

(H) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 8**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 8**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 8** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 8**;

(I) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 9**, or **hPD-1 mAb 9 VH2**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 9**, or **hPD-1 mAb 9 VL2**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 9**, or **hPD-1 mAb 9 VH2** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 9**, or **hPD-1 mAb 9 VL2**;

(4) the VH Domain of **hPD-1 mAb 9 VH1**, or **hPD-1 mAb 9 VH2**;

(5) the VL Domain of **hPD-1 mAb 9 VL1**, or **hPD-1 mAb 9 VL2**;

(6) the VH and VL Domains of the **hPD-1 mAb 9(1.1)**, or **hPD-1 mAb 9(1.2)**, or **hPD-1 mAb 9(2.1)**, or **hPD-1 mAb 9(2.2)**;

(J) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 10**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 10**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 10** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 10**;

(K) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 11**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 11**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 11** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 11**;

(L) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 12**;

(2) the three CDR_{LS} of the VL Domain of the **PD-1 mAb 12**;

(3) the three CDR_{HS} of the VH Domain of the **PD-1 mAb 12** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 12**;

(M) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 13**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 13**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 13** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 13**;

(N) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 14**;

(2) the three CDR_{LS} of the VL Domain of the **PD-1 mAb 14**;

(3) the three CDR_{HS} of the VH Domain of the **PD-1 mAb 14** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 14**;

(O) (1) the three CDR_{HS} of the VH Domain of **PD-1 mAb 15**;

(2) the three CDR_{LS} of the VL Domain of **PD-1 mAb 15**;

(3) the three CDR_{HS} of the VH Domain of **PD-1 mAb 15** and the three CDR_{LS} of the VL Domain of **PD-1 mAb 15**;

(4) the VH Domain of **hPD-1 mAb 15 VH1**;

(5) the VL Domain of **hPD-1 mAb 15 VL1**;

(6) the VH and VL Domains of **hPD-1 mAb 15**;

or

that binds, or competes for binding with, the same epitope as **PD-1 mAb 1**, **PD-1 mAb 2**, **PD-1 mAb 3**, **PD-1 mAb 4**, **PD-1 mAb 5**, **PD-1 mAb 6**, **PD-1 mAb 7**, **PD-1 mAb 8**, **PD-1 mAb 9**, **PD-1 mAb 10**, **PD-1 mAb 11**, **PD-1 mAb 12**, **PD-1 mAb 13**, **PD-1 mAb 14**, or **PD-1 mAb 15**.

(a) PD-1 mAb 1

[00186] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 1 (SEQ ID NO:46)** is shown below (CDR_H residues are shown underlined).

DVQLQESGPG RVKPSQSLSL TCTVTGFSIT **NDYAWN**WIRQ FPGNKLEWMG **HITYSGSTSY NPSLKS**RISI TRDTSKNHFF LQLSSVTPED TATYYCAR**DY GSGYPYTLDY** WGQGTSVTVS S

CDR$_H$1 of PD-1 mAb 1 **(SEQ ID NO:47)**: **NDYAWN**

CDR$_H$2 of PD-1 mAb 1 **(SEQ ID NO:48)**: **HITYSGSTSYNPSLKS**

CDR$_H$3 of PD-1 mAb 1 **(SEQ ID NO:49)**: **DYGSGYPYTLDY**

[00187] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 1 (SEQ ID NO:50)** is shown below (CDR_L residues are shown underlined):

QIVLTQSPAL MSASPGEKVT MTC**SATSIVS YVY**WYQQKPG SSPQPWIY**LT SNLAS**GVPAR FSGSGSGTSY SLTISSMEAE DAATYYC**QQW SDNPYT**FGGG TKLEIK

CDR$_L$1 of PD-1 mAb 1 **(SEQ ID NO:51)**: **SATSIVSYVY**

CDR$_L$2 of PD-1 mAb 1 **(SEQ ID NO:52)**: **LTSNLAS**

CDR$_L$3 of PD-1 mAb 1 **(SEQ ID NO:53)**: **QQWSDNPYT**

[00188] The above-described murine anti-human PD-1 antibody **PD-1 mAb 1** was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one humanized VH Domain, designated herein as "**hPD-1 mAb 1 VH1**," and one humanized VL Domain designated herein as "**hPD-1 mAb 1 VL1.**" Accordingly, an antibody comprising the humanized VL Domains paired with the humanized VH Domain is referred to as "**hPD-1 mAb 1**."

[00189] The amino acid sequence of the VH Domain of **hPD-1 mAb 1 VH1 (SEQ ID NO:54)** is shown below (CDR_H residues are shown underlined):

DVQLQESGPG LVKPSQTLSL TCTVSGFSIS **NDYAWN**WIRQ PPGKGLEWIG **HITYSGSTSY NPSLKS**RLTI TRDTSKNQFV LTMTNMDPVD TATYYCAR**DY GSGYPYTLDY** WGQGTTVTVS S

[00190] The amino acid sequence of the VL Domain of **hPD-1 mAb 1 VL1** (**SEQ ID NO:55**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSVSPGEKVT ITC**SATSIVS YVY**WYQQKPG QAPQPLIY**LT SNLAS**GIPAR FSGSGSGTDF TLTISSLEAE DAATYYC**QQW SDNPYT**FGGG TKVEIK

(b) PD-1 mAb 2

[00191] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 2** (**SEQ ID NO:56**) is shown below (CDR_H residues are shown underlined).

DVQLVESGGG LVQPGGSRKL SCAASGFVFS **SFGMH**WVRQA PEKGLEWVA**Y ISSGSMSISY ADTVKG**RFTV TRDNAKNTLF LQMTSLRSED TAIYYCAS**LS DYFDY**WGQGT TLTVSS

CDR_H1 of PD-1 mAb 2 (**SEQ ID NO:57**): **SFGMH**

CDR_H2 of PD-1 mAb 2 (**SEQ ID NO:58**): **YISSGSMSISYADTVKG**

CDR_H3 of PD-1 mAb 2 (**SEQ ID NO:59**): **LSDYFDY**

[00192] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 2** (**SEQ ID NO:60**) is shown below (CDR_L residues are shown underlined):

DVVMSQTPLS LPVSLGDQAS ISC**RSSQSLV HSTGNTYLH**W YLQKPGQSPK LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV FFC**SQTTHVP WT**FGGGTKLE IK

CDR_L1 of PD-1 mAb 2 (**SEQ ID NO:61**): **RSSQSLVHSTGNTYLH**

CDR_L2 of PD-1 mAb 2 (**SEQ ID NO:62**): **RVSNRFS**

CDR_L3 of PD-1 mAb 2 (**SEQ ID NO:63**): **SQTTHVPWT**

[00193] The above-described murine anti-human PD-1 antibody **PD-1 mAb 2** was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one humanized VH Domain, designated herein as "**hPD-1 mAb 2 VH1,**" and one humanized VL Domains designated herein as "**hPD-1 mAb 1 VL1**." Accordingly, any antibody comprising the humanized VL Domains paired with the humanized VH Domain is referred to as "**hPD-1 mAb 2**."

[00194] The amino acid sequence of the VH Domain of **hPD-1 mAb 2 VH1 (SEQ ID NO:64)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFVFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSGSMSISY ADTVKGRFTI SRDNAKNTLY LQMNSLRTED TALYYCAS**LS**
DYFDYWGQGT TVTVSS

[00195] The amino acid sequence of the VL Domain of **hPD-1 mAb 2 VL1 (SEQ ID NO:65)** is shown below (CDR_H residues are shown underlined):

DVVMTQSPLS LPVTLGQPAS ISC**RSSQSLV HSTGNTYLH**W YLQKPGQSPQ
LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**SQTTHVP**
WTFGQGTKLE IK

(c) PD-1 mAb 3

[00196] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 3 (SEQ ID NO:66)** is shown below (CDR_H residues are shown underlined).

QVQLQQSGAE LVRPGASVTL SCKASGYTFT **DYVMH**WVKQT PVHGLEWIG**T**
IDPETGGTAY NQKFKGKAIL TADKSSNTAY MELRSLTSED SAVYYFTR**EK**
ITTIVEGTYW YFDVWGTGTT VTVSS

CDR_H1 of PD-1 mAb 3 **(SEQ ID NO:67)**: **DYVMH**

CDR_H2 of PD-1 mAb 3 **(SEQ ID NO:68)**: **TIDPETGGTAYNQKFKG**

CDR_H3 of PD-1 mAb 3 **(SEQ ID NO:69)**: **EKITTIVEGTYWYFDV**

[00197] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 3 (SEQ ID NO:70)** is shown below (CDR_L residues are shown underlined):

DVLLTQTPLS LPVSLGDQAS ISC**RSSQNIV HSNGDTYLE**W YLQKPGQSPK
LLIY**KVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYC**FQGSHLP**
YTFGGGTKLE IK

CDR_L1 of PD-1 mAb 3 **(SEQ ID NO:71)**: **RSSQNIVHSNGDTYLE**

CDR_L2 of PD-1 mAb 3 **(SEQ ID NO:72)**: **KVSNRFS**

CDR_L3 of PD-1 mAb 3 **(SEQ ID NO:73)**: **FQGSHLPYT**

(d) PD-1 mAb 4

[00198] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 4 (SEQ ID NO:74)** is shown below (CDR_H residues are shown underlined).

DVQLVESGGG LVQPGGSRKL SCAASGFVFS **SFGMH**WVRQA PEKGLEWVA**Y**
ISSGSMSISY ADTVKGRFTV TRDNAKNTLF LQMTSLRSED TAIYYCAS**LT**
DYFDYWGQGT TLTVSS

CDR_H1 of PD-1 mAb 4 **(SEQ ID NO:75)**: SFGMH

CDR_H2 of PD-1 mAb 4 **(SEQ ID NO:76)**: YISSGSMSISYADTVKG

CDR_H3 of PD-1 mAb 4 **(SEQ ID NO:77)**: LTDYFDY

[00199] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 4 (SEQ ID NO:78)** is shown below (CDR_L residues are shown underlined):

DVVMSQTPLS LPVSLGDQAS ISC**RSSQSLV HSTGNTYFH**W YLQKPGQSPK LLIY**RVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQTTHVP WT**FGGGTKLE IK

CDR_L1 of PD-1 mAb 4 **(SEQ ID NO:79)**: RSSQSLVHSTGNTYFH

CDR_L2 of PD-1 mAb 4 **(SEQ ID NO:80)**: RVSNRFS

CDR_L3 of PD-1 mAb 4 **(SEQ ID NO:81)**: SQTTHVPWT

(e) PD-1 mAb 5

[00200] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 5 (SEQ ID NO:82)** is shown below (CDR_H residues are shown underlined).

QVQLQQPGVE LVRPGASVKL SCKASGYSFT **AYWMN**WMKQR PGQGLEWIG**V IHPSDSETWL NQKFKD**KATL TVDKSSSTAY MQLISPTSED SAVYYCAR**EH YGSSPFAY**WG QGTLVTVSA

CDR_H1 of PD-1 mAb 5 **(SEQ ID NO:83)**: AYWMN

CDR_H2 of PD-1 mAb 5 **(SEQ ID NO:84)**: VIHPSDSETWLNQKFKD

CDR_H3 of PD-1 mAb 5 **(SEQ ID NO:85)**: EHYGSSPFAY

[00201] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 5 (SEQ ID NO:86)** is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**RANESVD NYGMSFMN**WF QQKPGQPPKL LIY**AASNQGS** GVPARFSGSG SGTDFSLNIH PMEEDDTAMY FC**QQSKEVPY T**FGGGTKLEI K

CDR_L1 of PD-1 mAb 5 **(SEQ ID NO:87)**: RANESVDNYGMSFMN

CDR_L2 of PD-1 mAb 5 **(SEQ ID NO:88)**: AASNQGS

CDR_L3 of PD-1 mAb 5 **(SEQ ID NO:89)**: QQSKEVPYT

(f) PD-1 mAb 6

[00202] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 6 (SEQ ID NO:90)** is shown below (CDR_H residues are shown underlined).

EVKLVESGGG LVNPGGSLKL SCAASGFTFS **SYGMS**WVRQT PEKRLEWVA**T**
ISGGGSDTYY PDSVKGRFTI SRDNAKNNLY LQMSSLRSED TALYYCAR**QK**
ATTWFAYWGQ GTLVTVST

CDR_H1 of PD-1 mAb 6 **(SEQ ID NO:91)**: **SYGMS**

CDR_H2 of PD-1 mAb 6 **(SEQ ID NO:92)**: **TISGGGSDTYYPDSVKG**

CDR_H3 of PD-1 mAb 6 **(SEQ ID NO:93)**: **QKATTWFAY**

[00203] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 6 (SEQ ID NO:94)** is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**RASESVD NYGISFMN**WF QQKPGQPPKL
LIY**PASNQGS** GVPARFSGSG SGTDFSLNIH PMEEDDAAMY FC**QQSKEVPW**
TFGGGTKLEI K

CDR_L1 of PD-1 mAb 6 **(SEQ ID NO:95)**: **RASESVDNYGISFMN**

CDR_L2 of PD-1 mAb 6 **(SEQ ID NO:96)**: **PASNQGS**

CDR_L3 of PD-1 mAb 6 **(SEQ ID NO:97)**: **QQSKEVPWT**

(g) PD-1 mAb 7

[00204] The amino acid sequence of the VH Domain of murine anti-human anti-human **PD-1 mAb 7 (SEQ ID NO:98)** is shown below (CDR_H residues are shown underlined).

QVQLQQPGAE LVRPGASVKL SCKASGYSFT **SYWMN**WVKQR PGQGLEWIG**V**
IHPSDSETWL DQKFKDKATL TVDKSSTTAY MQLISPTSED SAVYYCAR**EH**
YGTSPFAYWG QGTLVTVSS

CDR_H1 of PD-1 mAb 7 **(SEQ ID NO:99)**: **SYWMN**

CDR_H2 of PD-1 mAb 7 **(SEQ ID NO:100)**: **VIHPSDSETWLDQKFKD**

CDR_H3 of PD-1 mAb 7 **(SEQ ID NO:101)**: **EHYGTSPFAY**

[00205] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 7 (SEQ ID NO:102)** is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**RANESVD NYGMSFMN**WF QQKPGQPPKL
LIH**AASNQGS** GVPARFSGSG FGTDFSLNIH PMEEDDAAMY FC**QQSKEVPY**
TFGGGTKLEI K

CDR$_L$1 of PD-1 mAb 7 (**SEQ ID NO:103**): RANESVDNYGMSFMN

CDR$_L$2 of PD-1 mAb 7 (**SEQ ID NO:104**): AASNQGS

CDR$_L$3 of PD-1 mAb 7 (**SEQ ID NO:105**): QQSKEVPYT

[00206] The above-described murine anti-human PD-1 antibody **PD-1 mAb 7** was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded two humanized VH Domains, designated herein as "**hPD-1 mAb 7 VH1,**" and "**hPD-1 mAb 7 VH2**," and three humanized VL Domains designated herein as "**hPD-1 mAb 7 VL1**," "**hPD-1 mAb 7 VL2**," and "**hPD-1 mAb 7 VL3**." Any of the humanized VL Domains may be paired with either of the humanized VH Domains. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hPD-1 mAb 7**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising **hPD-1 mAb 7 VH1** and **hPD-1 mAb 1 VL2** is specifically referred to as "**hPD-1 mAb 7(1.2)**."

[00207] The amino acid sequence of the VH Domain of **hPD-1 mAb 7 VH1** (**SEQ ID NO:106**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWIGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSS

[00208] The amino acid sequence of the VH Domain of **hPD-1 mAb 7 VH2** (**SEQ ID NO:107**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT SYWMNWVRQA PGQGLEWAGV
IHPSDSETWL DQKFKDRVTI TVDKSTSTAY MELSSLRSED TAVYYCAREH
YGTSPFAYWG QGTLVTVSS

[00209] The amino acid sequence of the VL Domain of **hPD-1 mAb 7 VL1** (**SEQ ID NO:108**) is shown below (CDR$_H$ residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRANESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI K

[00210] The amino acid sequence of the VL Domain of **hPD-1 mAb 7 VL2** (**SEQ ID NO:109**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY TFGGGTKVEI K

[00211] The amino acid sequence of the VL Domain of **hPD-1 mAb 7 VL3** (**SEQ ID NO:110**) is shown below (CDR_H residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL LIHAASNRGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY TFGGGTKVEI K

[00212] The CDR_L1 of the VL Domain of both **hPD-1 mAb 7 VL2** and **hPD-1 mAb 7 VL3** comprises an asparagine to serine amino acid substitution and has the amino acid sequence: RASESVDNYGMSFMN (**SEQ ID NO:111**), the substituted serine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 7 CDR_L1 Domains described above.

[00213] In addition, the CDR_L2 of the VL Domain of **hPD-1 mAb 7 VL3** comprises a glutamine to arginine amino acid substitution and has the amino acid sequence: AASNRGS (**SEQ ID NO:112**), the substituted arginine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the PD-1 mAb 7 CDR_L2 Domains described above.

(h) PD-1 mAb 8

[00214] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 8** (**SEQ ID NO:113**) is shown below (CDR_H residues are shown underlined).

EGQLQQSGPE LVKPGASVKI SCKASGYTFT DYYMNWVKQN HGKSLEWIGD INPKNGDTHY NQKFKGEATL TVDKSSTTAY MELRSLTSED SAVYYCASDF DYWGQGTTLT VSS

CDR_H1 of PD-1 mAb 8 (**SEQ ID NO:114**): DYYMN

CDR_H2 of PD-1 mAb 8 (**SEQ ID NO:115**): DINPKNGDTHYNQKFKG

CDR_H3 of PD-1 mAb 8 (**SEQ ID NO:116**): DFDY

[00215] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 8 (SEQ ID NO:117)** is shown below (CDR_L residues are shown underlined):

DVVMTQTPLS LPVGLGDQAS ISC**RSSQTLV YSNGNTYLN**W FLQKPGQSPK LLIY**KVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQSTHVP FT**FGSGTKLE IK

CDR_L1 of PD-1 mAb 8 **(SEQ ID NO:118)**: **RSSQTLVYSNGNTYLN**

CDR_L2 of PD-1 mAb 8 **(SEQ ID NO:119)**: **KVSNRFS**

CDR_L3 of PD-1 mAb 8 **(SEQ ID NO:120)**: **SQSTHVPFT**

(i) PD-1 mAb 9

[00216] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 9 (SEQ ID NO:121)** is shown below (CDR_H residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFTFS **SYLVS**WVRQT PEKRLEWVA**T ISGGGGNTYY SDSVKG**RFTI SRDNAKNTLY LQISSLRSED TALYYCAR**YG FDGAWFAY**WG QGTLVTVSS

CDR_H1 of PD-1 mAb 9 **(SEQ ID NO:122)**: **SYLVS**

CDR_H2 of PD-1 mAb 9 **(SEQ ID NO:123)**: **TISGGGGNTYYSDSVKG**

CDR_H3 of PD-1 mAb 9 **(SEQ ID NO:124)**: **YGFDGAWFAY**

[00217] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 9 (SEQ ID NO:125)** is shown below (CDR_L residues are shown underlined):

DIQMTQSPAS LSASVGDIVT ITC**RASENIY SYLA**WYQQKQ EKSPQLLVY**N AKTLAA**GVPS RFSGSGSGTQ FSLTINSLQP EDFGNYYC**QH HYAVPWT**FGG GTRLEIT

CDR_L1 of PD-1 mAb 9 **(SEQ ID NO:126)**: **RASENIYSYLA**

CDR_L2 of PD-1 mAb 9 **(SEQ ID NO:127)**: **NAKTLAA**

CDR_L3 of PD-1 mAb 9 **(SEQ ID NO:128)**: **QHHYAVPWT**

[00218] The above-described murine anti-human PD-1 antibody **PD-1 mAb 9** was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded two humanized VH Domains, designated herein as "**hPD-1 mAb 9 VH1**," and "**hPD-1 mAb 9 VH2**," and two humanized VL Domains designated herein as "**hPD-1 mAb 9 VL1**," and "**hPD-1 mAb 9 VL2**." Any of the humanized VL Domains may be paired with the

humanized VH Domains. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hPD-1 mAb 9**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising **hPD-1 mAb 9 VH1** and **hPD-1 mAb 9 VL2** is specifically referred to as "**hPD-1 mAb 9(1.2)**."

[00219] The amino acid sequence of the VH Domain of **hPD-1 mAb 9 VH1** (**SEQ ID NO:129**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVRPGGSLKL SCAASGFTFS **SYLVS**WVRQA PGKGLEWVA**T**
ISGGGGNTYY SDSVKGRFTI SRDNAKNSLY LQMNSLRAED TATYYCAR**YG**
FDGAWFAYWG QGTLVTVSS

[00220] The amino acid sequence of the VH Domain of **hPD-1 mAb 9 VH2** (**SEQ ID NO:130**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LARPGGSLKL SCAASGFTFS **SYLVG**WVRQA PGKGLEWTA**T**
ISGGGGNTYY SDSVKGRFTI SRDNAKNSLY LQMNSARAED TATYYCAR**YG**
FDGAWFAYWG QGTLVTVSS

[00221] The CDR_H1 of the VH Domain of **hPD-1 mAb 9 VH2** comprises a serine to glycine amino acid substitution and has the amino acid sequence: SYLV**G** (**(SEQ ID NO:131**), the substituted glycine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the **PD-1 mAb 9** CDR_H1 Domains described above.

[00222] The amino acid sequence of the VL Domain of **hPD-1 mAb 9 VL1** (**SEQ ID NO:132**) is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASENIY SYLA**WYQQKP GKAPKLLIY**N**
AKTLAAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYAVPWT**FGQ
GTKLEIK

[00223] The amino acid sequence of the VL Domain of **hPD-1 mAb 9 VL2** (**SEQ ID NO:133**) is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASENIY NYLA**WYQQKP GKAPKLLIY**D**
AKTLAAGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYAVPWT**FGQ
GTKLEIK

[00224] The CDR_L1 of the VL Domain of **hPD-1 mAb 9 VL2** comprises a serine to asparagine amino acid substitution and has the amino acid sequence: RASENIY**N**YLA (**SEQ ID NO:134**), the substituted asparagine is shown underlined). It is contemplated that

a similar substitution may be incorporated into any of the **PD-1 mAb 9** CDR_L1 Domains described above.

[00225] The CDR_L2 of the VL Domain of **hPD-1 mAb 9 VL2** comprises an asparagine to aspartate amino acid substitution and has the amino acid sequence: DAKTLAA (**(SEQ ID NO:135)**, the substituted aspartate is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the **PD-1 mAb 7** CDR_L2 Domains described above.

(j) PD-1 mAb 10

[00226] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 10 (SEQ ID NO:136)** is shown below (CDR_H residues are shown underlined).

EVILVESGGG LVKPGGSLKL SCAASGFTFS **NYLMS**WVRQT PEKRLEWVA**S**
ISGGGSNIYY PDSVKGRFTI SRDNAKNTLY LQMNSLRSED TALYYCAR**QE**
LAFDYWGQGT TLTVSS

CDR_H1 of **PD-1 mAb 10 (SEQ ID NO:137)**: **NYLMS**

CDR_H2 of **PD-1 mAb 10 (SEQ ID NO:138)**: **SISGGGSNIYYPDSVKG**

CDR_H3 of **PD-1 mAb 10 (SEQ ID NO:139)**: **QELAFDY**

[00227] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 10 (SEQ ID NO:140)** is shown below (CDR_L residues are shown underlined):

DIQMTQTTSS LSASLGDRVT ISC**RTSQDIS NFLN**WYQQKP DGTIKLLIY**Y**
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFC**QQ GSTLPWT**FGG
GTKLEII

CDR_L1 of **PD-1 mAb 10 (SEQ ID NO:141)**: **RTSQDISNFLN**

CDR_L2 of **PD-1 mAb 10 (SEQ ID NO:142)**: **YTSRLHS**

CDR_L3 of **PD-1 mAb 10 (SEQ ID NO:143)**: **QQGSTLPWT**

(k) PD-1 mAb 11

[00228] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 11 (SEQ ID NO:144)** is shown below (CDR_H residues are shown underlined).

EVQLQQSGTV LARPGASVKM SCKTSGYTFT **GYWMH**WVKQR PGQGLKWMG**A**
IYPGNSDTHY NQKFKGKAKL TAVTSASTAY MELSSLTNED SAIYYCTT**GT**
YSYFDVWGTG TTVTVSS

CDR_H1 of **PD-1 mAb 11 (SEQ ID NO:145)**: GYWMH

CDR_H2 of **PD-1 mAb 11 (SEQ ID NO:146)**: AIYPGNSDTHYNQKFKG

CDR_H3 of **PD-1 mAb 11 (SEQ ID NO:147)**: GTYSYFDV

[00229] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 11 (SEQ ID NO:148)** is shown below (CDR_L residues are shown underlined):

DILLTQSPAI LSVSPGERVS FSCRASQSIG TSIHWYQHRT NGSPRLLIKY ASESISGIPS RFSGSGSGTD FTLSINSVES EDIADYYCQQ SNSWLTFGAG TKLELK

CDR_L1 of **PD-1 mAb 11 (SEQ ID NO:149)**: RASQSIGTSIH

CDR_L2 of **PD-1 mAb 11 (SEQ ID NO:150)**: YASESIS

CDR_L3 of **PD-1 mAb 11 (SEQ ID NO:151)**: QQSNSWLT

(l) PD-1 mAb 12

[00230] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 12 (SEQ ID NO:152)** is shown below (CDR_H residues are shown underlined).

QGHLQQSGAE LVRPGASVTL SCKASGFTFT DYEMHWVKQT PVHGLEWIGT IDPETGGTAY NQKFKGKAIL TVDKSSTTTY MELRSLTSED SAVFYCSRER ITTVVEGAYW YFDVWGTGTT VTVSS

CDR_H1 of **PD-1 mAb 12 (SEQ ID NO:153)**: DYEMH

CDR_H2 of **PD-1 mAb 12 (SEQ ID NO:154)**: TIDPETGGTAYNQKFKG

CDR_H3 of **PD-1 mAb 12 (SEQ ID NO:155)**: ERITTVVEGAYWYFDV

[00231] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 12 (SEQ ID NO:156)** is shown below (CDR_L residues are shown underlined):

DVLMTQTPLS LPVSLGDQAS ISCRSSQNIV HSNGNTYLEW YLQKPGQSPK LLICKVSTRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP YTFGGGTKLE IK

CDR_L1 of **PD-1 mAb 12 (SEQ ID NO:157)**: RSSQNIVHSNGNTYLE

CDR_L2 of **PD-1 mAb 12 (SEQ ID NO:158)**: KVSTRFS

CDR_L3 of **PD-1 mAb 12 (SEQ ID NO:159)**: FQGSHVPYT

(m) PD-1 mAb 13

[00232] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 13 (SEQ ID NO:160)** is shown below (CDR_H residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFTFS **SHTMS**WVRQT PEKRLEWVA**T**
ISGGGSNIYY PDSVKGRFTI SRDNAKNTLY LQMSSLRSED TALYYCAR**QA**
YYGNYWYFDV WGTGTTVTVS S

CDR_H1 of **PD-1 mAb 13 (SEQ ID NO:161)**: **SHTMS**

CDR_H2 of **PD-1 mAb 13 (SEQ ID NO:162)**: **TISGGGSNIYYPDSVKG**

CDR_H3 of **PD-1 mAb 13 (SEQ ID NO:163)**: **QAYYGNYWYFDV**

[00233] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 13 (SEQ ID NO:164)** is shown below (CDR_L residues are shown underlined):

DIQMTQSPAT QSASLGESVT ITC**LASQTIG TWLA**WYQQKP GKSPQLLIY**A**
ATSLADGVPS RFSGSGSGTK FSFKISSLQA EDFVSYYC**QQ LDSIPWT**FGG
GTKLEIK

CDR_L1 of **PD-1 mAb 13 (SEQ ID NO:165)**: **LASQTIGTWLA**

CDR_L2 of **PD-1 mAb 13 (SEQ ID NO:166)**: **AATSLAD**

CDR_L3 of **PD-1 mAb 13 (SEQ ID NO:167)**: **QQLDSIPWT**

(n) PD-1 mAb 14

[00234] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 14 (SEQ ID NO:168)** is shown below (CDR_H residues are shown underlined).

QVQLQQPGAE LVKPGASVKM SCKASGYNFI **SYWIT**WVKQR PGQGLQWIG**N**
IYPGTDGTTY NEKFKSKATL TVDTSSSTAY MHLSRLTSED SAVYYCAT**GL**
HWYFDVWGTG TTVTVSS

CDR_H1 of **PD-1 mAb 14 (SEQ ID NO:169)**: **SYWIT**

CDR_H2 of **PD-1 mAb 14 (SEQ ID NO:170)**: **NIYPGTDGTTYNEKFKS**

CDR_H3 of **PD-1 mAb 14 (SEQ ID NO:171)**: **GLHWYFDV**

[00235] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 14 (SEQ ID NO:172)** is shown below (CDR_L residues are shown underlined):

DIVMTQSQKF MSTSVGDRVS VTC**KASQSVG TNVA**WYQQKP GQSPKALIY**S**
ASSRFSGVPD RFTGSGSGTD FTLTISNVQS EDLAEYFC**QQ YNSYPYT**FGG
GTKLEIK

CDR$_L$1 of **PD-1 mAb 14 (SEQ ID NO:173)**: KASQSVGTNVA

CDR$_L$2 of **PD-1 mAb 14 (SEQ ID NO:174)**: SASSRFS

CDR$_L$3 of **PD-1 mAb 14 (SEQ ID NO:175)**: QQYNSYPYT

(o) PD-1 mAb 15

[00236] The amino acid sequence of the VH Domain of murine anti-human **PD-1 mAb 15 (SEQ ID NO:176)** is shown below (CDR$_H$ residues are shown underlined).

EVMLVESGGG LVKPGGSLKL SCAASGFIFS **SYLIS**WVRQT PEKRLEWVA**A**
ISGGGADTYY ADSVKGRFTI SRDNAKNTLY LQMSSLRSED TALYYCTR**RG**
TYAMDYWGQG TSVTVSS

CDR$_H$1 of **PD-1 mAb 15 (SEQ ID NO:177)**: SYLIS

CDR$_H$2 of **PD-1 mAb 15 (SEQ ID NO:178)**: AISGGGADTYYADSVKG

CDR$_H$3 of **PD-1 mAb 15 (SEQ ID NO:179)**: RGTYAMDY

[00237] The amino acid sequence of the VL Domain of murine anti-human **PD-1 mAb 15 (SEQ ID NO:180)** is shown below (CDR$_L$ residues are shown underlined):

DIQMTQSPAS QSASLGESVT ITC**LASQTIG TWLA**WYQQKP GKSPQLLIY**A**
ATSLADGVPS RFSGSGSGTK FSFKISSLQA EDFVNYYC**QQ LYSIPWT**FGG
GTKLEIK

CDR$_L$1 of **PD-1 mAb 15 (SEQ ID NO:181)**: LASQTIGTWLA

CDR$_L$2 of **PD-1 mAb 15 (SEQ ID NO:182)**: AATSLAD

CDR$_L$3 of **PD-1 mAb 15 (SEQ ID NO:183)**: QQLYSIPWT

[00238] The above-described murine anti-human PD-1 antibody **PD-1 mAb 15** was humanized and further deimmunized when antigenic epitopes were identified in order to demonstrate the capability of humanizing an anti-human PD-1 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded one humanized VH Domain, designated herein as "**hPD-1 mAb 15 VH1**," and one humanized VL Domain designated herein as "**hPD-1 mAb 15 VL1**." An antibody comprising the humanized VL Domain paired with the humanized VH Domain is referred to as "**hPD-1 mAb 15**."

[00239] The amino acid sequence of the VH Domain of **hPD-1 mAb 15 VH1 (SEQ ID NO:184)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVRPGGSLRL SCAASGFTFS **SYLIS**WVRQA PGKGLEWVA**A**
ISGGGADTYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TATYYCAR**RG**
TYAMDYWGQG TLVTVSS

[00240] The amino acid sequence of the VL Domain of **hPD-1 mAb 15 VL1 (SEQ ID NO:185)** is shown below (CDR_H residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**LASQTIG TWLA**WYQQKP GKAPKLLIY**A**
ATSLADGVPS RFSGSGSGTD FTFTISSLQP EDFATYYC**QQ LYSIPWT**FGQ
GTKLEIK

(p) Additional Anti-PD-1 Antibodies

[00241] Alternative anti-PD-1 antibodies useful in the generation of molecules capable of binding PD-1 or a natural ligand of PD-1 possess the VL and/or VH Domains of the anti-human PD-1 monoclonal antibody **nivolumab** (CAS Reg. No.:946414-94-4, also known as 5C4, BMS-936558, ONO-4538, MDX-1106, and marketed as OPDIVO® by Bristol-Myers Squibb); **pembrolizumab** (formerly known as lambrolizumab), CAS Reg. No.:1374853-91-4, also known as MK-3475, SCH-900475, and marketed as KEYTRUDA® by Merck); **EH12.2H7** (Dana Farber); **pidilizumab** (CAS Reg. No.: 1036730-42-3 also known as CT-011, CureTech,), or any of the anti-PD-1 antibodies in **Table 8**; and more preferably possess 1, 2 or all 3 of the CDR_Ls of the VL Region and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of such anti-PD-1 monoclonal antibodies. The amino acid sequences of the complete Heavy and Light Chains of nivolumab (WHO Drug Information, 2013, Recommended INN: List 69, 27(1):68-69), pembrolizumab (WHO Drug Information, 2014, Recommended INN: List 75, 28(3):407) and pidilizumab (WHO Drug Information, 2013, Recommended INN: List 70, 27(3):303-304) are known in the art. Additional anti-PD-1 antibodies possessing unique binding characteristics useful in the methods and compositions of the instant inventions have recently been identified (see, United States Patent Application Nos. 62/198,867; 62/239,559; 62/255,140).

Table 8: Additional Anti-PD-1 Antibodies	
PD-1 Antibodies	**Reference / Source**
PD1-17; PD1-28; PD1-33; PD1-35; and PD1-F2	US Patents No. 7,488,802; 7,521,051 and 8,088,905; PCT Patent Publication WO 2004/056875
17D8; 2D3; 4H1; 5C4; 4A11; 7D3; and 5F4	US Patents No. 8,008,449; 8,779,105 and 9,084,776; PCT Patent Publication WO 2006/121168
hPD-1.08A; hPD-1.09A; 109A; K09A; 409A; h409A11; h409A16; h409A17; Codon optimized 109A; and Codon optimized 409A	US Patents No. 8,354,509; 8,900,587 and 5,952,136; PCT Patent Publication WO 2008/156712
1E3; 1E8; and 1H3	US Patent Publication 2014/0044738; PCT Patent Publication WO 2012/145493
9A2; 10B11; 6E9; APE1922; APE1923; APE1924; APE1950; APE1963; and APE2058	PCT Patent Publication WO 2014/179664
GA1; GA2; GB1; GB6; GH1; A2; C7; H7; SH-A4; SH-A9; RG1H10; RG1H11; RG2H7; RG2H10; RG3E12; RG4A6; RG5D9; RG1H10-H2A-22-1S; RG1H10-H2A-27-2S; RG1H10-3C; RG1H10-16C; RG1H10-17C; RG1H10-19C; RG1H10-21C; and RG1H10-23C2	US Patent Publication 2014/0356363; PCT Patent Publication WO 2014/194302
H1M7789N; H1M7799N; H1M7800N; H2M7780N; H2M7788N; H2M7790N; H2M7791N; H2M7794N; H2M7795N; H2M7796N; H2M7798N; H4H9019P; H4xH9034P2; H4xH9035P2; H4xH9037P2; H4xH9045P2; H4xH9048P2; H4H9057P2; H4H9068P2; H4xH9119P2; H4xH9120P2; H4Xh9128p2; H4Xh9135p2; H4Xh9145p2; H4Xh8992p; H4Xh8999p; and H4Xh9008p;	US Patent Publication 2015/0203579; PCT Patent Publication WO 2015/112800
PD-1 mAb 1; PD-1 mAb 2; hPD-1 mAb 2; PD-1 mAb 3; PD-1 mAb 4; PD-1 mAb 5; PD-1 mAb 6; PD-1 mAb 7; hPD-1 mAb 7; PD-1 mAb 8; PD-1 mAb 9; hPD-1 mAb 9; PD-1 mAb 10; PD-1 mAb 11; PD-1 mAb 12; PD-1 mAb 13; PD-1 mAb 14; PD-1 mAb 15; and hPD-1 mAb 15	US Patent Applications No. 62/198,867 and 62/239,559

(q) Exemplary IgG4 PD-1 Antibodies

[00242] In certain embodiments anti-PD-1 antibodies useful in the methods and compositions of the instant inventions comprise the VL and VH Domains of any of the antibodies provided above (*e.g.*, PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, *etc.*, or any of the anti-PD-1

antibodies in **Table 6**), a kappa CL Domain (**SEQ ID NO:12**), and an IgG4 Fc Domain, optionally lacking the C-terminal lysine residue. Such antibodies will preferably comprise an IgG4 CH1 Domain (**SEQ ID NO:3**) and a Hinge Domain, and more preferably comprise a stabilized IgG4 Hinge comprising an S228P substitution (wherein the numbering is according to the EU index as in Kabat, **SEQ ID NO:7**), and IgG4 CH2-CH3 Domains (**SEQ ID NO:7**).

[00243] An exemplary anti-PD-1 antibody designated "**hPD-1 mAb 7 (1.2) IgG4 (P)**" is a humanized anti-human PD-1 antibody. As indicated above, **hPD-1 mAb 7(1.2)** comprises the VH Domain of **hPD-1 mAb 7 VH1** and the VL Domain of antibody **hPD-1 mAb 7 VL2**.

[00244] The amino acid sequence of the complete Heavy Chain of **hPD-1 mAb7 (1.2) IgG4 (P)** is **SEQ ID NO:186** (CDR_H residues and the S228P residue are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYSFT **SYWMN**WVRQA PGQGLEWIG**V**
IHPSDSETWL **DQKFKD**RVTI TVDKSTSTAY MELSSLRSED TAVYYCAR**EH**
YGTSPFAYWG QGTLVTVSSA STKGPSVFPL APCSRSTSES TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTKTY
TCNVDHKPSN TKVDKRVESK YGPPCP**P**CPA PEFLGGPSVF LFPPKPKDTL
MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR
VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAKG QPREPQVYTL
PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD
GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLG

[00245] In **SEQ ID NO:186**, residues 1-119 correspond to the VH Domain of **hPD-1 mAb 7 VH1 (SEQ ID NO:106)**, amino acid residues 120-217 correspond to the human IgG4 CH1 Domain is (**SEQ ID NO:3**), amino acid residues 218-229 correspond to the human IgG4 Hinge Domain comprising the S228P substitution (**SEQ ID NO:7**), amino acid residues 230-245 correspond to the human IgG4 CH2-CH3 Domains (**SEQ ID NO:11**, wherein X is absent).

[00246] The amino acid sequence of the complete Light Chain of antibody **hPD-1 mAb7 (1.2) IgG4 (P)** possesses a kappa constant region and is (**SEQ ID NO:187**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[00247] In **SEQ ID NO:187**, amino acid residues 1-111 correspond to the VL Domain of **hPD-1 mAb 7 VL2 (SEQ ID NO:109)**, and amino acid residues 112-218 correspond to the Light Chain kappa constant region (**SEQ ID NO:12**).

[00248] Other exemplary anti-PD-1 antibodies having IgG4 constant regions are **nivolumab**, which is a human antibody, and **pembrolizumab**, which is a humanized antibody. Each comprise a kappa CL Domain, an IgG4 CH1 Domain, a stabilized IgG4 Hinge, and an IgG4 CH2-CH3 Domain as described above.

(r) Exemplary Bispecific Molecules Capable Of Binding PD-1 And LAG-3

[00249] As provided herein, the molecule capable of binding PD-1 or a natural ligand of PD-1 may a bispecific molecule. In certain embodiments, bispecific molecules will preferably comprise the VL and VH Domains of any of the anti-PD-1 antibodies provided above (*e.g.*, PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, *etc.*, or any of the anti-PD-1 antibodies in **Table 6**), and the VL and VH Domains of an antibody that binds an epitope of CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA. Such bispecific molecules may be diabodies, BITEs®, bispecific antibodies, or trivalent binding molecules.

[00250] An exemplary bispecific molecule capable of binding PD-1 and LAG-3 designated "**DART-1**" is a diabody comprising four polypeptide chains. **DART-1** is a bispecific, four chain, Fc Region-containing diabody having two binding sites specific for PD-1, two binding sites specific for LAG-3, a variant IgG4 Fc Region engineered for extended half-life, and cysteine-containing E/K-coil Heterodimer-Promoting Domains (see, *e.g.*, **Figure 3B**). The first and third polypeptide chains of DART-1 comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to LAG-3 (underlined in **SEQ ID NO:274**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of **hPD-1 mAb 7 VH1** (**SEQ ID NO:106**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:29**)); a stabilized IgG4 Hinge region (**SEQ ID NO:7**); a variant IgG4 CH2-CH3 Domain (**SEQ ID NO:11**) further comprising amino acid substitutions M252Y/S254T/T256E and lacking the C-terminal residue); and a C-

terminus. The amino acid sequence of the first and third polypeptide chains of **DART-1** is (**SEQ ID NO:274**):

DIQMTQSPSS LSASVGDRVT ITCRASQDVS SVVAWYQQKP GKAPKLLIYS
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ HYSTPWTFGG
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYSFTSYWMN
WVRQAPGQGL EWIGVIHPSD SETWLDQKFK DRVTITVDKS TSTAYMELSS
LRSEDTAVYY CAREHYGTSP FAYWGQGTLV TVSSGGCGGG EVAACEKEVA
ALEKEVAALE KEVAALEKES KYGPPCPPCP APEFLGGPSV FLFPPKPKDT
LYITREPEVT CVVVDVSQED PEVQFNWYVD GVEVHNAKTK PREEQFNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS SIEKTISKAK GQPREPQVYT
LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE ALHNHYTQKS LSLSLG

[00251] The second and fourth polypeptide chains of **DART-1** comprise, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of **hPD-1 mAb 7 VL2** (**SEQ ID NO:109**); an intervening linker peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:14**)); a VH Domain of a monoclonal antibody capable of binding LAG-3 (underlined in **SEQ ID NO:275**); a cysteine-containing intervening linker peptide (**Linker 2:** GGCGGG (**SEQ ID NO:15**)); a cysteine-containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:30**); and a C-terminus. The amino acid sequence of the second and fourth polypeptide chains of **DART-1** is (**SEQ ID NO:275**):

EIVLTQSPAT LSLSPGERAT LSCRASESVD NYGMSFMNWF QQKPGQPPKL
LIHAASNQGS GVPSRFSGSG SGTDFTLTIS SLEPEDFAVY FCQQSKEVPY
TFGGGTKVEI KGGGSGGGGQ VQLVQSGAEV KKPGASVKVS CKASGYTFTD
YNMDWVRQAP GQGLEWMGDI NPDNGVTIYN QKFEGRVTMT TDTSTSTAYM
ELRSLRSDDT AVYYCAREAD YFYFDYWGQG TTLTVSSGGC GGGKVAACKE
KVAALKEKVA ALKEKVAALK E

[00252] Another exemplary bispecific molecule capable of binding PD-1 and LAG-3 designated "**DART-2**" has the same structure as **DART-1** but incorporates alternative LAG-3 VL and VH Domains.

2. Binding Molecules Immunospecific For Natural Ligands of PD-1

[00253] As discussed above, natural ligands of PD-1, for example, B7-H1 (PD-L1) and B7-DC (PD-L2), have been described (Ohigashi *et al.* (2005) "*Clinical Significance Of Programmed Death-1 Ligand-1 And Programmed Death-1 Ligand-2 Expression In Human Esophageal Cancer*," Clin. Cancer Res. 11:2947-2953; Dong, H. *et al.* (1999) "*B7-H1, A Third Member Of The B7 Family, Co-Stimulates Cell Proliferation And Interleukin-10 Secretion*," Nat. Med. 5:1365-1369; Freeman, G.J. *et al.* (2000) "*Engagement Of The PD-1*

Immunoinhibitory Receptor By A Novel B7 Family Member Leads To Negative Regulation Of Lymphocyte Activation," J. Exp. Med. 192:1027-1034; Tseng, S.Y. *et al.* (2001) "*B7-DC, A New Dendritic Cell Molecule With Potent Costimulatory Properties For T Cells*," J. Exp. Med 193:839-846; Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T Cell Activation*," Nat. Immunol. 2:261-268; Iwai *et al.* (2002) "*Involvement Of PD-L1 On Tumor Cells In The Escape From Host Immune System And Tumor Immunotherapy By PD-L1 Blockade*," Proc. Natl. Acad. Sci. (U.S.A.) 99:12293-12297).

[00254] A representative human B7-H1 (PD-L1) polypeptide (NCBI Sequence NP_001254635.1, including a predicted 18 amino acid signal sequence) has the amino acid sequence (**SEQ ID NO:188**):

MRIFAVFIFM TYWHLLNAPY NKINQRILVV DPVTSEHELT CQAEGYPKAE
VIWTSSDHQV LSGKTTTTNS KREEKLFNVT STLRINTTTN EIFYCTFRRL
DPEENHTAEL VIPELPLAHP PNERTHLVIL GAILLCLGVA LTFIFRLRKG
RMMDVKKCGI QDTNSKKQSD THLEET

[00255] A representative human B7-DC (PD-L2) polypeptide (NCBI Sequence NP_079515.2; including a predicted 18 amino acid signal sequence) has the amino acid sequence (**SEQ ID NO:189**):

MIFLLLMLSL ELQLHQIAAL FTVTVPKELY IIEHGSNVTL ECNFDTGSHV
NLGAITASLQ KVENDTSPHR ERATLLEEQL PLGKASFHIP QVQVRDEGQY
QCIIIYGVAW DYKYLTLKVK ASYRKINTHI LKVPETDEVE LTCQATGYPL
AEVSWPNVSV PANTSHSRTP EGLYQVTSVL RLKPPPGRNF SCVFWNTHVR
ELTLASIDLQ SQMEPRTHPT WLLHIFIPFC IIAFIFIATV IALRKQLCQK
LYSSKDTTKR PVTTTKREVN SAI

[00256] Although B7-H1 and B7-DC share 34% identity of amino acid sequence, their expression has been suggested to be differentially regulated (Youngnak, P. *et al.* (2003) "*Differential Binding Properties Of B7-H1 And B7-DC To Programmed Death*-1," Biochem. Biophys. Res. Commun. 307:672-677; Loke, P. *et al.* (2003) "*PD-L1 And PD-L2 Are Differentially Regulated By Th1 And Th2 Cells*," Proc. Natl. Acad. Sci. (U.S.A.) 100:5336-5341). PD-L1 has been suggested to play a role in tumor immunity by increasing apoptosis of antigen-specific T-cell clones (Dong *et al.* (2002) "*Tumor-Associated B7-H1 Promotes T-Cell Apoptosis: A Potential Mechanism Of Immune Evasion*," Nat Med 8:793-800). It has also been suggested that B7-H1 might be involved in intestinal mucosal inflammation and inhibition of B7-H1 suppresses wasting disease associated with colitis (Kanai *et al.* (2003) "*Blockade Of B7-H1 Suppresses The Development Of Chronic Intestinal Inflammation*," J. Immunol. 171:4156-4163). B7-H1 expression has been

reported in human carcinoma of lung, ovary, and colon and in melanomas (Dong *et al.* (2002) "*Tumor-Associated B7-H1 Promotes T-Cell Apoptosis: A Potential Mechanism Of Immune Evasion*," Nat Med 8:793-800). On the other hand, the function of B7-DC in tumors remains largely unknown (Liu, X. *et al.* (2003) "*B7-DC/PD-L2 Promotes Tumor Immunity By A PD-1-Independent Mechanism*," J. Exp. Med. 197:1721-1730; Radhakrishnan, S. *et al.* (2004) "*Immunotherapeutic Potential Of B7-DC (PD-L2) Cross-Linking Antibody In Conferring Antitumor Immunity*," Cancer Res 64:4965-4972. B7-DC expression on the cancer cells has been shown to promote CD8 T-cell-mediated rejection at both the induction and effector phase of antitumor immunity (Liu, X. *et al.* (2003) "*B7-DC/PD-L2 Promotes Tumor Immunity By A PD-1-Independent Mechanism*," J. Exp. Med. 197:1721-1730).

[00257] Anti-B7-H1 antibodies may be obtained using proteins having the above-provided B7-H1 amino acid sequence as an immunogen. Alternatively, anti-B7-H1 antibodies useful in the generation of molecules capable of binding a natural ligand of PD-1 may possess the VL and/or VH Domains of the anti-human B7-H1 antibody **atezolizumab** (CAS Reg No. 1380723-44-3, also known as MPDL3280A), **durvalumab** (CAS Reg No. 1428935-60-7, also known as MEDI-4736), **avelumab**, MDX1105 (CAS Reg No. 1537032-82-8, also known as BMS-936559), 5H1); (also see, US Patents No. 9,273,135, 9,062,112, 8,981,063, 8,779,108, 8,609,089 and 8,460,927; McDermott, D.F. *et al.* (2016) "*Atezolizumab, an Anti-Programmed Death-Ligand 1 Antibody, in Metastatic Renal Cell Carcinoma: Long-Term Safety, Clinical Activity, and Immune Correlates From a Phase Ia Study*," J. Clin. Oncol. 34(8):833-842; Antonia, S. *et al.* (2016) "*Safety And Antitumour Activity Of Durvalumab Plus Tremelimumab In Non-Small Cell Lung Cancer: A Multicentre, Phase 1b Study*," Lancet Oncol. 17(3):299-308; Boyerinas, B. *et al.* (2015) "*Antibody-Dependent Cellular Cytotoxicity Activity of a Novel Anti-PD-L1 Antibody Avelumab (MSB0010718C) on Human Tumor Cells*," Cancer Immunol Res. 3(10):1148-1157; Katy, K. *et al.* (2014) "*PD-1 And PD-L1 Antibodies For Melanoma*," Hum. Vaccin. Immunother. 10(11):3111-3116; Voena, C. *et al.* (2016) "*Advances In Cancer Immunology And Cancer Immunotherapy*," Discov. Med. 21(114):125-133) and/or of a commercially available antibody (*e.g.*, rabbit anti-human PDL-1 monoclonal, 1:25, clone SP142; Ventana, Tuscon, AZ).

[00258] Exemplary anti-human B7-H1 antibodies that may be used in accordance with the present invention include atezolizumab, durvalumab and avelumab. The amino acid sequences of the complete heavy and Light Chains of atezolizumab (WHO Drug Information, 2015, Recommended INN: List 74, 29(3):387), durvalumab (WHO Drug Information, 2015, Recommended INN: List 74, 29(3):393-394) and avelumab (WHO Drug Information, 2016, Recommended INN: List 74, 30(1):100-101) are known in the art.

[00259] Anti-B7-DC antibodies may likewise be obtained using proteins having the above-provided B7-DC amino acid sequence as an immunogen. Alternatively, previously described anti-B7-DC antibodies (*e.g.*, 2C9, MIH18, *etc.*) or commercially available anti-B7-DC antibodies (*e.g.*, MIH18, Affymetrix eBioscience) may be employed in accordance with the present invention (see, U.S. Patent Publication No. 2015/0299322; Ritprajak, P. *et al.* (2012) "*Antibodies Against B7-DC With Differential Binding Properties Exert Opposite Effects*," Hybridoma (Larchmt). 31(1):40-47; Tsushima, F. *et al.* (2003) "*Preferential Contribution Of B7-H1 To Programmed Death-1-Mediated Regulation Of Hapten-Specific Allergic Inflammatory Responses*," Eur. J. Immunol. 33(10):2773-2782).

[00260] An exemplary anti-human anti-B7-DC antibody that may be used in accordance with the present invention is the commercially available anti-B7-DC antibody **MIH18** (eBioscience, Inc.)

B. Molecules Capable Of Mediating The Redirected Killing Of A Target Cell

[00261] 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) will preferably have two binding affinities. First, such molecules will have the ability to immunospecifically bind an epitope of a cell surface molecule of an effector cell. Second, such molecules will have the ability to immunospecifically bind an epitope of a Disease Antigen (e.g., a Cancer Antigen or a Pathogen-Associated Antigen) that is arrayed on the surface of the target cell. The combined presence of both such binding affinities serves to localize the 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.

1. Exemplary Cell Surface Molecules Of An Effector Cell

[00262] 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.

(a) CD2 Binding Capabilities

[00263] 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**."

[00264] The amino acid sequence of the VH Domain of **CD2 mAb Lo-CD2a** (ATCC Accession No: 11423); **SEQ ID NO:190**) is shown below (CDR_H residues are shown underlined):

EVQLQQSGPE LQRPGASVKL SCKASGYIFT **EYYMY**WVKQR PKQGLELVG**R**
IDPEDGSIDY **VEKFKK**KATL TADTSSNTAY MQLSSLTSED TATYFCAR**GK**
FNYRFAYWGQ GTLVTVSS

[00265] The amino acid sequence of the VL Domain of **CD2 mAb Lo-CD2a** (ATCC Accession No: 11423; **SEQ ID NO:191**) is shown below (CDR_L residues are shown underlined):

DVVLTQTPPT LLATIGQSVS ISC**RSSQSLL** **HSSGNTYLN**W LLQRTGQSPQ
PLIY**LVSKLE** **S**GVPNRFSGS GSGTDFTLKI SGVEAEDLGV YYC**MQFTHYP**
YTFGAGTKLE LK

(b) CD3 Binding Capabilities

[00266] 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 CD3 present on the surface of such effector cell.

Molecules that specifically binds CD3 include the anti-CD3 antibodies "**CD3 mAb 1**" and "**OKT3**." The anti-CD3 antibody **CD3 mAb 1** is capable of binding non-human primates (*e.g.*, cynomolgus monkey).

[00267] The amino acid sequence of the VH Domain of **CD3 mAb 1 (SEQ ID NO:192)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT YYADSVKDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTL VTVSS

[00268] The amino acid sequence of the VL Domain of **CD3 mAb 1 (SEQ ID NO:193)** is shown below (CDR_L residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TC**RSSTGAVT TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWV**F
GGGTKLTVLG

[00269] A preferred variant of such antibody is termed "**CD3 mAb 1 (D65G),**" and comprises a **CD3 mAb 1** VH Domain having a D65G substitution (Kabat position 65, corresponding to residue 68 of **SEQ ID NO:192**) and the VL Domain of **CD3 mAb 1 (SEQ ID NO:193)**. The amino acid sequence of the VH Domain of **CD3 mAb 1 (D65G) (SEQ ID NO:194)** is shown below (CDR_H residues are shown underlined, the substituted position (D65G) is shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTL VTVSS

[00270] Alternatively, an affinity variant of **CD3 mAb 1** may be employed. Variants include a low affinity variant designated "**CD3 mAb 1 Low**" and a variant having a faster off rate designated "**CD3 mAb 1 Fast.**" The amino acid sequences of the VH Domains of each of **CD3 mAb 1 Low** and **CD3 mAb1 Fast** are provided below.

[00271] The amino acid sequence of the VH Domain of anti-human **CD3 mAb 1 Low (SEQ ID NO:195)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVT WFAYWGQGTL VTVSS

[00272] The amino acid sequence of the VH Domain of anti-human **CD3 mAb 1 Fast (SEQ ID NO:196)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR
IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HKNFGNSYVT WFAYWGQGTL VTVSS

[00273] The VL Domain of **CD3 mAb 1 (SEQ ID NO:193)** is common to **CD3 mAb 1 Low** and **CD3 mAb1 Fast** and is provided above.

[00274] Another anti-CD3 antibody that may be utilized is antibody Muromonab-CD3 "**OKT3**" (Xu *et al.* (2000) *"In Vitro Characterization Of Five Humanized OKT3 Effector Function Variant Antibodies,"* Cell. Immunol. 200:16-26); Norman, D.J. (1995) *"Mechanisms Of Action And Overview Of OKT3,"* Ther. Drug Monit. 17(6):615-620; Canafax, D.M. *et al.* (1987) *"Monoclonal Antilymphocyte Antibody (OKT3)* Treatment *Of Acute Renal Allograft Rejection,"* Pharmacotherapy 7(4):121-124; Swinnen, L.J. *et al.* (1993) *"OKT3 Monoclonal Antibodies Induce Interleukin-6 And Interleukin-10: A Possible Cause Of Lymphoproliferative Disorders Associated With Transplantation,"* Curr. Opin. Nephrol. Hypertens. 2(4):670-678).

[00275] The amino acid sequence of the VH Domain of **OKT3 (SEQ ID NO:197)** is shown below (CDR_H residues are shown underlined):

QVQLQQSGAE LARPGASVKM SCKASGYTFT RYTMHWVKQR PGQGLEWIGY
INPSRGYTNY NQKFKDKATL TTDKSSSTAY MQLSSLTSED SAVYYCARYY
DDHYCLDYWG QGTTLTVSS

[00276] The amino acid sequence of the VL Domain of **OKT3 (SEQ ID NO:198)** is shown below (CDR_L residues are shown underlined):

QIVLTQSPAI MSASPGEKVT MTCSASSSVS YMNWYQQKSG TSPKRWIYDT
SKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYCQQW SSNPFTFGSG
TKLEINR

[00277] Additional anti-CD3 antibodies that may be utilized include, but are not limited to, those described in PCT Publication Nos. WO 2008/119566; and WO 2005/118635.

(c) CD8 Binding Capabilities

[00278] 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**."

(i) OKT8

[00279] The amino acid sequence of the VH Domain of **OKT8 (SEQ ID NO:199)** is shown below (CDR_H residues are shown underlined):

QVQLLESGPE LLKPGASVKM SCKA**SGYTFT** **DYNMH**WVKQS HGKSLEWIG**Y**
IYPYTGGTGY **NQKFKN**KATL TVDSSSSTAY MELRSLTSED SAVYYCARNF
RYTYWYFDVW GQGTTVTVSS

[00280] The amino acid sequence of the VL Domain of **OKT8 (SEQ ID NO:200)** is shown below (CDR_L residues are shown underlined):

DIVMTQSPAS LAVSLGQRAT ISCRASESVD **SYDNSLMH**WY QQKPGQPPKV
LIY**LASNLES** GVPARFSGSG SRTDFTLTID PVEADDAATY YC**QQNNEDPY**
TFGGGTKLEI KR

(ii) TRX2

[00281] The amino acid sequence of the VH Domain of **TRX2 (SEQ ID NO:201)** is shown below (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS **DFGMN**WVRQA PGKGLEWVA**L**
IYYDGSNKFY **ADSVKG**RFTI SRDNSKNTLY LQMNSLRAED TAVYYCAK**PH**
YDGYYHFFDS WGQGTLVTVS S

[00282] The amino acid sequence of the VL Domain of **TRX2 (SEQ ID NO:202)** is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**KGSQDIN** **NYLA**WYQQKP GKAPKLLIY**N**
TDILHTGVPS RFSGSGSGTD FTFTISSLQP EDIATYYC**YQ** **YNNGYT**FGQG
TKVEIK

(d) CD16 Binding Capabilities

[00283] 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 CD16 present on the surface of such effector cell. Molecules that specifically bind CD16 include the anti-CD16 antibodies "**3G8**" and "**A9**." Humanized A9 antibodies are described in PCT Publication WO 03/101485.

(i) 3G8

[00284] The amino acid sequence of the VH Domain of **3G8 (SEQ ID NO:203)** is shown below (CDR_H residues are shown underlined):

QVTLKESGPG ILQPSQTLSL TCSFSGFSLR **TSGMGVG**WIR QPSGKGLEWL
A**HIWWDDDKR** **YNPALKS**RLT ISKDTSSNQV FLKIASVDTA DTATYYCAQ**I**
NPAWFAYWGQ GTLVTVSA

[00285] The amino acid sequence of the VL Domain of **3G8 (SEQ ID NO:204)** is shown below (CDR_L residues are shown underlined):

DTVLTQSPAS LAVSLGQRAT ISC**KASQSVD** **FDGDSFMN**WY QQKPGQPPKL
LIY**TTSNLES** GIPARFSASG SGTDFTLNIH PVEEEDTATY YC**QQSNEDPY**
TFGGGTKLEI K

(ii) A9

[00286] The amino acid sequence of the VH Domain of **A9 (SEQ ID NO:205)** is shown below (CDR_H residues are shown underlined):

QVQLQQSGAE LVRPGTSVKI SCKASGYTFT **NYWLG**WVKQR PGHGLEWIG**D**
IYPGGGYTNY **NEKFKG**KATV TADTSSRTAY VQVRSLTSED SAVYFCAR**SA**
SWYFDVWGAR TTVTVSS

[00287] The amino acid sequence of the VL Domain of **A9 (SEQ ID NO:206)** is shown below (CDR_L residues are shown underlined):

DIQAVVTQES ALTTSPGETV TLTC**RSNTGT** **VTTSNYAN**WV QEKPDHLFTG
LIG**HTNNRAP** GVPARFSGSL IGDKAALTIT GAQTEDEAIY FC**ALWYNNHW**
VFGGGTKLTVL

[00288] Additional anti-CD19 antibodies that may be utilized include but are not limited to those described in PCT Publication Nos. WO 03/101485; and WO 2006/125668.

(e) TCR Binding Capabilities

[00289] 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 TCR present on the surface of such effector cell.

[00290] Molecules that specifically bind the T Cell Receptor include the anti-TCR antibody "**BMA 031**" (EP 0403156; Kurrle, R. *et al.* (1989) "*BMA 031 – A TCR-Specific Monoclonal Antibody For Clinical Application*," Transplant Proc. 21(1 Pt 1):1017-1019; Nashan, B. *et al.* (1987) "*Fine Specificity Of A Panel Of Antibodies Against The TCR/CD3 Complex*," Transplant Proc. 19(5):4270-4272; Shearman, C.W. *et al.* (1991) "*Construction,*

Expression, And Biologic Activity Of Murine/Human Chimeric Antibodies With Specificity For The Human α/β T Cell," J. Immunol. 146(3):928-935; Shearman, C.W. *et al.* (1991) "*Construction, Expression And Characterization of Humanized Antibodies Directed Against The Human α/β T Cell Receptor*," J. Immunol. 147(12):4366-4373).

[00291] The amino acid sequence of a VH Domain of **BMA 031** (**SEQ ID NO:207**) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYKFT **SYVMH**WVRQA PGQGLEWIG**Y**
INPYNDVTKY NEKFKGRVTI TADKSTSTAY LQMNSLRSED TAVHYCAR**GS**
YYDYDGFVYW GQGTLVTVSS

[00292] The amino acid sequence of the VL Domain of **BMA 031 (SEQ ID NO:208)** is shown below (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**SATSSVS YMH**WYQQKPG KAPKRWIY**DT**
SKLASGVPSR FSGSGSGTEF TLTISSLQPE DFATYYC**QQW SSNPLT**FGQG
TKLEIK

(f) NKG2D Binding Capabilities

[00293] 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 the NKG2D receptor present on the surface of such effector cell. Molecules that specifically bind the NKG2D receptor include the anti-NKG2D antibodies "**KYK-1.0**" and "**KYK-2.0**" (Kwong, KY *et al.* (2008) "*Generation, Affinity Maturation, And Characterization Of A Human Anti-Human NKG2D Monoclonal Antibody With Dual Antagonistic And Agonistic Activity*," J. Mol. Biol. 384:1143-1156; and PCT/US09/54911).

(i) KYK-1.0

[00294] The amino acid sequence of the VH Domain of **KYK-1.0 (SEQ ID NO:209)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG VVQPGGSLRL SCAASGFTFS **SYGMH**WVRQA PGKGLEWVA**F**
IRYDGSNKYY ADSVKGRFTI SRDNSKNTKY LQMNSLRAED TAVYYCAK**DR**
FGYYLDYWGQ GTLVTVSS

[00295] The amino acid sequence of the VL Domain of **KYK-1.0 (SEQ ID NO:210)** is shown below (CDR_L residues are shown underlined):

QPVLTQPSSV SVAPGETARI PC**GGDDIETK SVH**WYQQKPG QAPVLVIY**DD**
DDRPSGIPER FFGSNSGNTA TLSISRVEAG DEADYYC**QVW DDNNDEWV**FG
GGTQLTVL

(ii) KYK-2.0

[00296] The amino acid sequence of a VH Domain of **KYK-2.0** (**SEQ ID NO:211**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYGMH**WVRQA PGKGLEWVA**F**
IRYDGSNKYY **ADSVKG**RFTI SRDNSKNTLY LQMNSLRAED TAVYYCAK**DR**
GLGDGTYFDY WGQGTTVTVS S

[00297] The amino acid sequence of a VL Domain of **KYK-2.0** (**SEQ ID NO:212**) is shown below (CDR_L residues are shown underlined):

QSALTQPASV SGSPGQSITI SC**SGSSSNIG** **NNAVN**WYQQL PGKAPKLLIY
YDDLLPSGVS DRFSGSKSGT SAFLAISGLQ SEDEADYYC**A** **AWDDSLNGPV**
FGGGTKLTVL

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

[00298] 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**; **A33** (a colorectal carcinoma antigen; Almqvist, Y. (2006) "*In vitro and in vivo Characterization of 177Lu-huA33: A Radioimmunoconjugate Against Colorectal Cancer*," Nucl. Med. Biol. 33(8):991-998); **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-A 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-Idiotype 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**; **$D_1$56-22**; **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); **E_1 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** (G_{D2}; Saleh *et al.* (1993) *"Generation Of A Human Anti-Idiotypic Antibody That Mimics The GD2 Antigen,"* J. Immunol., 151, 3390-3398); **ganglioside GD3** (G_{D3}; Shitara *et al.* (1993) *"A Mouse/Human Chimeric Anti-(Ganglioside GD3) Antibody With Enhanced Antitumor Activities,"* Cancer Immunol. Immunother. 36:373-380); **ganglioside GM2** (G_{M2}; 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** (G_{M3}; 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) *"Idiotype Vaccines Against Human T Cell Leukemia. II. Generation And Characterization Of A Monoclonal Idiotype 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); **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;

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17:359-363); **PEMA (polymorphic epithelial mucin antigen)**; **PIPA** (United States Patent No. 7,405,061; PCT Publication No. WO 04/043239); **PSA** (prostate-specific antigen; Henttu *et al.* (1989) *"cDNA Coding For The Entire Human Prostate Specific Antigen Shows High Homologies To The Human Tissue Kallikrein Genes,"* Biochem. Biophys. Res. Comm. 10(2):903-910; Israeli *et al.* (1993) *"Molecular Cloning Of A Complementary DNA Encoding A Prostate-Specific Membrane Antigen,"* Cancer Res. 53:227-230; Cracco, C.M. *et al.* (2005) "*Immune Response In Prostate Cancer*," Minerva Urol. Nefrol. 57(4):301-311); **PSMA** (prostate-specific membrane antigen; Ragupathi, G. (2005) "*Antibody Inducing Polyvalent Cancer Vaccines*," Cancer Treat. Res. 123:157-180); **prostatic acid 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); **R_{24}** 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_5A_7** 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-ß 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 10**. 5T4, B7-H3, CEACAM5/CEACAM6, CD123, DR5, EGFR, an Ephrin receptor, gpA33,HER2/neu, IL13Rα2, ROR1, and VEGF are particularly preferred "**Cancer Antigens**" of the present invention.

Table 10
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

Table 10
Antibody and Antibody-Based Molecules

Antibody Name	Cancer Antigens	Therapeutic Target Application
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
Cc49	Tag-72	Tumor Detection
Cetuximab	EGFR	Metastatic Colorectal Cancer and Head and Neck Cancer
Ch.14.18	Undetermined	Neuroblastoma
Citatuzumab	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

Table 10
Antibody and Antibody-Based Molecules

Antibody Name	Cancer Antigens	Therapeutic Target Application
Ertumaxomab	HER2/Neu, CD3	Breast Cancer
Etaracizumab	Integrin $A_V\beta_3$	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
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-1β	Diabetes
Girentuximab	Carbonic Anhydrase 9 (CA-IX)	Clear Cell Renal Cell Carcinoma
Glembatumumab Vedotin	GPNMB	Melanoma, Breast Cancer
Golimumab	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	Cldn18.2	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

Table 10
Antibody and Antibody-Based Molecules

Antibody Name	Cancer Antigens	Therapeutic Target Application
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
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

Table 10
Antibody and Antibody-Based Molecules

Antibody Name	Cancer Antigens	Therapeutic Target Application
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
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	*E. Coli* 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

Table 10
Antibody and Antibody-Based Molecules

Antibody Name	Cancer Antigens	Therapeutic Target Application
Seribantumab	ERBB3	Cancer
Setoxaximab	*E. Coli* 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
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

Table 10 Antibody and Antibody-Based Molecules		
Antibody Name	**Cancer Antigens**	**Therapeutic Target Application**
Ziralimumab	CD147	Cancer

D. Exemplary Antibodies Capable Of Binding A Cancer Antigen

[00299] Exemplary antibodies, whose VH and VL Domains 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 listed in **Table 10** 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. Antibodies that Bind B7-H3

[00300] **B7-H3** is a Cancer Antigen that is over-expressed 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 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).

[00301] 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-κB (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).

[00302] Preferred B7-H3-binding molecules possess the VL and/or VH Domains, of the anti-human B7-H3 monoclonal antibody "**B7-H3 mAb 1**," "**B7-H3 mAb 2**," or "**B7-H3 mAb 3**," or any of the anti-B7-H3 antibodies provided herein; and more preferably possess 1, 2 or all 3 of the CDR_{L}s of the VL Region and/or 1, 2 or all 3 of the CDR_{H}s of the VH Domain of such anti-B7-H3 monoclonal antibodies. 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).

[00303] The present invention specifically includes and encompasses B7-H3 x CD3 bispecific binding molecules that are capable of binding to B7-H3 and to CD3, and particularly such bispecific binding molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDR_{L}s of the VL Region and/or 1, 2 or all 3 of the CDR_{H}s of the VH Domain of any of anti-B7-H3 monoclonal antibodies **B7-H3 mAb 1**, **B7-H3 mAb 2**, or **B7-H3 mAb 3**, or of any of the B7-H3 x CD3 bispecific binding molecules provided herein, or of any of the B7-H3 x CD3 bispecific binding molecules provided in WO 2017/030926.

(a) B7-H3 mAb 1

[00304] The amino acid sequence of the VH Domain of **B7-H3 mAb 1** (**SEQ ID NO:213**) is shown below (CDR_{H} residues are shown underlined).

QVQLQQSGAE LARPGASVKL SCKASGYTFT **SYWMQ**WVKQR PGQGLEWIG**T**
IYPGDGDTRY **TQKFKG**KATL TADKSSSTAY MQLSSLASED SAVYYCAR**RG**
IPRLWYFDVW GAGTTVTVSS

[00305] The amino acid sequence of the VL Domain of **B7-H3 mAb 1** (**SEQ ID NO:214**) is shown below (CDR_{L} residues are shown underlined).

DIQMTQTTSS LSASLGDRVT ISC**RASQDIS** **NYLN**WYQQKP DGTVKLLIY**Y**
TSRLHSGVPS RFSGSGSGTD YSLTIDNLEQ EDIATYFC**QQ** **GNTLPPT**FGG
GTKLEIK

[00306] Two exemplary humanized VH Domains of **B7-H3 mAb 1**designated herein as "**hB7-H3 mAb 1 VH1**," and "**hB7-H3 mAb 1 VH2**," and two exemplary humanized VL Domains of **B7-H3 mAb 1** designated herein as "**hB7-H3 mAb 1 VL1**," and "**hB7-H3 mAb 1 VL2**," are provided below. It will be noted that **hB7-H3 mAb 1 VL2** includes amino acid substitutions in CDR_L1 and CDR_L2, and that **hB7-H3 mAb 1 VH2** includes amino acid substitutions in CDR_H2. Any of the humanized VL Domains may be paired with any of the humanized VH Domains to generate a B7-H3 binding domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hB7-H3 mAb 1**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising **hB7-H3 mAb 1 VH1** and **hB7-H3 mAb 1 VL2** is specifically referred to as "**hB7-H3 mAb 1 (1.2)**."

[00307] The amino acid sequence of the VH Domain of **hB7-H3 mAb 1 VH1** is **(SEQ ID NO:215)** (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYWMQ**WVRQA PGQGLEWMG**T**
IYPGDGDTRY **TQKFKG**RVTI TADKSTSTAY MELSSLRSED TAVYYCAR**RG**
IPRLWYFDVW GQGTTVTVSS

[00308] The amino acid sequence of the VH Domain of **hB7-H3 mAb 1 VH2** is **(SEQ ID NO:216)** (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYWMQ**WVRQA PGQGLEWMG**T**
IYPGGGDTRY **TQKFQG**RVTI TADKSTSTAY MELSSLRSED TAVYYCAR**RG**
IPRLWYFDVW GQGTTVTVSS

[00309] The amino acid sequence of the VL Domain of **hB7-H3 mAb 1 VL1 (SEQ ID NO:217)** is shown below (CDR_L residues are shown underlined).

DIQMTQSPSS LSASVGDRVT ITC**RASQDIS** **NYLN**WYQQKP GKAPKLLIY**Y**
TSRLHSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYC**QQ** **GNTLPPT**FGG
GTKLEIK

[00310] The amino acid sequence of the VL Domain of **hB7-H3 mAb 1 VL2 (SEQ ID NO:218)** is shown below (CDR_L residues are shown underlined).

DIQMTQSPSS LSASVGDRVT ITC**RASQSIS** **SYLN**WYQQKP GKAPKLLIY**Y**
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYC**QQ** **GNTLPPT**FGG
GTKLEIK

(b) B7-H3 mAb 2

[00311] The amino acid sequence of the VH Domain of **B7-H3 mAb 2 (SEQ ID NO:219)** is shown below (CDR_H residues are shown underlined).

DVQLVESGGG LVQPGGSRKL SCAASGFTFS **SFGMH**WVRQA PEKGLEWVA**Y**
ISSDSSAIYY ADTVKGRFTI SRDNPKNTLF LQMTSLRSED TAMYYCGR**GR**
ENIYYGSRLD YWGQGTTLTV SS

[00312] The amino acid sequence of the VL Domain of **B7-H3 mAb 2 (SEQ ID NO:220)** is shown below (CDR_L residues are shown underlined).

DIAMTQSQKF MSTSVGDRVS VTC**KASQNVD TNVA**WYQQKP GQSPKALIY**S**
ASYRYSGVPD RFTGSGSGTD FTLTINNVQS EDLAEYFC**QQ YNNYPFT**FGS
GTKLEIK

[00313] Four exemplary humanized VH Domains of **B7-H3 mAb 2**, designated herein as "**hB7-H3 mAb 2 VH1**," "**hB7-H3 mAb 2 VH2**," "**hB7-H3 mAb 2 VH3**," and "**hB7-H3 mAb 2 VH4**," and six exemplary humanized VL Domains of **B7-H3 mAb 2**, designated herein as "**hB7-H3 mAb 2 VL1,**" "**hB7-H3 mAb 2 VL2**," "**hB7-H3 mAb 2 VL3**," "**hB7-H3 mAb 2 VL4**," "**hB7-H3 mAb 2 VL5**," and "**hB7-H3 mAb 2 VL6**," and are provided below. Any of the humanized VL Domains may be paired with any of the humanized VH Domains to generate a B7-H3 binding domain. Accordingly, any antibody comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hB7-H3 mAb 2**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising **hB7-H3 mAb 2 VH1** and **hB7-H3 mAb 2 VL2** is specifically referred to as "**hB7-H3 mAb 2 (1.2)**."

[00314] The amino acid sequence of the VH Domain of **hB7-H3 mAb 2 VH1 (SEQ ID NO:221)** is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCAR**GR**
ENIYYGSRLD YWGQGTTVTV SS

[00315] The amino acid sequence of the VH Domain of **hB7-H3 mAb 2 VH2 (SEQ ID NO:222)** is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCGR**GR**
ENIYYGSRLD YWGQGTTVTV SS

[00316] The amino acid sequence of the VH Domain of **hB7-H3 mAb 2 VH3 (SEQ ID NO:223)** is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRDED TAMYYCGR**GR**
ENIYYGSRLD YWGQGTTVTV SS

[00317] The amino acid sequence of the VH Domain of **hB7-H3 mAb 2 VH4 (SEQ ID NO:224)** is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRSED TAVYYCAR**GR**
ENIYYGSRLD YWGQGTTVTV SS

[00318] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL1 (SEQ ID NO:225)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVT ITC**KASQNVD TNVA**WYQQKP GKAPKLLIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YNNYPFT**FGQ
GTKLEIK

[00319] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL2 (SEQ ID NO:226)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVT ITC**KASQNVD TNVA**WYQQKP GKAPKALIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YNNYPFT**FGQ
GTKLEIK

[00320] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL3 (SEQ ID NO:227)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVS VTC**KASQNVD TNVA**WYQQKP GKAPKLLIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YNNYPFT**FGQ
GTKLEIK

[00321] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL4 (SEQ ID NO:228)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVT ITC**KASQNVD TNVA**WYQQKP GQAPKLLIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YNNYPFT**FGQ
GTKLEIK

[00322] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL5 (SEQ ID NO:229)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVT ITC**KASQNVD TNVA**WYQQKP GQAPKALIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YNNYPFT**FGQ
GTKLEIK

[00323] The amino acid sequence of the VL Domain of **hB7-H3 mAb 2 VL6 (SEQ ID NO:230)** is shown below (CDR_L residues are shown underlined).

DIQLTQSPSF LSASVGDRVT ITC**KASQNVD** **TNVA**WYQQKP GKAPKLLIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYYC**QQ** **YNNYPFT**FGQ
GTKLEIK

(c) B7-H3 mAb 3

[00324] The amino acid sequence of the VH Domain of **B7-H3 mAb 3 (SEQ ID NO:231)** is shown below (CDR_H residues are shown underlined).

EVQQVESGGD LVKPGGSLKL SCAASGFTFS **SYGMS**WVRQT PDKRLEW**VAT**
INSGGSNTYY **PDSLKG**RFTI SRDNAKNTLY LQMRSLKSED TAMYYCAR**HD**
GGAMDYWGQG TSVTVSS

[00325] The amino acid sequence of the VL Domain of **B7-H3 mAb 3 (SEQ ID NO:232)** is shown below (CDR_L residues are shown underlined).

DIQMTQSPAS LSVSVGETVT ITC**RASESIY** **SYLA**WYQQKQ GKSPQLLVY**N**
TKTLPEGVPS RFSGSGSGTQ FSLKINSLQP EDFGRYYC**QH** **HYGTPPWT**FG
GGTNLEIK

(d) Other Anti-B7-H3 Binding Molecules

[00326] 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 Patents No. 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 Patents No. 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. Antibodies That Bind CEACAM5 and CEACAM6

[00327] 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 Pubmication 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).

[00328] 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 Pubmication 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).

(a) Antibody 16C3

[00329] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / ANTI-CEACAM6 antibody **16C3** (EP 2585476) (**SEQ ID NO:233**) is shown below (CDR_H residues are shown underlined):

QVQLQQSGPE VVRPGVSVKI SCKGSGYTFT **DYAMH**WVKQS HAKSLEWIG**L**
ISTYSGDTKY NQNFKGKATM TVDKSASTAY MELSSLRSED TAVYYCAR**GD**
YSGSRYWFAY WGQGTLVTVS S

[00330] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / ANTI-CEACAM6 antibody **16C3** (EP 2585476) (**SEQ ID NO:234**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**GASENIY GALN**WYQRKP GKSPKLLIW**G**
ASNLADGMPS RFSGSGSGRQ YTLTISSLQP EDVATYY**CQN VLSSPYT**FGG
GTKLEIK

(b) Antibody hMN15

[00331] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) (**SEQ ID NO:235**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCSSSGFALT **DYYMS**WVRQA PGKGLEWLG**F**
IANKANGHTT DYSPSVKGRF TISRDNSKNT LFLQMDSLRP EDTGVYFCAR
DMGIRWNFDV WGQGTPVTVS S

[00332] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) (**SEQ ID NO:236**) is shown below (CDR_L residues are shown underlined):

DIQLTQSPSS LSASVGDRVT MTC**SASSRVS YIH**WYQQKPG KAPKRWIY**GT**
STLASGVPAR FSGSGSGTDF TFTISSLQPE DIATYYC**QQW SYNPPT**FGQG
TKVEIKR

[00333] 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 Region 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. Antibodies That Bind EGFR

[00334] 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 EGRF 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 (Erbitux)*," 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).

(a) Cetuximab

[00335] The amino acid sequence of the VH Domain of the chimeric anti-EGFR antibody **Cetuximab (SEQ ID NO:237)** is shown below (CDR_H residues are shown underlined):

QVQLKQSGPG LVQPSQSLSI TCTVS**GFSLT NYGVH**WVRQS PGKGLEWLG**V**
IWSGGNTDYN TPFTSRLSIN KDNSKSQVFF KMNSLQSNDT AIYYCAR**ALT**
YYDYEFAYWG QGTLVTVSA

[00336] The amino acid sequence of the VL Domain of the chimeric anti-EGFR antibody **Cetuximab (SEQ ID NO:238)** is shown below (CDR_L residues are shown underlined):

DILLTQSPVI LSVSPGERVS FSC**RASQSIG TNIH**WYQQRT NGSPRLLIK**Y**
ASESISGIPS RFSGSGSGTD FTLSINSVES EDIADYYC**QQ NNNWPTT**FGA
GTKLELKR

(b) Panitumumab

[00337] The amino acid sequence of the VH Domain of Panitumumab **(SEQ ID NO:239)** is shown below (CDR_H residues are shown underlined):

QVQLQESGPG LVKPSETLSL TCTVS**GGSVS SGDYY**WTWIR QSPGKGLEWI
G**HIYYSGNTN YNPSLKS**RLT ISIDTSKTQF SLKLSSVTAA DTAIYYCVR**D**
RVTGAFDIWG QGTMVTVSS

[00338] The amino acid sequence of the VL Domain of Panitumumab **(SEQ ID NO:240)** is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**QASQDIS NYLN**WYQQKP GKAPKLLIY**D**
ASNLETGVPS RFSGSGSGTD FTFTISSLQP EDIATYFC**QH FDHLPLA**FGG
GTKVEIKR

[00339] 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 Region 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. Antibodies That Bind EphA2

[00340] 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**."

(a) EphA2 mAb 1

[00341] The amino acid sequence of the VH Domain of **EphA2 mAb 1 (SEQ ID NO:241)** is shown below (CDR_H residues are shown underlined):

QVQLKESGPG LVAPSQSLSI TCTVSGFSLS **RYSVH**WVRQP PGKGLEWLG**M**
IWGGGSTDYN SALKSRLSIS KDNSKSQVFL KMNSLQTDDT AMYYCAR**KHG**
NYYTMDYWGQ GTSVTVSS

[00342] The amino acid sequence of the VL Domain of **EphA2 mAb 1 (SEQ ID NO:242)** is shown below (CDR_L residues are shown underlined):

DIQMTQTTSS LSASLGDRIT ISC**RASQDIS NYLN**WYQQKP DGTVKLLIY**Y**
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFC**QQ GYTLYT**FGGG
TKLEIK

(b) EphA2 mAb 2

[00343] The amino acid sequence of the VH Domain of **EphA2 mAb 2** (**SEQ ID NO:243**) is shown below (CDR_H residues are shown underlined):

QIQLVQSGPE LKKPGETVKI SCKASGFTFT **NYGMN**WVKQA PGKGLKWMG**W**
INTYIGEPTY ADDFKGRFVF SLETSASTAY LQINNLKNED MATYFCAR**EL**
GPYYFDYWGQ GTTLTVSS

[00344] The amino acid sequence of the VL Domain of **EphA2 mAb 2** (**SEQ ID NO:244**) is shown below (CDR_L residues are shown underlined):

DVVMTQTPLS LPVSLGDQAS ISC**RSSQSLV HSSGNTYLH**W YLQKPGQSPK
LLIY**KVSNRF S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFC**SQSTHVP**
TFGSGTKLEI K

(c) EphA2 mAb 3

[00345] The amino acid sequence of the VH Domain of **EphA2 mAb 3** (**SEQ ID NO:245**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG SVKPGGSLKL SCAASGFTFT **DHYMY**WVRQT PEKRLEWVA**T**
ISDGGSFTSY PDSVKGRFTI SRDIAKNNLY LQMSSLKSED TAMYYCTR**DE**
SDRPFPYWGQ GTLVTVSS

[00346] The amino acid sequence of the VL Domain of **EphA2 mAb 3** (**SEQ ID NO:246**) is shown below (CDR_L residues are shown underlined):

DIVLTQSHRS MSTSVGDRVN ITC**KASQDVT TAVA**WYQQKP GQSPKLLIF**W**
ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYC**QQ HYSTPYT**FGG
GTKLEIK

[00347] 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 Region 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. Antibodies That Bind gpA33

[00348] 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**."

[00349] The amino acid sequence of the VH Domain of **gpA33 mAb 1** (**SEQ ID NO:247**) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **GSWMN**WVRQA PGQGLEWIG**R**
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCAR**IY**
GNNVYFDVWG QGTTVTVSS

[00350] The amino acid sequence of the VL Domain of **gpA33 mAb 1** (**SEQ ID NO:248**) is shown below (CDR_L residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITC**SARSSIS FMY**WYQQKPG KAPKLLIY**DT**
SNLASGVPSR FSGSGSGTEF TLTISSLEAE DAATYYC**QQW SSYPLT**FGQG
TKLEIK

[00351] 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 Region 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. Antibodies That Bind HER2/neu

[00352] 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 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 PERJETA™; CAS Reg No 380610-27-5; see for example, WO2001/000245) is a humanized version of antibody 2C4 having IgG1/kappa constant regions.

[00353] 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 Region 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.

(a) Margetuximab

[00354] The amino acid sequence of the VH Domain of Margetuximab is (**SEQ ID NO:249**) (CDR_H residues are shown underlined):

QVQLQQSGPE LVKPGASLKL SCTASGFNIK **DTYIH**WVKQR PEQGLEWIG**R**
IYPTNGYTRY **DPKFQ**DKATI TADTSSNTAY LQVSRLTSED TAVYYCSR**WG**
GDGFYAMDYW GQGASVTVSS

[00355] The amino acid sequence of the VL Domain of Margetuximab is (**SEQ ID NO:250**) (CDR_L residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITC**KASQDVN** **TAVA**WYQQKP GHSPKLLIY**S**
ASFRYTGVPD RFTGSRSGTD FTFTISSVQA EDLAVYYC**QQ** **HYTTPPT**FGG
GTKVEIK

[00356] 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).

(b) Trastuzumab

[00357] The amino acid sequence of the VH Domain of Trastuzumab is (**SEQ ID NO:251**) (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFNIK **DTYIH**WVRQA PGKGLEWVA**R**
IYPTNGYTRY **ADSVKG**RFTI SADTSKNTAY LQMNSLRAED TAVYYCSR**WG**
GDGFYAMDYW GQGTLVTVSS

[00358] The amino acid sequence of the VL Domain of Trastuzumab is (**SEQ ID NO:252**) (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASQDVN** **TAVA**WYQQKP GKAPKLLIY**S**
ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYC**QQ** **HYTTPPT**FGQ
GTKVEIK

(c) Pertuzumab

[00359] The amino acid sequence of the VH Domain of Pertuzumab is (**SEQ ID NO:253**) (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFT **DYTMD**WVRQA PGKGLEWVA**D**
VNPNSGGSIY **NQRFKG**RFTL SVDRSKNTLY LQMNSLRAED TAVYYCAR**NL**
GPSFYFDYWG QGTLVTVSS

[00360] The amino acid sequence of the VL Domain of Pertuzumab is (**SEQ ID NO:254**) (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**KASQDVS** **IGVA**WYQQKP GKAPKLLIY**S**
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ** **YYIYPYT**FGQ
GTKVEIK

(d) Other Anti-HER2/neu Antibodies

[00361] 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 Region 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 No. 8,350,011; 8,858,942; and PCT Patent Publication WO 2008/019290); **F5** and **C1** (US Patents No. 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 Publications 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. Antibodies That Bind VEGF

[00362] 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).

[00363] The amino acid sequence of the VH Domain of **Bevacizumab (SEQ ID NO:255)** is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGYTFT **NYGMN**WVRQA PGKGLEWVG**W**
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCA**KYP**
HYYGSSHWYF DVWGQGTLVT VSS

[00364] The amino acid sequence of the VL Domain of **Bevacizumab (SEQ ID NO:256)** is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**SASQDIS NYLN**WYQQKP GKAPKVLIY**F**
TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QQ YSTVPWT**FGQ
GTKVEIKR

[00365] 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 Region and/or 1, 2 or all 3 of the CDR_Hs of the VH Domain of the anti-VEGF monoclonal antibody Bevacizumab.

8. Antibodies That Bind 5T4

[00366] 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**."

(a) 5T4 mAb 1

[00367] The amino acid sequence of the VH Domain of **5T4 mAb 1 (SEQ ID NO:257)** is shown below (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKAS**GYTFT SFWMH**WVRQA PGQGLEWMG**R**
IDPNRGGTEY NEKAKSRVTM TADKSTSTAY MELSSLRSED TAVYYCAG**GN**
PYYPMDYWGQ GTTVTVSS

[00368] The amino acid sequence of the VL Domain of an exemplary **5T4 mAb 1** (**SEQ ID NO:258**) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASQGIS** **NYLA**WFQQKP GKAPKSLIY**R**
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYC**LQ** **YDDFPWT**FGQ
GTKLEIK

(b) 5T4 mAb 2

[00369] The amino acid sequence of the VH Domain of **5T4 mAb 2** (**SEQ ID NO:259**) is shown below (CDR residues are shown underlined):

QVQLQQPGAE LVKPGASVKM SCKAS**GYTFT** **SYWIT**WVKQR PGQGLEWIG**D**
IYPGSGRANY **NEKFKS**KATL TVDTSSSTAY MQLSSLTSED SAVYNCAR**YG**
PLFTTVVDPN **SYAMDY**WGQG TSVTVSS

[00370] The amino acid sequence of the VL Domain of **5T4 mAb 2** (**SEQ ID NO:260**) is shown below (CDR residues are shown underlined):

DVLMTQTPLS LPVSLGDQAS ISC**RSSQSIV** **YSNGNTYLE**W YLQKPGQSPK
LLIY**KVSNRF** **S**GVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYC**FQGSHVP**
FTFGSGTKLE IK

[00371] 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 Region 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 additional includes and encompasses the exemplary 5T4 x CD3 bispecific binding molecules provided in WO 2015/184203.

[00372] 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 Region 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 Region 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. The present invention additional includes and encompasses the exemplary 5T4 x CD3 x CD8 trispecific molecules provided in WO 2015/184203.

9. Antibodies That Bind IL13Rα2

[00373] Interleukin-13 Receptor α2 (**IL13Rα2**) is overexpressed in a variety of cancers, including glioblastoma, colorectal cancer, cervical cancer, pancreatic cencer, multiple melanoma, osteosarcoma, leukemia, lymphoma, prostate cancer and lung cancer (PCT Pubmication 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-13Ralpha2 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 IL13Rα2 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 IL13Rα2 include "**hu08**" (see, *e.g.*, PCT Publication No. WO 2014/072888).

[00374] The amino acid sequence of the VH Domain of **hu08** (**SEQ ID NO:261**) is shown below (CDR residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAAS**GFTFS RNGMS**WVRQA PGKGLEWVA**T**
VSSGGSYIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR**QG**
TTALATRFFD VWGQGTLVTV SS

[00375] The amino acid sequence of the VL Domain of **hu08** (**SEQ ID NO:262**) is shown below (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**KASQDVG TAVA**WYQQKP GKAPKLLIY**S**
ASYRSTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYSAPWT**FGG
GTKVEIK

[00376] The present application specifically includes and encompasses IL13Rα2 binding molecules (*e.g.*, IL13Rα2 x CD3 bispecific binding molecules) that are capable of binding to IL13Rα2, 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 Region and/or 1, 2 or all 3 of the CDR_{HS} of the VH Domain of the anti- IL13Rα2 monoclonal antibody **hu08**.

10. Antibodies That Bind CD123

[00377] 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) 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).

[00378] 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).

[00379] The amino acid sequence of the VH Domain of **CD123 mAb 1 (SEQ ID NO:263)** is shown below (CDR_H residues are shown underlined):

EVQLVQSGAE LKKPGASVKV SCKASGYTFT **DYYMK**WVRQA PGQGLEWIG**D**
IIPSNGATFY NQKFKGRVTI TVDKSTSTAY MELSSLRSED TAVYYCAR**SH**
LLRASWFAYW GQGTLVTVSS

[00380] The amino acid sequence of the VL Domain of **CD123 mAb 1 (SEQ ID NO:264)** is shown below (CDR_L residues are shown underlined):

DFVMTQSPDS LAVSLGERVT MSC**KSSQSLL NSGNQKNYLT** WYQQKPGQPP
KLLIY**WASTR ES**GVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYC**QNDYSY**
PYTFGQGTKL EIK

[00381] 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 Region 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. Antibodies That Bind CD19

[00382] 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).

[00383] An exemplary antibody that binds to human CD19, and that may be employed in the present invention, is the anti-CD19 antibody diclosed in WO 2016/048938 (referred to herein as "**CD19 mAb 1**").

[00384] The amino acid sequence of the VH Domain of **CD19 mAb 1 (SEQ ID NO:265)** is shown below (CDR_H residues are shown underlined):

QVTLRESGPA LVKPTQTLTL TCTFSGFSLS **TSGMGVG**WIR QPPGKALEWL
A**HIWWDDDKR YNPALKS**RLT ISKDTSKNQV FLTMTNMDPV DTATYYCAR**M**
ELWSYYFDYW GQGTTVTVSS

[00385] The amino acid sequence of the VL Domain of **CD19 mAb 1 (SEQ ID NO:266)** is shown below (CDR_L residues are shown underlined):

ENVLTQSPAT LSVTPGEKAT ITC**RASQSVS YMH**WYQQKPG QAPRLLIY**DA**
SNRASGVPSR FSGSGSGTDH TLTISSLEAE DAATYYC**FQG SVYPFT**FGQG
TKLEIK

[00386] 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 Region 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).

E. Exemplary Pathogen-Associated Antigens

[00387] 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, 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 schenkii, Paracoccidioides brasiliensis, Coccidioides immitis, Histoplasma capsulatum, Leptospirosis, Borrelia burgdorferi*, helminth parasite (hookworm, tapeworms, flukes, flatworms (*e.g. Schistosomia*), *Giardia lambia, 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.

F. Exemplary Antibodies Capable Of Binding A Pathogen-Associated Antigen

[00388] 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.

[00389] 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.

[00390] 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; Electrofusion 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).

[00391] The amino acid sequence of the VH Domain of **7B2** (**SEQ ID NO:267**) is shown below (CDR residues are shown underlined):

QVQLVQSGGG VFKPGGSLRL SCEASGFTFT **EYYMT**WVRQA PGKGLEWLAY
ISKNGEYSKY SPSSNGRFTI SR**DNAKNS**VF LQLDRLSADD TAVYYCAR**AD**
GLTYFSELLQ YIFDLWGQGA RVTVSS

[00392] The amino acid sequence of the VL Domain of **7B2** (**SEQ ID NO:268**) is shown below (CDR residues are shown underlined):

DIVMTQSPDS LAVSPGERAT IHCKSSQTLL YSSNNRHSIA WYQQRPGQPP KLLLYWASMR LSGVPDRFSG SGSGTDFTLT INNLQAEDVA IYYCHQYSSH PPTFGHGTRV EIK

[00393] The amino acid sequence of the VH Domain of **A32** (**SEQ ID NO:269**) is shown below (CDR residues are shown underlined):

QVQLQESGPG LVKPSQTLSL SCTVSGGSSS SGAHYWSWIR QYPGKGLEWI GYIHYSGNTY YNPSLKSRIT ISQHTSENQF SLKLNSVTVA DTAVYYCARG TRLRTLRNAF DIWGQGTLVT VSS

[00394] The amino acid sequence of the VL Domain of **A32** (**SEQ ID NO:270**) is shown below (CDR residues are shown underlined):

QSALTQPPSA SGSPGQSVTI SCTGTSSDVG GYNYVSWYQH HPGKAPKLII SEVNNRPSGV PDRFSGSKSG NTASLTVSGL QAEDEAEYYC SSYTDIHNFV FGGGTKLTVL

[00395] 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 Region 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.

[00396] 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 Region 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 Region 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. The present invention

specifically includes and encompasses the exemplary HIV x CD3 x CD8 trispecific binding molecules provided in WO 2015/184203, WO 2017/011413, and WO 2017/011414.

G. Exemplary Binding Molecules of the Present Invention

[00397] As discussed below, the present invention is illustrated using a combination therapy of two administered molecules: a molecule capable of binding PD-1 (*e.g.*, **hPD-1 mAb7 (1.2) IgG4 (P)**, **DART-1** or **DART-2,** described above), and a molecule capable of mediating the redirected killing of a tumor cell (*e.g.*, "**DART-A**," or "**DART-B**," described below).

[00398] **DART-A** is a bispecific diabody capable of binding the CD3 cell surface molecule of an effector cell and the B7-H3 Cancer Antigen. It is an Fc Region-containing diabody composed of three polypeptide chains having one binding site for B7-H3, one binding site for B7-H3, Knob and Hole bearing IgG1 Fc Regions, and E/K-coil Heterodimer-Promoting Domains (see, *e.g.*, **Figure 4A**).

[00399] The first polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL Domain of a monoclonal antibody capable of binding B7-H3 (**hB7-H3 mAb 2 VL2**) (**SEQ ID NO:226**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:14**)), a VH Domain of a monoclonal antibody capable of binding CD3 (**CD3 mAb 1 VH**) (**SEQ ID NO:192**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:15**)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:27**)), an intervening linker peptide (**Spacer-Linker 3**; GGGDKTHTCPPCP (**SEQ ID NO:39**)), a "**knob-bearing**" Fc Domain (**SEQ ID NO:42**), and a C-terminus. Thus, the first polypeptide chain of **DART-A** is composed of: **SEQ ID NO:226 — SEQ ID NO:14 — SEQ ID NO:192 — SEQ ID NO:15 — SEQ ID NO:27 — SEQ ID NO:39 — SEQ ID NO:42**. The amino acid sequence of the first polypeptide chain of **DART-A** is (**SEQ ID NO:271**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW 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

[00400] The second polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL Domain of a monoclonal antibody capable of binding CD3 (**CD3 mAb 1 VL**) (**SEQ ID NO:193**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:14**)), a VH Domain of a monoclonal antibody capable of binding B7-H3 (**hB7-H3 mAb 2 VH2**) (**SEQ ID NO:222**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:15**)), a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:28**)), and a C-terminus. Thus, the second polypeptide of **DART-A** is composed of: **SEQ ID NO:193 — SEQ ID NO:14 — SEQ ID NO:222 — SEQ ID NO:15 — SEQ ID NO:28**. The amino acid sequence of the second polypeptide chain of **DART-A** is (**SEQ ID NO:272**):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFSSF GMHWVRQAPG KGLEWVAYIS SDSSAIYYAD TVKGRFTISR DNAKNSLYLQ MNSLRDEDTA VYYCGRGREN IYYGSRLDYW GQGTTVTVSS GGCGGGKVAA LKEKVAALKE KVAALKEKVA ALKE

[00401] The third polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a peptide (**Linker 3**; DKTHTCPPCP (**SEQ ID NO:38**)), a "**hole-bearing**" Fc Domain (**SEQ ID NO:43**), and a C-terminus. Thus, the third polypeptide of **DART-A** is composed of: **SEQ ID NO:38 — SEQ ID NO:43**. The amino acid sequence of the third polypeptide of **DART-A** is (**SEQ ID NO:273**):

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

[00402] Another exemplary molecule capable of mediating the redirected killing of a tumor cell is **DART-B. DART-B** is a bispecific diabody capable of binding the CD3 cell surface molecule of an effector cell and the IL13Rα2 Cancer Antigen. **DART-B** is composed of three polypeptide chain and has the same general structure as **DART-A.**

[00403] Additional, exemplary molecules capable of mediating the redirected killing of 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; PCT Publication WO 2015/026892); **gpA33 and CD3** (*e.g.*, WO 2015/026894); **CEA and CD3** (*e.g.*, WO 2013/012414); **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 trispecific molecules (see, *e.g.*, WO 2015/184203; and WO 2015/184207).

VI. Methods of Production

[00404] 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.

[00405] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "S*olid 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).

[00406] 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. Patents No. 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).

[00407] 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.

[00408] 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.

[00409] 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.

[00410] 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 post-translational 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.

[00411] The invention encompasses fusion proteins comprising one or more of the VH and/or VL Domains of an antibody that binds to PD-1 (or a natural ligand of PD-1) or of an antibody that binds to a cell surface molecule of an effector cell or of an antibody that binds to a Disease Antigen (e.g., a Cancer Antigen or a Pathogen-Associated Antigen). 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 PD-1 (or a natural ligand of PD-1) or to a cell surface molecule of an effector cell, 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.

[00412] 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 thebinding molecule or indirectly, through an intermediate (*e.g.,* a linker) using techniques known in the art.

[00413] 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, Diptheria 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.

VII. Uses of the Binding Molecules of the Present Invention

[00414] As discussed above molecules capable of binding PD-1 or a natural ligand of PD-1 and molecules capable of mediating the redirected cell killing of a target cell (*i.e.,* a cancer cell, or a pathogen-infected cell) 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.

[00415] In particular, the present invention encompasses such methods wherein the molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that is capable of binding PD-1 or an epitope-binding domain of an antibody that is capable of binding a natural ligand of PD-1 and wherein the molecule capable of mediating redirected killing comprises an eptiope-binding domain capable of binding a cell surface molecule (*e.g.*, CD2, CD3, CD8, CD16, TCR, NKG2D, *etc.*) of an effector cell (*e.g.*, a helper T Cell, a cytotoxic T Cell, a Natural Killer (NK) cell, a plasma cell (an antibody-secreting B cell), a macrophage and a granulocyte) 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)).

[00416] In a specific embodiment, the molecule capable of binding PD-1 or a natural ligand of PD-1 is an antibody and the molecule capable of mediating redirected cell killing is a diabody. In another specific embodiment, the molecule capable of binding PD-1 or a natural ligand of PD-1 is an antibody and the molecule capable of mediating redirected cell killing is a trivalent binding molecule.

[00417] In a specific embodiment, the molecule capable of binding PD-1 or a natural ligand of PD-1 is a diabody and the molecule capable of mediating redirected cell killing is a diabody. In another specific embodiment, the molecule capable of binding PD-1 or a natural ligand of PD-1 is an diabody and the molecule capable of mediating redirected cell killing is a trivalent binding molecule.

[00418] In one embodiment, the molecule capable of binding PD-1 or a natural ligand of PD-1, and the molecule capable of mediating redirected cell killing are administered concurrently. As used herein, such "concurrent" administration is intended to denote:

(A) the administration of a single pharmaceutical composition that contains both a molecule capable of binding PD-1 or a natural ligand of PD-1, and a molecule capable of mediating redirected cell killing; or

(B) the separate administration of two or more pharmaceutical compositions, one composition of which contains the molecule capable of binding PD-1 or a natural ligand of PD-1, and another composition of which contains a molecule capable of mediating redirected cell killing, wherein the compositions are administered within a 48 hour period.

[00419] In a second embodiment, the molecules are administered "sequentially" (*e.g.*, a molecule capable of binding PD-1 or a natural ligand of PD-1 is administered and, at a later time, a molecule capable of mediating redirected cell killing is administered, or vice versa). In such sequential administration, the second administered composition is administered at least 48 hours, or more after the administration of the first administered composition.

[00420] "**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.

[00421] 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.

[00422] 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 a molecule capable of binding PD-1 or a natural ligand of PD-1 and a molecule capable of mediating the redirected killing of a target cell (*e.g.*, a tumor cell, a pathogen-infected cell or a foreign cell). The combination of such molecules is 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.

[00423] 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: **an adrenal gland cancer**, including but not limited to, a pheochromocytom or an adrenocortical carcinoma; **an AIDS-associated cancer**; **an alveolar soft part sarcoma**; **an astrocytic tumor**; **a basal cancer**; **a bladder cancer**, including but not limited to, a transitional cell carcinoma, a squamous cell cancer, an adenocarcinoma, or a carcinosarcoma; **a bone and connective tissue sarcoma**, such as but not limited to, a bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, or a synovial sarcoma; **a brain cancer**, including, but not limited to, a glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, or a primary brain lymphoma; **a brain and spinal cord cancer**; **a breast cancer**, including, but not limited to, an adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, or an inflammatory breast cancer; **a carotid body tumor**; **a cervical cancer**, including but not limited to, a squamous cell carcinoma, or a adenocarcinoma; **a cholangiocarcinoma**, including but not limited to, a papillary, nodular, or diffuse cholangiocarcinoma; **a chondrosarcoma**; **a chordoma**; **a chromophobe renal cell carcinoma**; **a clear cell carcinoma**; **a colon cancer**; **a colorectal**

cancer; **a cutaneous benign fibrous histiocytoma**; **a desmoplastic small round cell tumor**; **an ependymoma**; **an eye cancer**, including, but not limited to, an ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; **an esophageal cancer**, including but not limited to, a squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and an oat cell (small cell) carcinoma; **a Ewing's tumor**; **an extraskeletal myxoid chondrosarcoma**; **a fibrogenesis imperfecta ossium**; **a fibrous dysplasia of the bone**; **a gallbladder or bile duct cancer**, including but not limited to, an adenocarcinoma; **a gastric cancer**; **a gestational trophoblastic disease**; **a germ cell tumor**; **a head and neck cancer**; **a hepatocellular carcinoma**; **Heavy Chain disease**; **an islet cell tumor**; **a Kaposi's sarcoma**; **a leukemia**, including, but not limited to, an acute leukemia; acute lymphocytic leukemia; an acute myelocytic leukemia, such as, but not limited to, a myeloblastic, promyelocytic, myelomonocytic, monocytic, or erythroleukemia leukemia or a myelodysplastic syndrome; **a chronic leukemia**, such as but not limited to, a chronic myelocytic (granulocytic) leukemia, a chronic lymphocytic leukemia, a hairy cell leukemia; **a lipoma/benign lipomatous tumor**; **a liposarcoma/malignant lipomatous tumor**; **a liver cancer**, including but not limited to, a hepatocellular carcinoma, or a hepatoblastoma; **a lymphoma**, such as but not limited to, Hodgkin's disease; non-Hodgkin's disease; **a lung cancer**, including but not limited to, a non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma or a small-cell lung cancer; **a medulloblastoma**; **a melanoma**; **a meningioma**; **a benign monoclonal gammopathy**; **a monoclonal gammopathy of undetermined significance**; **a multiple endocrine neoplasia**; **a multiple myeloma**, such as but not limited to, a smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; **a myelodysplastic syndrome**; **a neuroblastoma**; **a neuroendocrine tumor**; **an oral cancer**, including but not limited to, a squamous cell carcinoma; **an ovarian cancer**; , including, but not limited to, an ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; **a pancreatic cancer**, including but not limited to, an insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, or a carcinoid or islet cell tumor; **a parathyroid tumor**; **a pediatric cancer**; **a penal cancer**; **a peripheral nerve sheath tumor**; **a phaeochromocytoma**; **a pharynx cancer**, including but not limited to, a squamous cell cancer, or a verrucous cancer; **a pituitary cancer**, including but not limited to, Cushing's

disease, a prolactin-secreting tumor, acromegaly, or a diabetes insipius tumor; **a prostate cancer**, including but not limited to, an adenocarcinoma, leiomyosarcoma, or rhabdomyosarcoma; **polycythemia vera**; **a posterious uveal melanoma**; **a rare hematologic disorder**; **a renal cancer**, including but not limited to, an adenocarcinoma, hypernephroma, fibrosarcoma, a renal metastatic cancer, or a transitional cell cancer (renal pelvis and/ or uterer); **a rhabdoid tumor**; **a rhabdomysarcoma**; **a salivary gland cancer**, including but not limited to, an adenocarcinoma, mucoepidermoid carcinoma, or an adenoidcystic carcinoma; **a sarcoma**; **a skin cancer**, including but not limited to, a basal cell carcinoma, a squamous cell carcinoma and melanoma, a superficial spreading melanoma, a nodular melanoma, a lentigo malignant melanoma, or an acral lentiginous melanoma; **a soft-tissue sarcoma**; **a squamous cell cancer**; **a stomach cancer**, including but not limited to, an adenocarcinoma, a fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, or malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; **a synovial sarcoma**; **a testicular cancer**, including but not limited to, a germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, or a choriocarcinoma (yolk-sac tumor); **a thymic carcinoma**; **a thymoma**; **a thyroid cancer**, such as but not limited to, papillary or follicular thyroid cancer, metastatic thyroid cancer, medullary thyroid cancer or anaplastic thyroid cancer; **a uterine cancer**, including but not limited to, an endometrial carcinoma or a uterine sarcoma; **a vaginal cancer**, including but not limited to, a squamous cell carcinoma, adenocarcinoma, or melanoma; **a vulvar cancer**, including but not limited to, a squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, or Paget's disease; a **Waldenström's macroglobulinemia**, or **Wilms' tumor**. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangio-endotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, see Fishman *et al.*, 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery, Viking Penguin, Penguin Books U.S.A., Inc.).

[00424] In particular, the binding molecules of the present invention may be used in the treatment of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer, acute lymphocytic

leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, testicular cancer, and uterine cancer.

[00425] 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 schenkii, Paracoccidioides brasiliensis, Coccidioides immitis*, *Histoplasma capsulatum*, *Leptospirosis, Borrelia burgdorferi*, helminth parasite (hookworm, tapeworms, flukes, flatworms (*e.g. Schistosomia*), *Giardia lambia*, *trichinella, Dientamoeba Fragilis, Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania donovani*).

VIII. Pharmaceutical Compositions

[00426] The present invention encompasses compositions comprising a molecule capable of binding PD-1 or a natural ligand of PD-1, a molecule capable of mediating the redirected killing of a tumor cell, or a combination of such molecules. 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 PD-1 or a natural ligand of PD-1, a molecule 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).

[00427] Where more than one therapeutic agent is to be administered the agents may be formulated together in the same formulation or may be formulated into separate compositions. Accordingly, in some embodiments, the molecule capable of binding PD-1 or a natural ligand of PD-1 and the molecule capable of mediating the redirected killing of a target cell (*e.g.*, a cancer cell, a pathogen-infected cell, *etc.*) are formulated together in the same pharmaceutical composition. In alternative embodiments, the molecules are formulated in separate pharmaceutical compositions.

[00428] Various formulations of a molecule capable of binding PD-1 or a natural ligand of PD-1, a molecule 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 molecules, 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.

[00429] 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.

[00430] 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.

[00431] 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.

IX. Methods of Administration

[00432] 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 a pharmaceutical composition comprising molecule capable of binding PD-1 or a natural ligand of PD-1 of the invention, and a pharmaceutical composition comprising a molecule capable of mediating the redirected killing of a tumor cell of the invention; or a pharmaceutical composition comprising a combination of such molecules of the 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.

[00433] 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.

[00434] 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.

[00435] 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.

[00436] 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.

[00437] 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.

[00438] 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 thepathogen-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.

[00439] 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.

[00440] 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.

[00441] 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.

[00442] 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.

[00443] 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).

[00444] Where the composition of the invention is a nucleic acid encoding a binding molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded binding molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.* (1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced

intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00445] 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.

EXAMPLES

[00446] 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
Combination Treatment Study: LOX-IMVI Tumor Model

[00447] To illustrate the principles of the present invention, a combination treatment study was undertaken using a reconstituted tumor model in which LOX-IMVI human metastatic melanoma cancer cells were subcutaneously injected into $MHCI^{-/-}$ mice reconstituted with human PBMCs. Mice were then administered vehicle or a treatment of:

(1) The humanized anti-human PD-1 antibody: **hPD-1 mAb7 (1.2) IgG4(P)**, such antibody being a molecule capable of binding PD-1; and/or

(2) The CD3 x B7-H3 bispecific diabody **DART-A**, such diabody being a molecule capable of binding a cell surface molecule of an effector cell (*i.e.*, CD3) and to a Cancer Antigen (*i.e.*, B7-H3), and thereby being able to mediate the redirected killing of cancer cells expressing B7-H3.

The amino acid sequences of such administered molecules are described above. **Table 11** shows the parameters of the study. Each group consisted of 6 female mice. For all groups, mice received 5 x 10^6 LOX-IMVI cancer cells (ID; administered at Study Day 33) and 10^6 human PBMC (IP; administered at Study Day 0). Treatment (administered molecule(s) or vehicle commencing at Study Day 42) was provided weekly for three doses (Q7Dx3); doses were administered by intravenous injection.

Table 11

Group	Administered Dose (mg/kg)	
	hPD-1 mAb7 (1.2) IgG4(P)	**DART-A**
1	0	0
2	0	0.5
3	1.0	0
4	1.0	0.5

[00448] Tumor volume was measured as a function of time. **Figure 7** shows the results of this study, and demonstrates the unexpected benefit of the combined therapy relative to administration of only **hPD-1 mAb7 (1.2) IgG4(P)** or of only **DART-A**.

Example 2
Combination Treatment Study: Detroit562 Tumor Model

[00449] To further illustrate the principles of the present invention, a combination treatment study was undertaken using a reconstituted tumor model in which Detroit562 human metastatic pharyngeal carcinoma cancer cells were subcutaneously injected into $MHCI^{-/-}$ mice reconstituted with human PBMCs. Mice were then administered vehicle control, 1 mg/kg **hPD-1 mAb7 (1.2) IgG4(P)**, 0.5 mg/kg **DART-A**, or both 1 mg/kg **hPD-1 mAb7 (1.2) IgG4(P)** and 0.5 mg/kg **DART-A**. **Table 12** shows the parameters of the study. Each group consisted of 8 male mice. For all groups, mice received 5 x 10^6 Detroit562 cancer cells (ID) and 10^6 human PBMC (IP; administered at Study Day 0). Treatment (administered molecule(s) or vehicle commencing at Study Day 7) was provided weekly for four weeks (Q7Dx4) or every 14 days for 2 doses (Q14Dx2); doses were administered by intravenous injection.

Table 12			
Group	Dosage Regimen	Administered Dose (mg/kg)	
		hPD-1 mAb7 (1.2) IgG4(P)	DART-A
1	Q7Dx4	0	0
2	Q7Dx4	1.0	0
3	Q7Dx4	0	0.5
4	Q14Dx2	0	0.5
5	Q7Dx4	1.0	0.5
6	Q14Dx2	1.0	0.5

[00450] Tumor volume was measured as a function of time. **Figures 8A-8B** show the results of this study, which again demonstrates the unexpected benefit of the combined therapy relative to administration of only **hPD-1 mAb7 (1.2) IgG4(P)** or of only **DART-A**. **Figure 8A** shows the results for Groups 1-3 and 5; **Figure 8B** shows the results for Groups 1-4 and 6.

[00451] The concentration of $CD3^+$ cells in the mice was determined at the conclusion of the study. It was surprisingly found that the concentration of such cells had increased in mice that had received the combination therapy (**Figure 9**), thus indicating that the therapy of the present invention had enhanced the animals' immune responses.

Example 3
Signaling Model

[00452] To further illustrate the principles of the present invention, cooperative T-cell signaling was examined in a T-cell/tumor cell co-culture system using a Jurkat-luc-NFAT / tumor cell luciferase reporter assay. Briefly, MDA-MB-231 tumor target cells expressing PD-1 and B7-H3 were mixed with MNFAT-luc2/PD-1 Jurkat T-cells at an effector:target cell ratio of 1:1 (**Figure 10A**) or 3:1 (**Figure 10B**) and cultured alone or with a fixed concentration (12.5 nM) of the PD-1 binding molecules **hPD-1 mAb7 (1.2) IgG4(P)**, **DART-1,** a control antibody, in the presence of increasing concentrations of **DART-A**. Luminescence was measured as an indicator of cell activation and signaling. **Figures 10A-10B** show the results of this study which demonstrate that the combination of a molecule capable of binding PD-1 (*e.g.*, **hPD-1 mAb7 (1.2) IgG4(P)**, **DART-1**) and a molecule capable of mediating the redirected killing of a target cell (*e.g.*, **DART-A**) enhances effector cell signaling activity.

Example 4
Combination Treatment Study: Comparing Normal to Anergic T-cell in a A375 Tumor Model

[00453] To further illustrate the principles of the present invention, a combination treatment study was undertaken using a reconstituted tumor model in which A375 human melanoma cells were subcutaneously injected into NOG mice reconstituted with activated or anergic human T-cells. Mice were then administered vehicle or a treatment of:

(1) The PD-1 x LAG-3 bispecific diabody: **DART-2** such diabody being a molecule capable of binding PD-1; and/or

(2) The CD3 x IL13Rα2 bispecific diabody **DART-B**, such diabody being a molecule capable of binding a cell surface molecule of an effector cell (*i.e.*, CD3) and to a Cancer Antigen (*i.e.*, IL13Rα2), and thereby being able to mediate the redirected killing of cancer cells expressing IL13Rα2.

[00454] Activated T-cells were prepared by two rounds of culturing purified human T-cells with CD3/CD28 activation beads in the presence of IL-2. Anergic T-cell were prepared by one round of culturing purified human T-cells with CD3/CD28 activation beads in the presence of IL-2 followed by one round of culturing with CD3/CD28 activation beads without IL-2. Groups of mice (n=8 female) received subcutaneous inoculation of 5 x 10^6 A375 melanoma cells (pretreated with 0.1 μg/mL IFNγ for 24 hours) and 5 x 10^6 human T-cells (activated or anergic) at Study Day 0. and were then administered vehicle control, 0.5 mg/kg **DART-2**, 0.5 mg/kg **DART-B**, or both 0.5 mg/kg **DART-2** and 0.5 mg/kg **DART-B**. Treatment (administered molecule(s) or vehicle) was provided weekly for four doses (Q7Dx4) or as a single treatment on Study Day 0 (QD (SD)); doses were administered by intravenous injection. **Table 13** shows the parameters of the study.

Table 13

Group	T-cells	Treatment	Dose (mg/kg)	Route/Schedule
1	Activated	Vehicle	0	IV/QD (SD0)
2	Activated	DART-B	0.01	IV/QD (SD0)
3	Activated	DART-B DART-2	0.01 0.5	IV/QD (SD0) IV/Q7Dx4
4	Activated	DART-2	0.5	IV/Q7Dx4
5	Anergic	Vehicle	0	IV/QD (SD0)
6	Anergic	DART-B	0.01	IV/QD (SD0)
7	Anergic	DART-B DART-2	0.01 0.5	IV/QD (SD0) IV/Q7Dx4
8	Anergic	DART-2	0.5	IV/Q7Dx4

[00455] Tumor volume was measured as a function of time. **Figures 11A-11B** show the results of this study, demonstrate that the combined therapy of a molecule capable of binding PD-1 (*e.g.*, **hPD-1 mAb7 (1.2) IgG4(P)**, **DART-1, DART-2**) and a molecule capable of mediating the redirected killing of a target cell (*e.g.*, **DART-A, DART-B**) reduces tumor recurrence in the presence of anergic T-cells. These results again demonstrate the unexpected benefit of the combined therapy of a molecule capable of binding PD-1 and a molecule capable of mediating the redirected killing of a target cell relative to administration of either molecule alone. **Figure 11A** shows the results for Groups 1-4 inoculated with normal active T-cells; **Figure 11B** shows the results for Groups 5-8 inoculated with anergic T-cells.

Example 5
Combination Treatment Study: A375 Tumor Model

[00456] To further illustrate the principles of the present invention, a combination treatment study was undertaken using a co-mix tumor model in which A375 melanoma cells were subcutaneously injected into NOG mice reconstituted with human T-cells. Mice were then administered Mice were then administered vehicle or a treatment of:

(1) The PD-1 x LAG-3 bispecific diabody: **DART-2** such diabody being a molecule capable of binding PD-1; and/or

(2) The CD3 x IL13Rα2 bispecific diabody **DART-B**, such diabody being a molecule capable of binding a cell surface molecule of an effector cell (*i.e.*, CD3) and to a Cancer Antigen (*i.e.*, IL13Rα2), and thereby being able to mediate the redirected killing of cancer cells expressing IL13Rα2.

[00457] **Table 14** shows the parameters of the study. Each group consisted of 8 female mice. For all groups, mice received 1.25 x 10^6 A375 melanoma cells (pretreated for 24 hours with 100 ng/ml IFNγ) co-mixed with 1.25 x10^6 human T-cells (pretreated with 120 μg/ml DART-2 for 20 min) (SC; administered at Study Day 0). Mice in groups 5-8 were pretreated with DART-2 (500 μg/kg) 24 hours prior to cell injections (Study Day -1) and received addition doses of DART-2 (500 μg/kg) every 7 days starting on Study Day 7, for a total of 10 doses. Mice in groups 2-4 and 6-8 received a single dose of DART-B (1, 5 or 10 μg/kg) on Study Day 0. Group 1, received vehicle alone. All doses were administered by intravenous injection.

Table 14				
Group	**N/sex**	**Treatment**	**Dose (μg/kg)**	**Route/ Schedule**
1	8/F	Vehicle	0	IV/QDx1
2	8/F	DART-B	1	IV/QDx1
3	8/F	DART-B	5	IV/QDx1
4	8/F	DART-B	10	IV/QDx1
5	8/F	DART-2	500	IV/Q7Dx7
6	8/F	DART-B	1	IV/QDx1
		DART-2	500	IV/Q7Dx7
7	8/F	DART-B	5	IV/QDx1
		DART-2	500	IV/Q7Dx7
8	8/F	DART-B	10	IV/QDx1
		DART-2	500	IV/Q7Dx7

[00458] Tumor volume was measured as a function of time and is plotted in **Figures 12A-12H**. **Figure 12A** shows the results for Groups 1, 2, 5 and 6 through day 50; **Figures 12B-12H** show the spider plots, through day 80, for the individual animals in Group 2 (**Figure 12B**), Group 5 (**Figure 12C**), Group 6 (**Figure 12D**), Group 3 (**Figure 12E**), Group 7 (**Figure 12F**), Group 4 (**Figure 12G**), and Group 8 (**Figure 12H**). The results of this study demonstrate the unexpected benefit of the combined therapy of a molecule capable of binding PD-1 and a molecule capable of mediating the redirected killing of a target cell relative to administration of either molecule alone.

[00459] 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 method for the treatment of cancer or a pathogen-associated disease, comprising administering to a subject in need thereof a therapeutically effective amount of:

(1) a molecule capable of binding PD-1 or a natural ligand of PD-1, and

(2) a molecule capable of mediating the redirected killing of a target cell, wherein said target cell is:

(a) a cancer cell that expresses a Cancer Antigen; or

(b) a pathogen-infected cell that expresses a Pathogen-Associated Antigen.

Claim 2. The method of claim 1, wherein said molecule capable of binding PD-1 or a natural ligand of PD-1 is capable of inhibiting binding between PD-1 and a natural ligand of PD-1.

Claim 3. The method of claim 1, wherein said method comprises administration of two binding molecules that cumulatively comprise three epitope-binding domains, said two binding molecules being:

(A) a binding molecule that comprises an epitope-binding domain of an antibody that is capable of binding PD-1, or an epitope-binding domain of an antibody that is capable of binding a natural ligand of PD-1; and

(B) a binding molecule that comprises:

(1) an epitope-binding domain of an antibody that is capable of binding a cell surface molecule of said effector cell; and

(2) an epitope-binding domain of an antibody that that is capable of binding said Cancer Antigen or said Pathogen Antigen of said target cell;

wherein said epitope-binding domain of said binding molecule (A) is capable of binding PD-1 or a natural ligand of PD-1, and said epitope-binding domains (1) and (2) of said binding molecule (B) are capable of mediating the redirected killing of said target cell.

Claim 4. The method of claim 3, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a diabody, scFv, antibody or

TandAb, and said binding molecule (B) comprises a bispecific diabody, a CAR, a BiTe, or bispecific antibody.

Claim 5. The method of any one of claims 3 or 4, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to PD-1.

Claim 6. The method of any one of claims 3 or 4, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to a natural ligand of PD-1.

Claim 7. The method of claim 5, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding PD-1, wherein such epitope-binding domains:

(a) compete for binding the same epitope of PD-1; or

(b) do not compete for binding the same epitope of PD-1.

Claim 8. The method of claim 7, wherein said PD-1-epitope-binding domains are capable of simultaneous binding to the same PD-1 molecule.

Claim 9. The method of claim 6, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding said natural ligand of PD-1, wherein such epitope-binding domains:

(a) compete for binding to the same epitope of such natural ligand of PD-1; or

(b) do not compete for binding to the same epitope of such natural ligand of PD-1.

Claim 10. The method of claim 9, wherein said PD-1 ligand-epitope-binding domains are capable of simultaneous binding the same molecule of said natural ligand of PD-1.

Claim 11. The method of claim 5 or 6, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding an epitope of a molecule that is not PD-1 or a natural ligand of PD-1.

Claim 12. The method of claim 11, wherein said second epitope-binding domain binds an epitope of CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA.

Claim 13. The method of any one of claims 1-12, wherein said binding molecule capable of mediating the redirected killing of said target cell comprises a third epitope-binding domain capable of binding a cell surface molecule of said effector cell.

Claim 14. The method of claim 13, wherein said third epitope-binding-domain of said binding molecule capable of mediating the redirected killing of said target cell is capable of binding a different cell surface molecule of said effector cell, such that said binding molecule capable of mediating said redirected killing is capable of binding two different cell surface molecules of said effector cell.

Claim 15. The method of any one of claims 2-12, wherein said binding molecule capable of mediating the redirected killing of said target cell comprises a third epitope-binding domain capable of binding to a Cancer Antigen or a Pathogen-Associated Antigen of said target cell.

Claim 16. The method of claim 15, wherein said third epitope-binding-domain of said binding molecule capable of mediating the redirected killing of said target cell is capable of binding a different Cancer Antigen or a different Pathogen Antigen of said target cell, such that said binding molecule capable of mediating said redirected killing is capable of binding to two different Cancer Antigens or two different Pathogen Antigens of said target cell.

Claim 17. The method of any one of claims 2-16, wherein said cell surface molecule of said effector cell is selected from the group consisting of: CD2, CD3, CD8, CD16, TCR, and NKG2D.

Claim 18. The method of any one of claims 2-17, wherein said Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, 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, $D_1$56-22, DR5, E_1 series, EGFR, an Ephrin receptor, 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, IL13Rα2, Integrin β6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6,L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R_{24}, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_5A_7, 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 19. The method of any one of claims 2-17, wherein said method comprises said administration of said pharmaceutical composition, and 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, *etc.*), 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 20. A pharmaceutical composition that comprises:

(A) therapeutically effective amounts of:

(1) a molecule capable of binding PD-1 or a natural ligand of PD-1, and

(2) a molecule capable of mediating the redirected killing of a target cell expressing a Cancer Antigen or a Pathogen Antigen; and

(B) a pharmaceutically acceptable carrier.

Claim 21. The pharmaceutical composition of claim 20, wherein said pharmaceutical composition comprises two binding molecules that cumulatively comprise three epitope-binding domains, said two binding molecules being:

(A) a binding molecule that comprises an epitope-binding domain of an antibody that is capable of binding PD-1, or an epitope-binding domain of an antibody that is capable of binding a natural ligand of PD-1; and

(B) a binding molecule that comprises:

(1) an epitope-binding domain of an antibody that is capable of binding a cell surface molecule of said effector cell; and

(2) an epitope-binding domain of an antibody that that is capable of binding a Cancer Antigen or a Pathogen-Associated Antigen of said target cell;

wherein said epitope-binding domain of said binding molecule (A) is capable of binding PD-1 or a natural ligand of PD-1, and said epitope-binding domains (1) and (2) of said binding molecule (B) are capable of mediating the redirected killing of said target cell.

Claim 22. The pharmaceutical composition of claim 21, wherein said binding molecule (A) comprises a diabody, scFv, antibody, or TandAb, and said binding molecule (B) comprises a diabody, a CAR, a BiTe, or bispecific antibody.

Claim 23. The pharmaceutical composition of any one of claims 21-22, wherein said molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to PD-1.

Claim 24. The pharmaceutical composition of any one of claims 21-22, wherein said molecule capable of binding PD-1 or a natural ligand of PD-1 comprises an epitope-binding domain of an antibody that binds to a natural ligand of PD-1.

Claim 25. The pharmaceutical composition of claim 23, wherein said molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding PD-1, wherein such PD-1-epitope-binding domains:

(a) compete for binding to the same epitope of PD-1; or

(b) do not compete for binding the same epitope of PD-1.

Claim 26. The pharmaceutical composition of claim 25, wherein said PD-1-epitope-binding domains are capable of simultaneous binding the same PD-1 molecule.

Claim 27. The pharmaceutical composition of claim 24, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding said natural ligand of PD-1, wherein such epitope-binding domains:

(a) compete for binding to the same epitope of such natural ligand of PD-1; or

(b) do not compete for binding to the same epitope of such natural ligand of PD-1.

Claim 28. The pharmaceutical composition of claim 27, wherein said PD-1 ligand-epitope-binding domains are capable of simultaneous binding the same molecule of said natural ligand of PD-1.

Claim 29. The method of claim 23 or 24, wherein said binding molecule capable of binding PD-1 or a natural ligand of PD-1 comprises a second epitope-binding domain capable of binding an epitope of a molecule that is not PD-1 or a natural ligand of PD-1.

Claim 30. The method of claim 29, wherein said second epitope-binding domain binds an epitope of CD137, LAG-3, OX40, TIGIT, TIM-3, or VISTA.

Claim 31. The pharmaceutical composition of any one of claims 20-29, wherein said molecule capable of mediating the redirected killing of said target cell comprises a third epitope-binding domain, wherein such three epitope-binding domains are capable of simultaneous binding, and wherein said third epitope-binding site is capable of binding an epitope of a cell surface molecule of said effector cell.

Claim 32. The pharmaceutical composition of claim 31, wherein said third epitope-binding-domain of said binding molecule capable of mediating the redirected killing of said target cell is capable of binding a different cell surface molecule of said effector cell, such that said binding molecule capable of mediating said redirected killing is capable of binding two different cell surface molecules of said effector cell.

Claim 33. The pharmaceutical composition of any one of claims 20-30, wherein said binding molecule capable of mediating the redirected killing of said target cell comprises a third epitope-binding domain capable of binding to a Cancer Antigen or a Pathogen-Associated Antigen of said target cell.

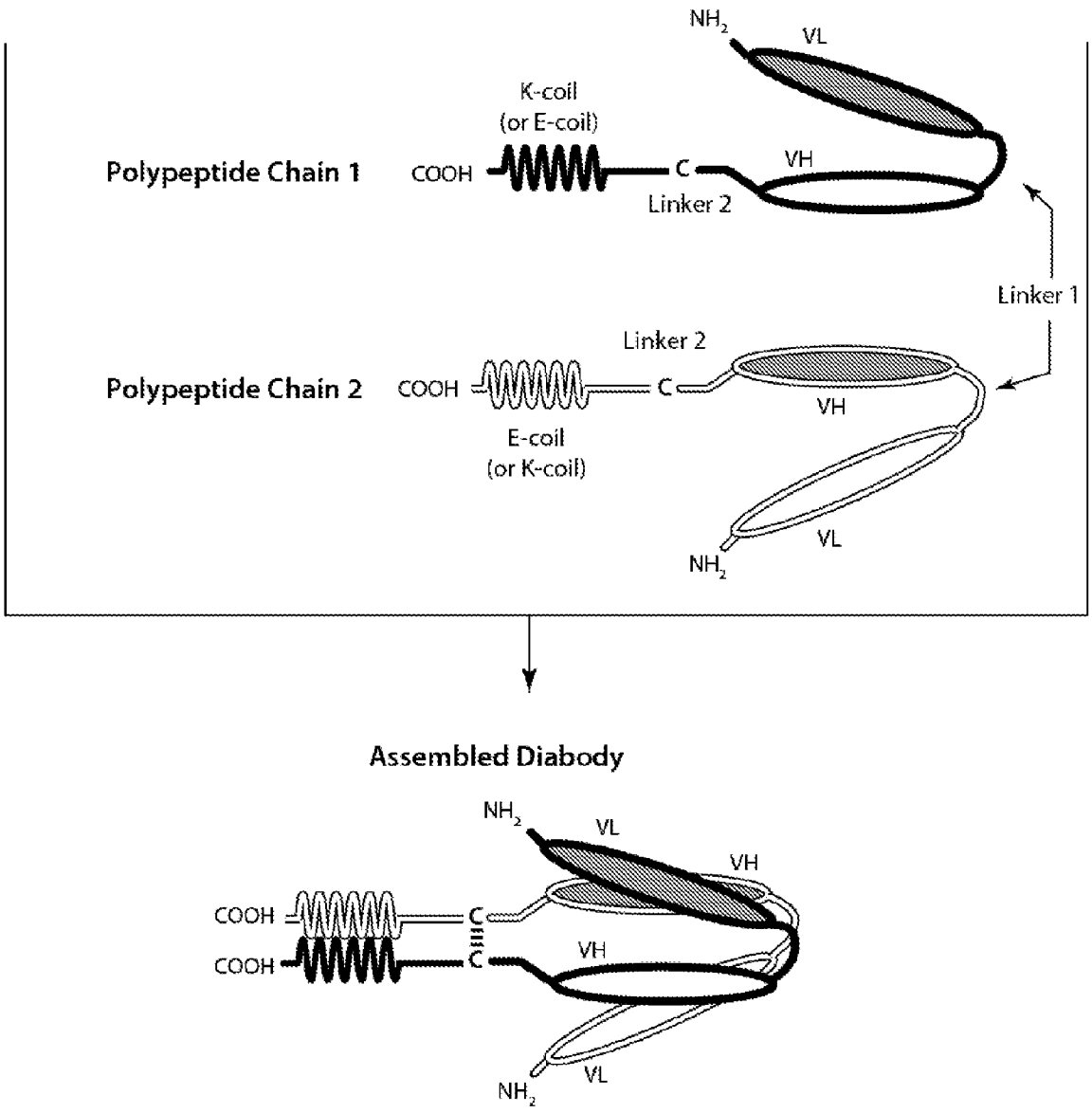
Claim 34. The pharmaceutical composition of claim 33, wherein said third epitope-binding-domain of said binding molecule capable of mediating the redirected killing of said target cell is capable of binding a different Cancer Antigen or a different Pathogen-Associated Antigen of said target cell, such that said binding molecule capable of mediating said redirected killing is capable of binding to two different Cancer Antigens or two different Pathogen-Associated Antigens of said target cell.

Claim 35. The pharmaceutical composition of any one of claims 21-34, wherein said cell surface molecule of said effector cell is selected from the group consisting of: CD2, CD3, CD8, CD16, TCR, and NKG2D.

Claim 36. The pharmaceutical composition of any one of claims 20-35, wherein said Cancer Antigen is selected from the group consisting of the Cancer Antigens: 19.9, 4.2, A33, 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, $D_1$56-22, DR5, E_1 series, EGFR, an Ephrin receptor, 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, IL13Rα2, Integrin β6, JAM-3, KID3, KID31, KS 1/4 pan-carcinoma antigen, L6,L20, LEA, LUCA-2, M1:22:25:8, M18, M39, MAGE, MART, mesothelin, MUC-1, MUM-1, Myl, N-acetylglucosaminyltransferase, neoglycoprotein, NS-10, OFA-1, OFA-2, Oncostatin M, p15, p97, PEM, PEMA, PIPA, PSA, PSMA, prostatic acid phosphate, R_{24}, ROR1, a sphingolipid, SSEA-1, SSEA-3, SSEA-4, sTn, the T cell receptor derived peptide, T_5A_7, 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 37. The pharmaceutical composition of any one of claims 20-35, wherein said Pathogen-Associated Antigen is selected from the group consisting of the Pathogen 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, *etc.*), 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 38. A kit comprising the pharmaceutical composition of any one of claims 20-37, wherein said binding molecules thereof are compartmentalized in one or more containers.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(or E-coil)
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C
VH
Linker 2
Linker 1
Linker 2
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COOH
C
VH
E-coil
(or K-coil)
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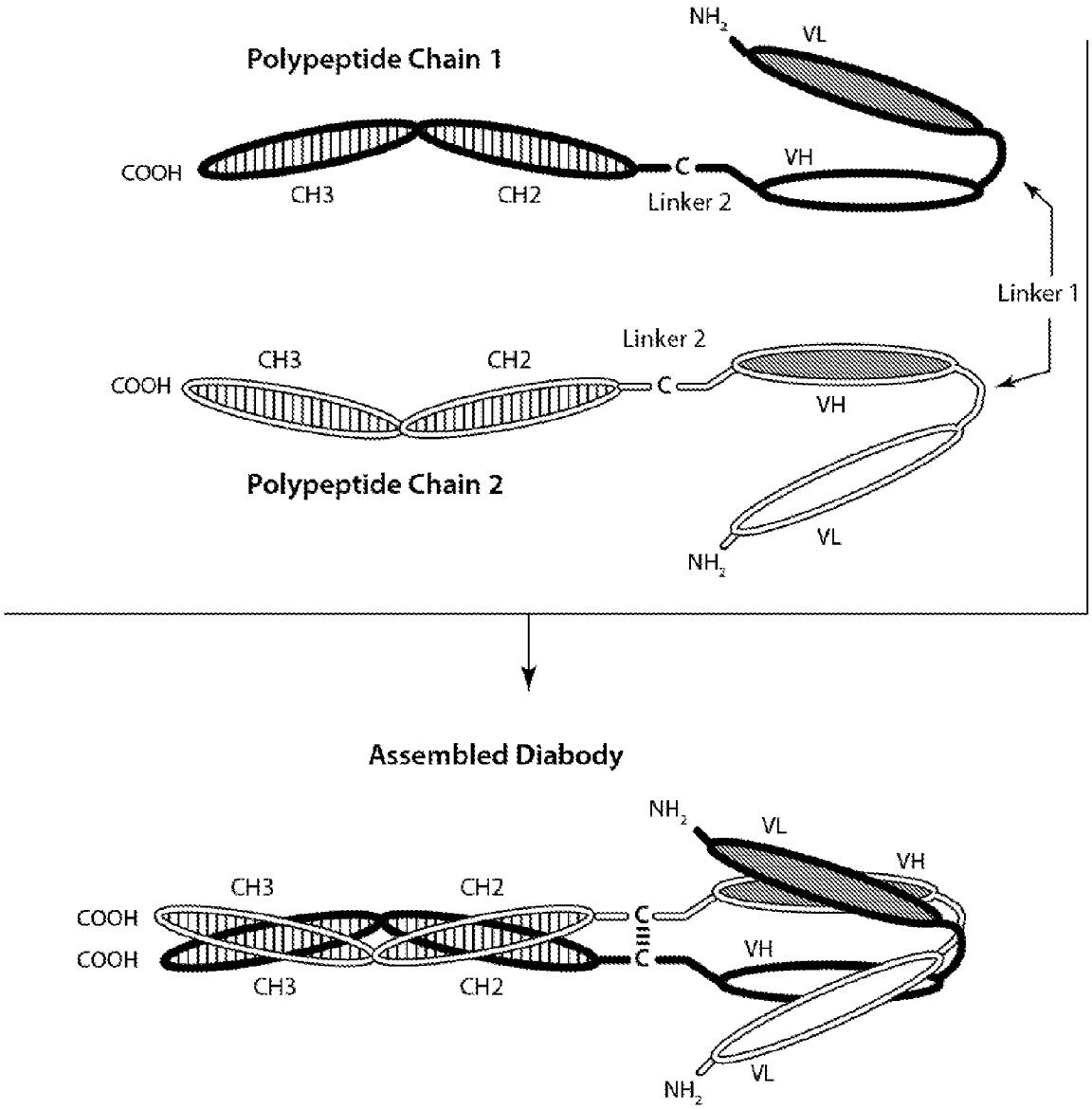
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1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COOH
CH3
CH2
C
VH
Linker 2
Linker 1
Linker 2
CH3
CH2
COOH
C
VH
Polypeptide Chain 2
VL
NH2
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
C
VH
COOH
C
CH3
CH2
VL
NH2

Figure 2

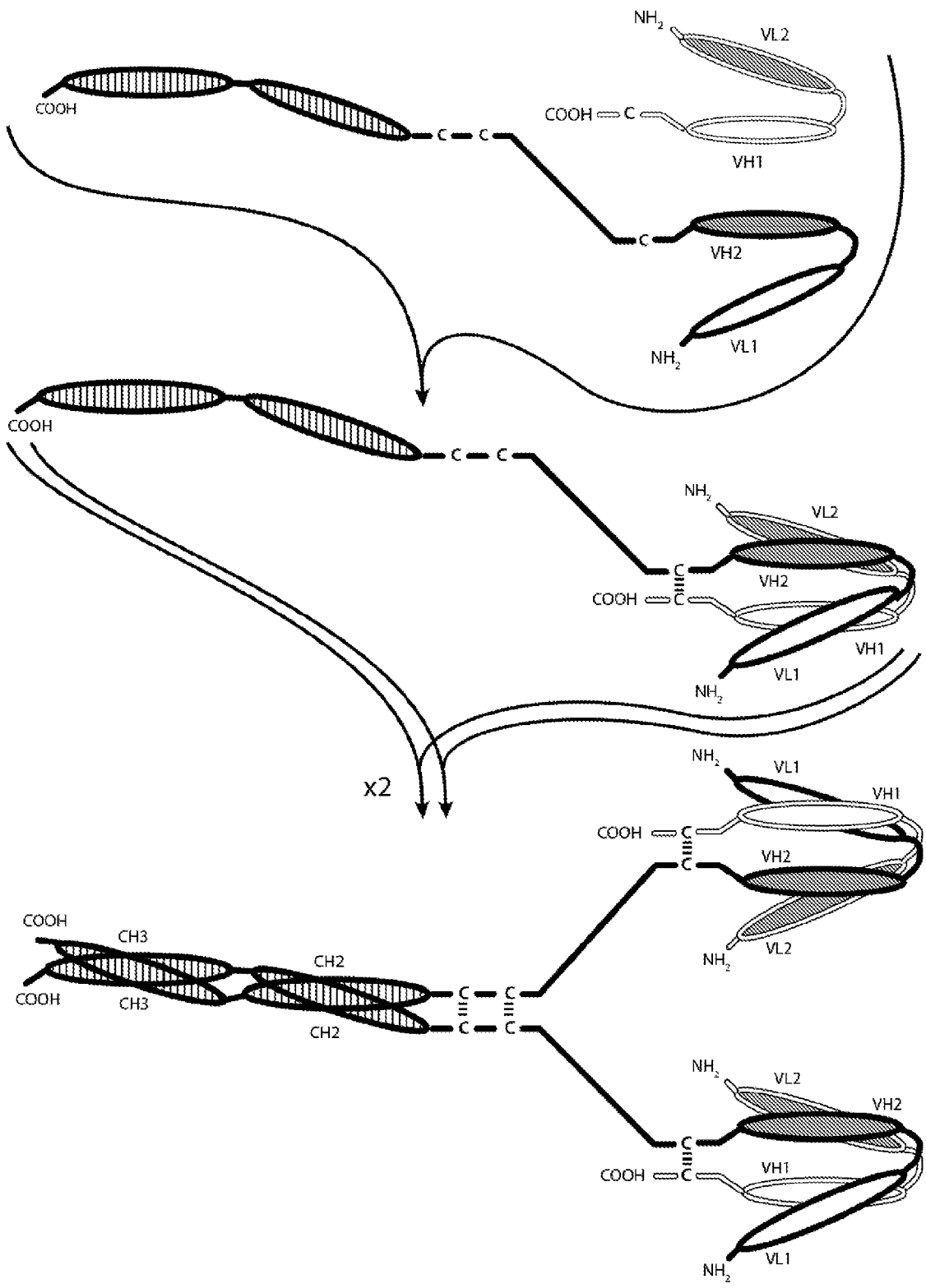
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
C
VH2
COOH
C
VH1
NH2
VL1
x2
NH2
VL1
VH1
COOH
C
C
VH2
NH2
VL2
COOH
CH3
CH2
COOH
CH3
C
C
CH2
C
C
NH2
VL2
VH2
COOH
C
C
VH1
NH2
VL1

Figure 3A

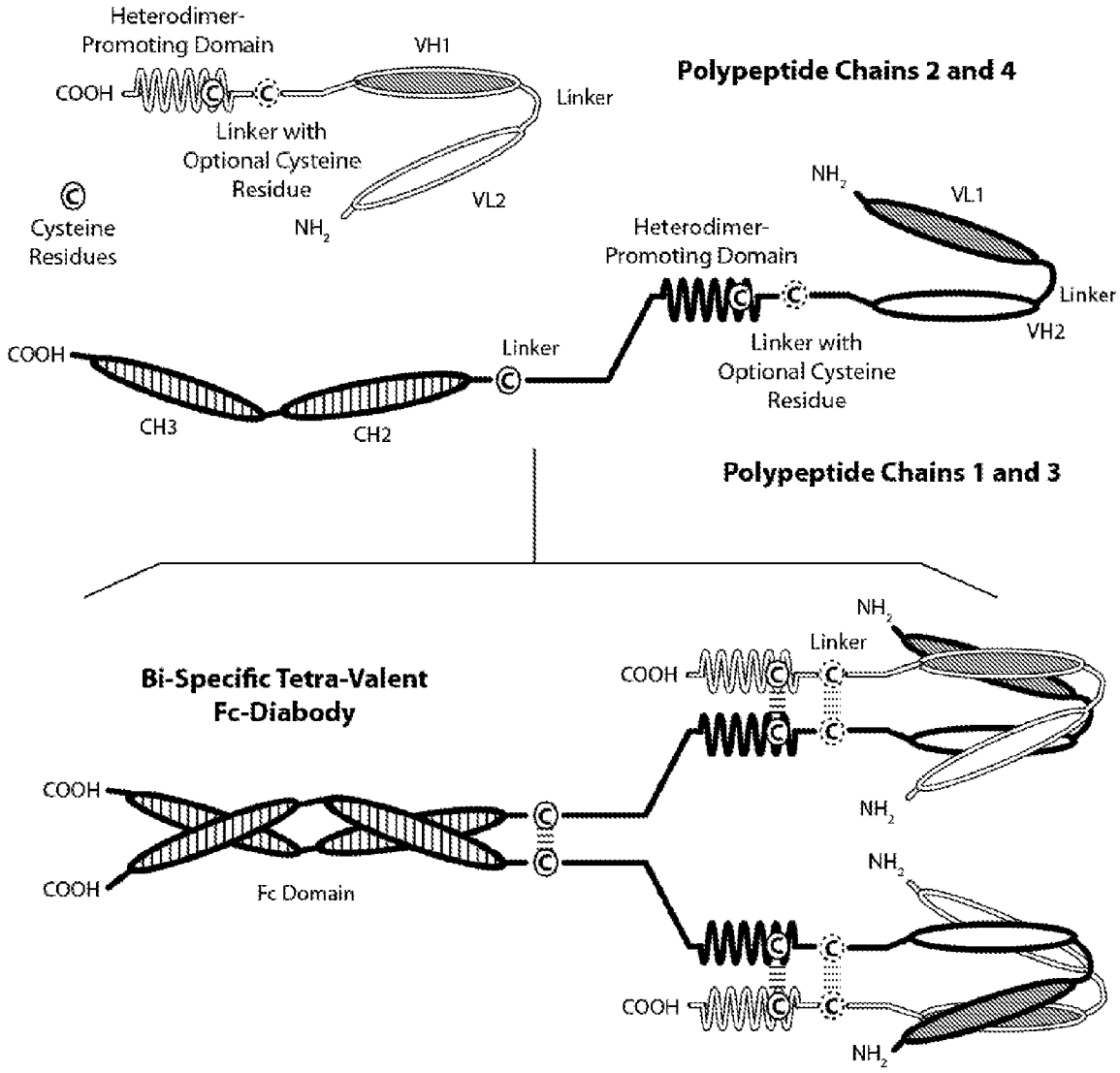
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody
NH2
Linker
COOH
COOH
Fc Domain
NH2
NH2
COOH
NH2

Figure 3B

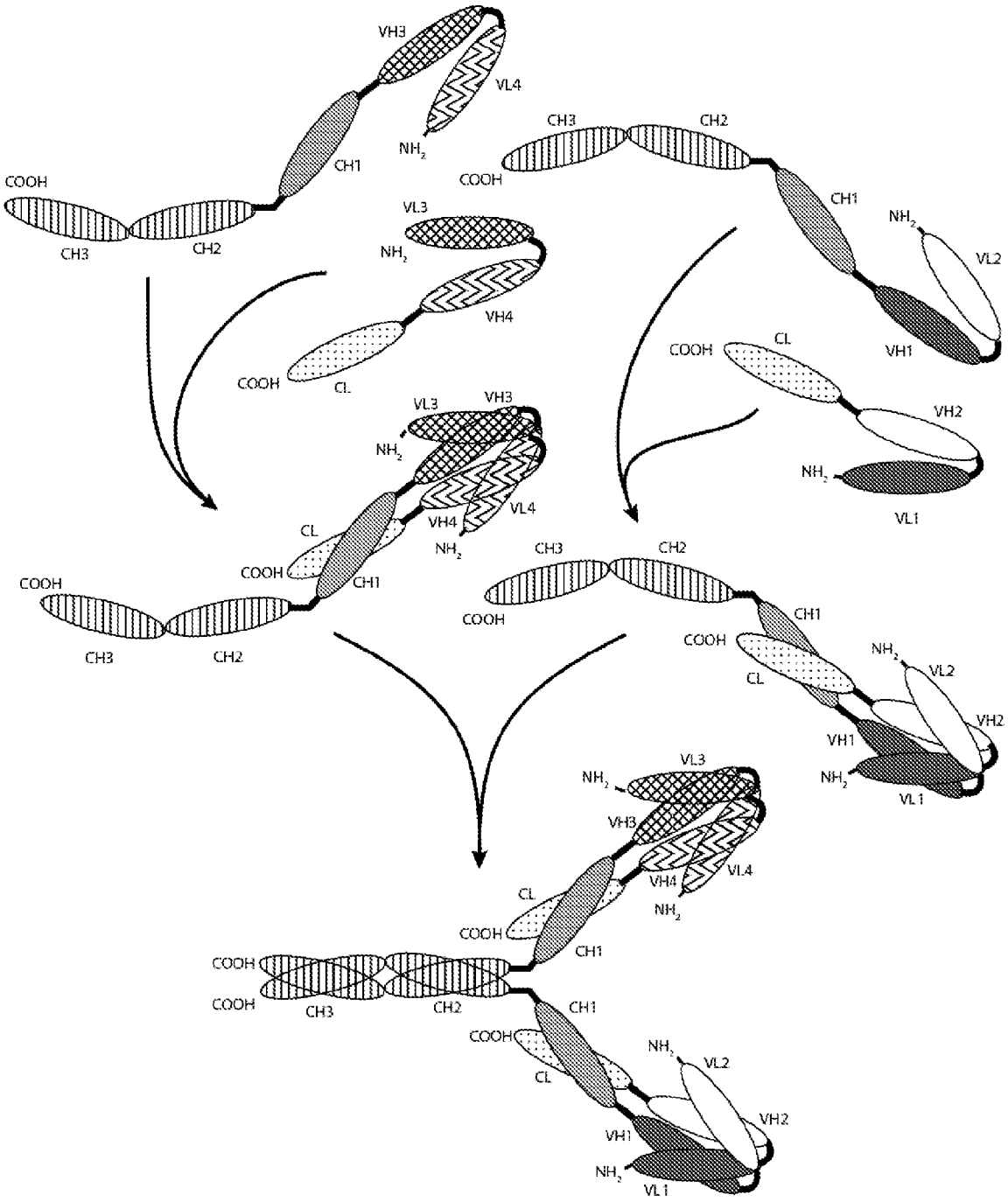
VH3
VL4
NH2
CH1
COOH
CH3
CH2
VL3
NH2
VH4
COOH
CL
CH3
CH2
COOH
CH1
NH2
VL2
VH1
COOH
CL
VH2
NH2
VL1
VL3
VH3
NH2
VH4
VL4
CL
COOH
CH1
COOH
CH3
CH2
CH3
CH2
COOH
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1
VL3
NH2
VH3
VH4
VL4
CL
COOH
CH1
COOH
COOH
CH3
CH2
CH1
COOH
CL
NH2
VL2
VH2
VH1
NH2
VL1

Figure 3C

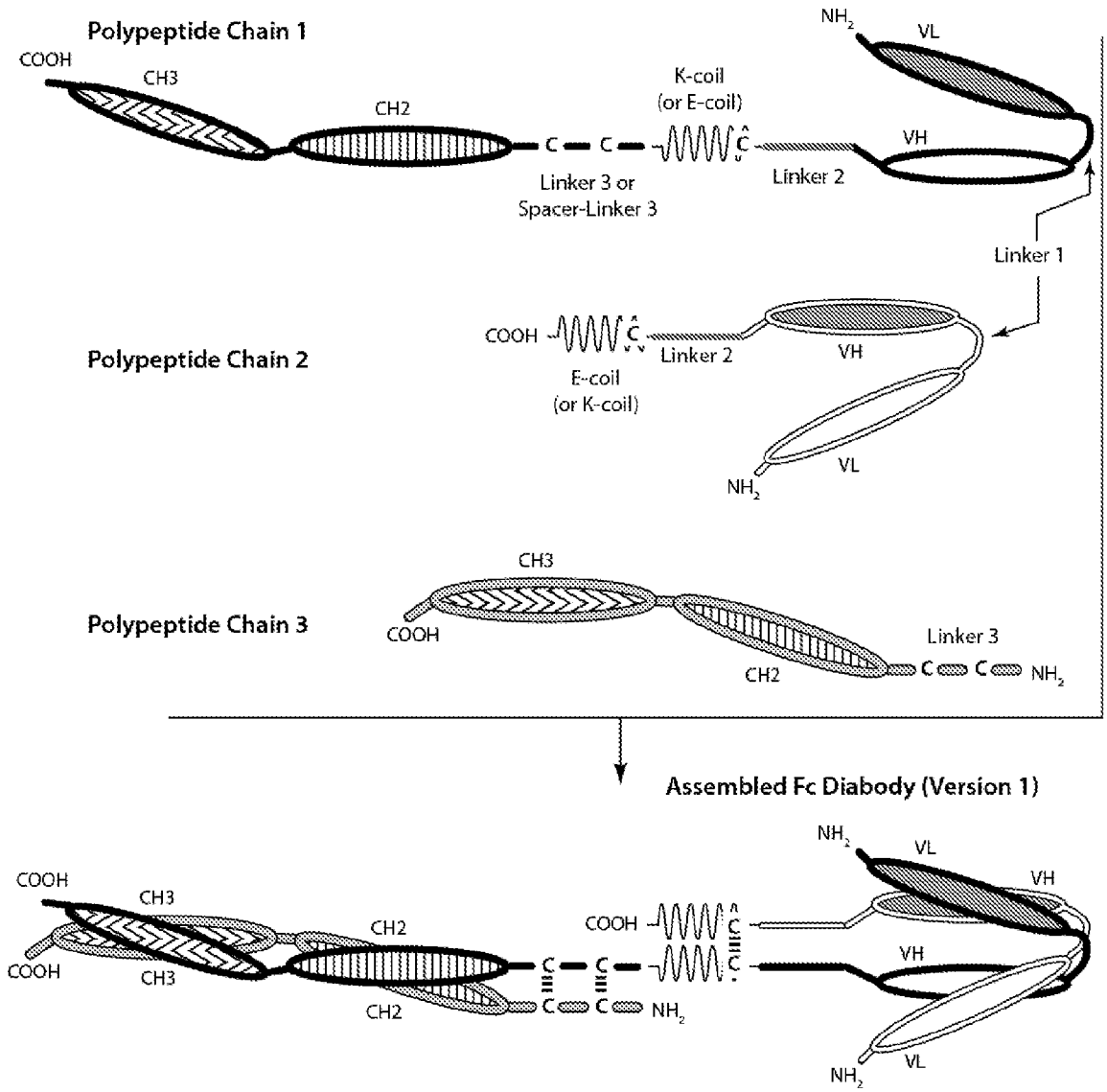
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
Linker 3 or
Spacer-Linker 3
Linker 2
NH2
VL
VH
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
CH2
Linker 3
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH2
COOH
CH3
CH2
NH2
VL
VH
COOH
VH
NH2
VL

Figure 4A

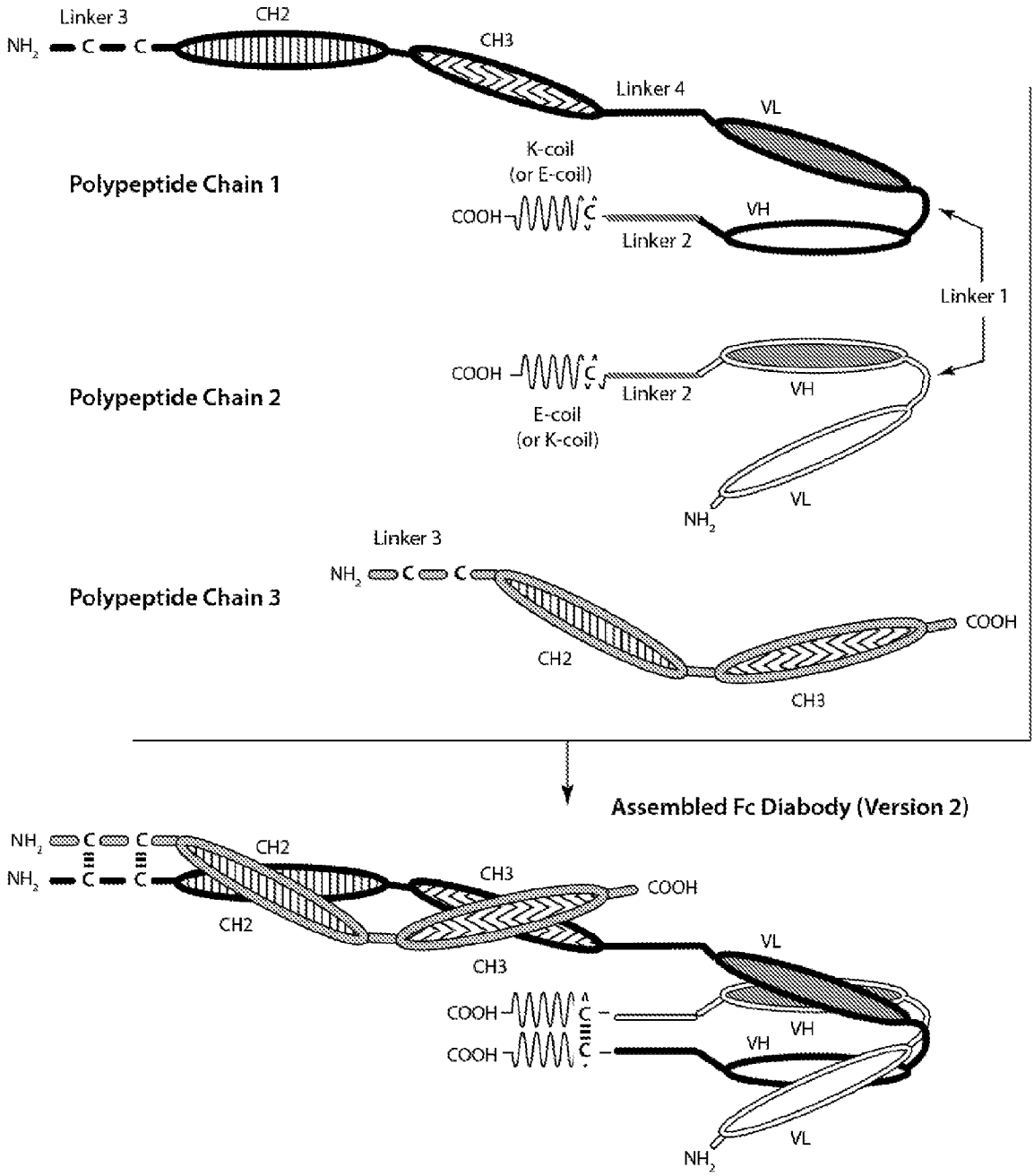
Linker 3
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
VH
Linker 2
Linker 1
Polypeptide Chain 2
COOH
Linker 2
VH
E-coil
(or K-coil)
VL
Linker 3
Polypeptide Chain 3
CH2
COOH
CH3
Assembled Fc Diabody (Version 2)
CH2
CH3
COOH
CH2
CH3
VL
COOH
VH
COOH
VH
VL

Figure 4B

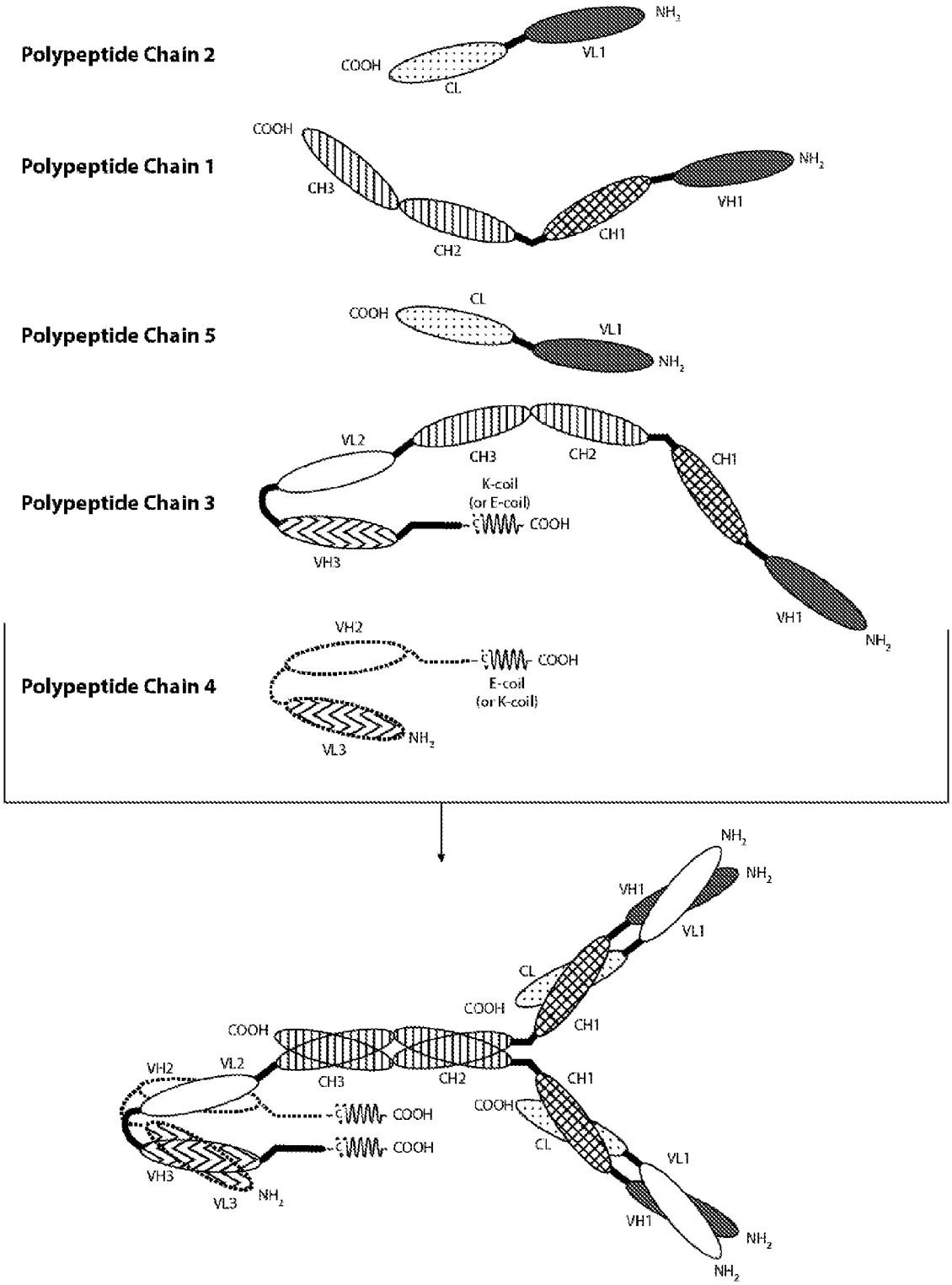
Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
NH2
VH1
CH2
CH1
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
VL3
NH2
NH2
NH2
VH1
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH
COOH
CL
VL1
VH3
VL3
NH2
VH1
NH2
NH2

Figure 5

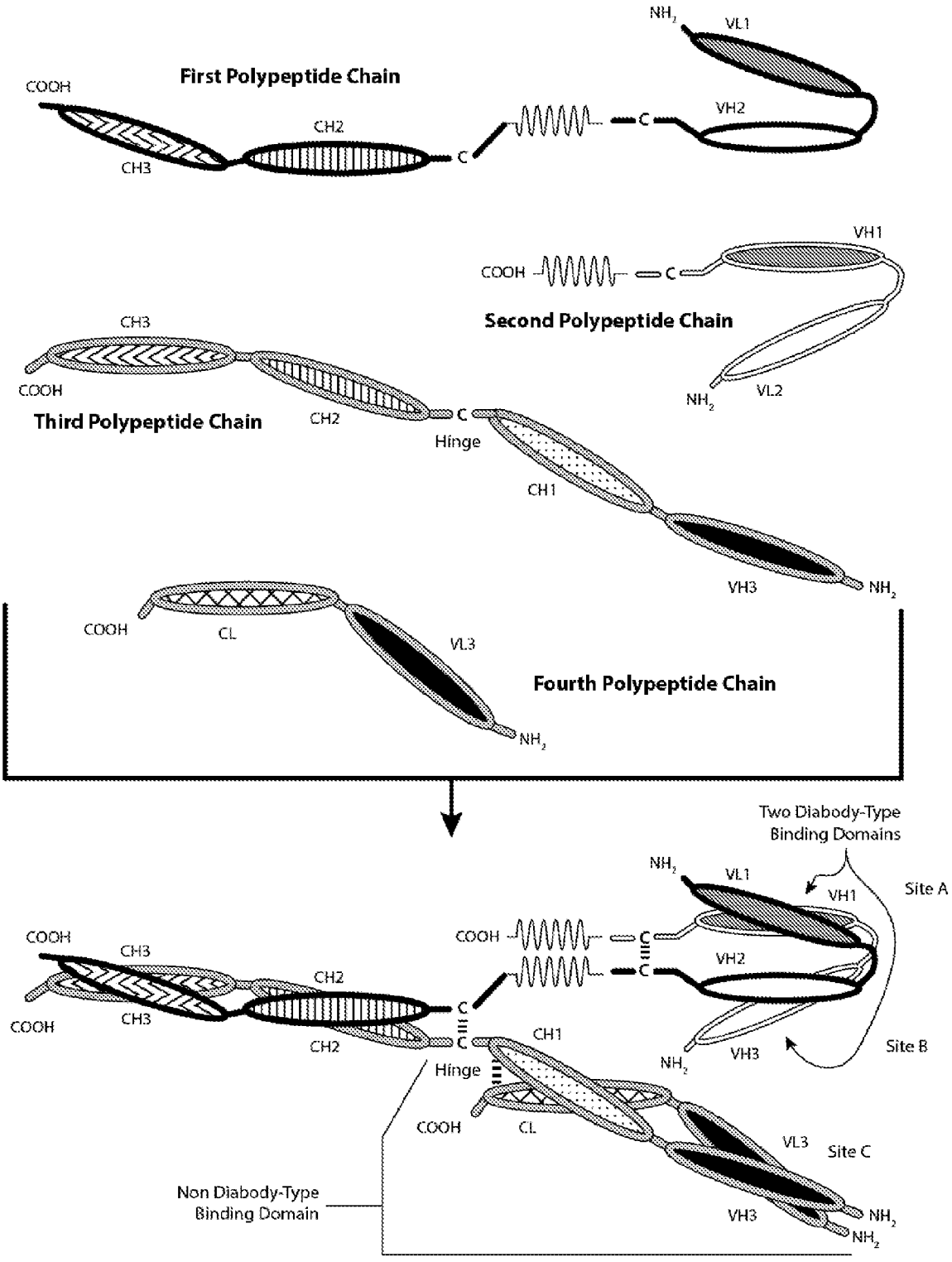
First Polypeptide Chain
COOH
CH2
CH3
C
NH2
VL1
VH2
Second Polypeptide Chain
VH1
VL2
Third Polypeptide Chain
Hinge
CH1
VH3
Fourth Polypeptide Chain
CL
VL3
Two Diabody-Type Binding Domains
Site A
Site B
Site C
Non Diabody-Type Binding Domain

Figure 6A

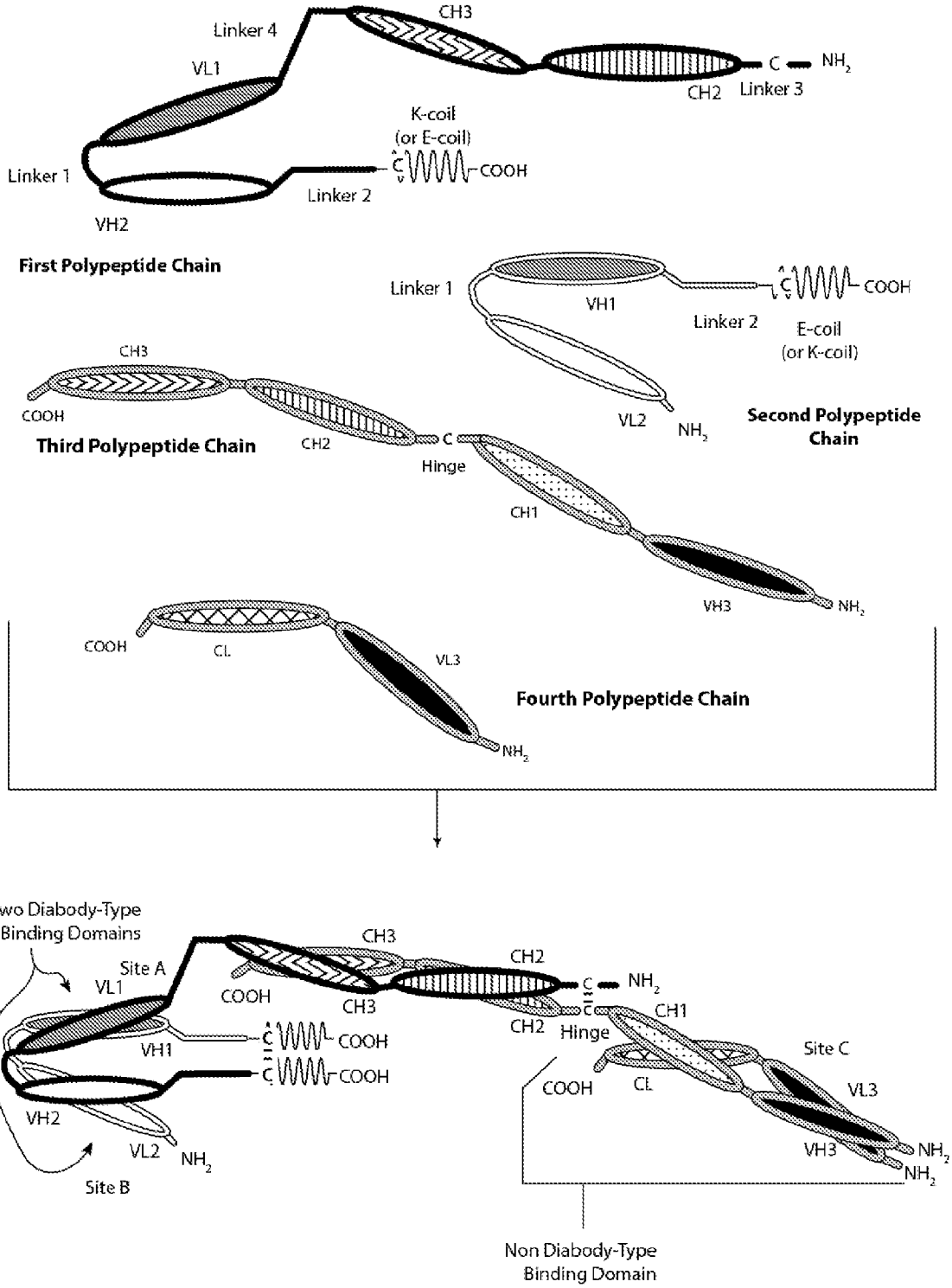
Linker 4
CH3
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
VL2
NH2
Second Polypeptide Chain
COOH
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding Domains
Site A
VL1
CH3
CH2
COOH
C
NH2
CH3
CH2
CH1
VH1
COOH
Hinge
Site C
COOH
CL
VL3
VH2
VH3
NH2
VL2
NH2
Site B
Non Diabody-Type
Binding Domain

Figure 6B

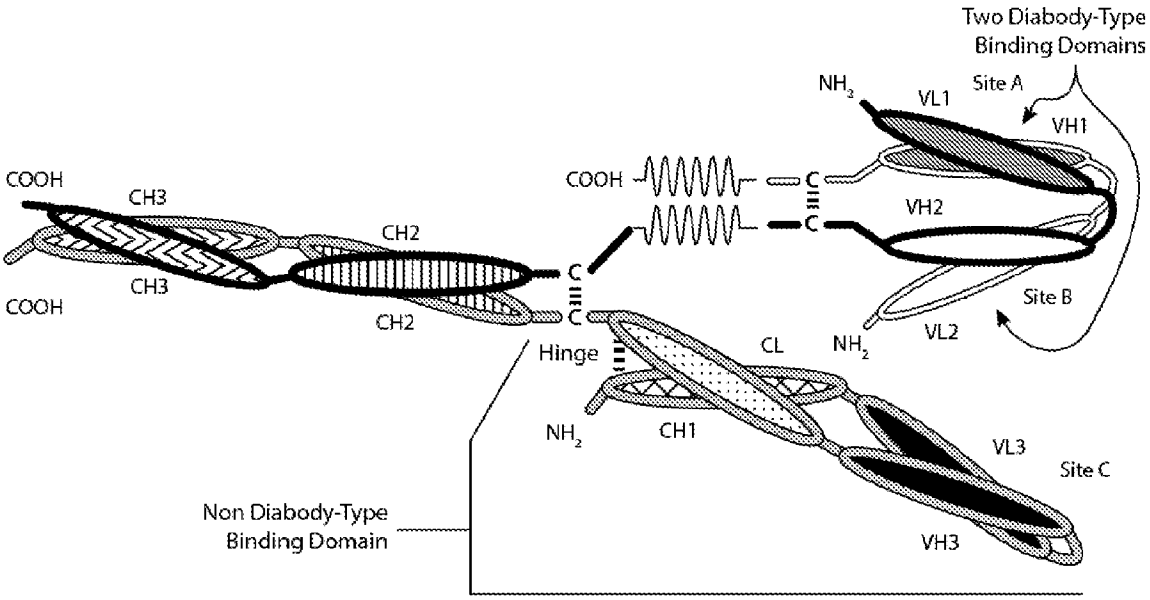

Figure 6C

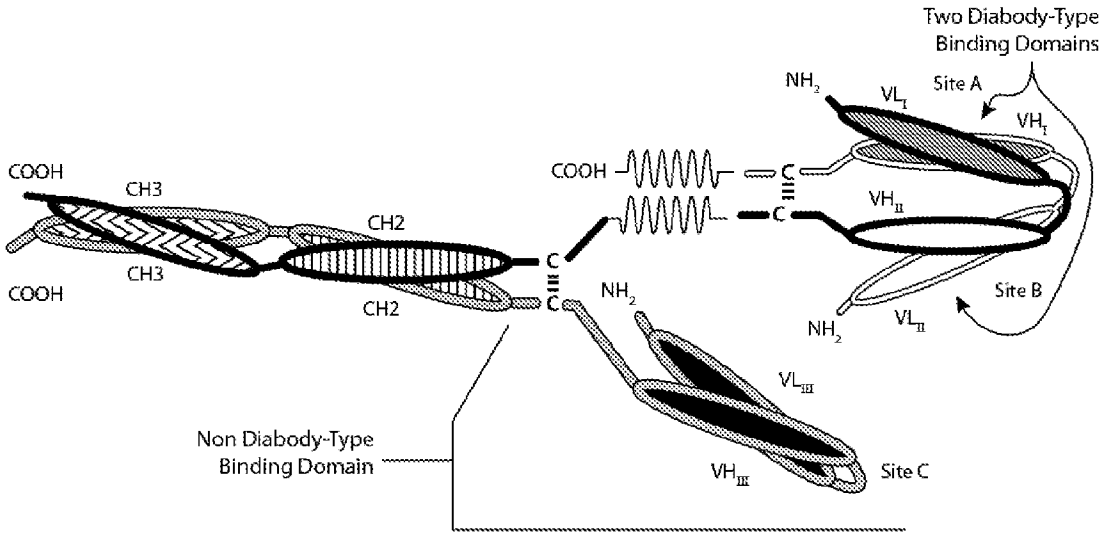

Figure 6D

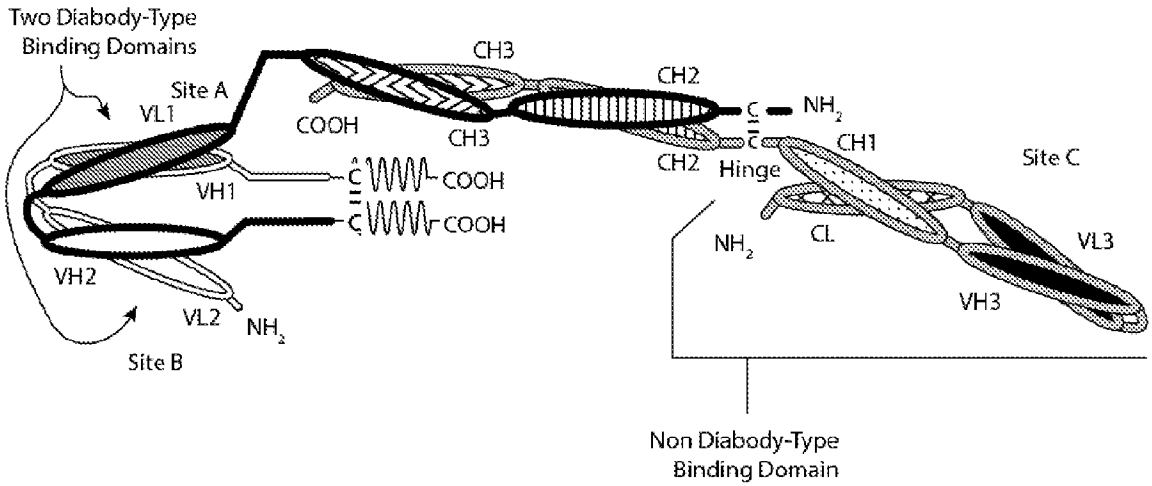

Figure 6E

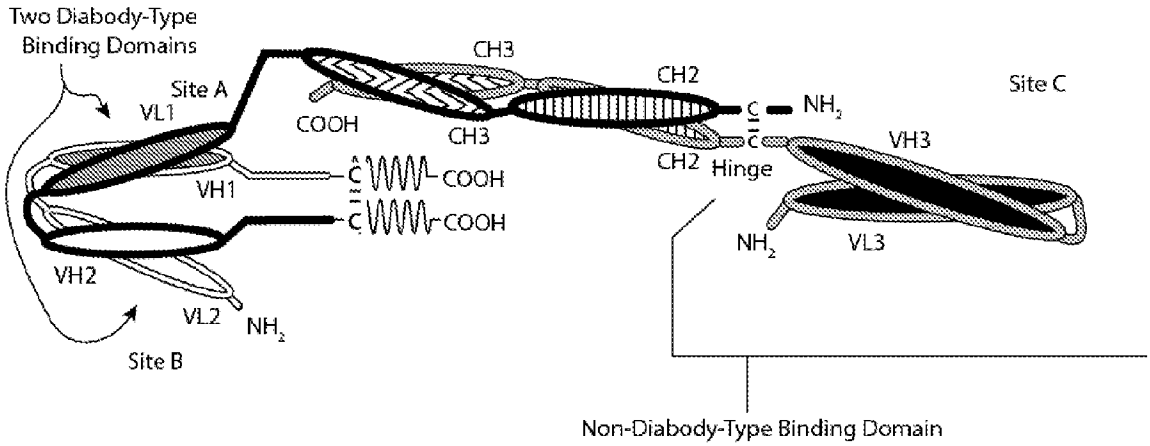

Figure 6F

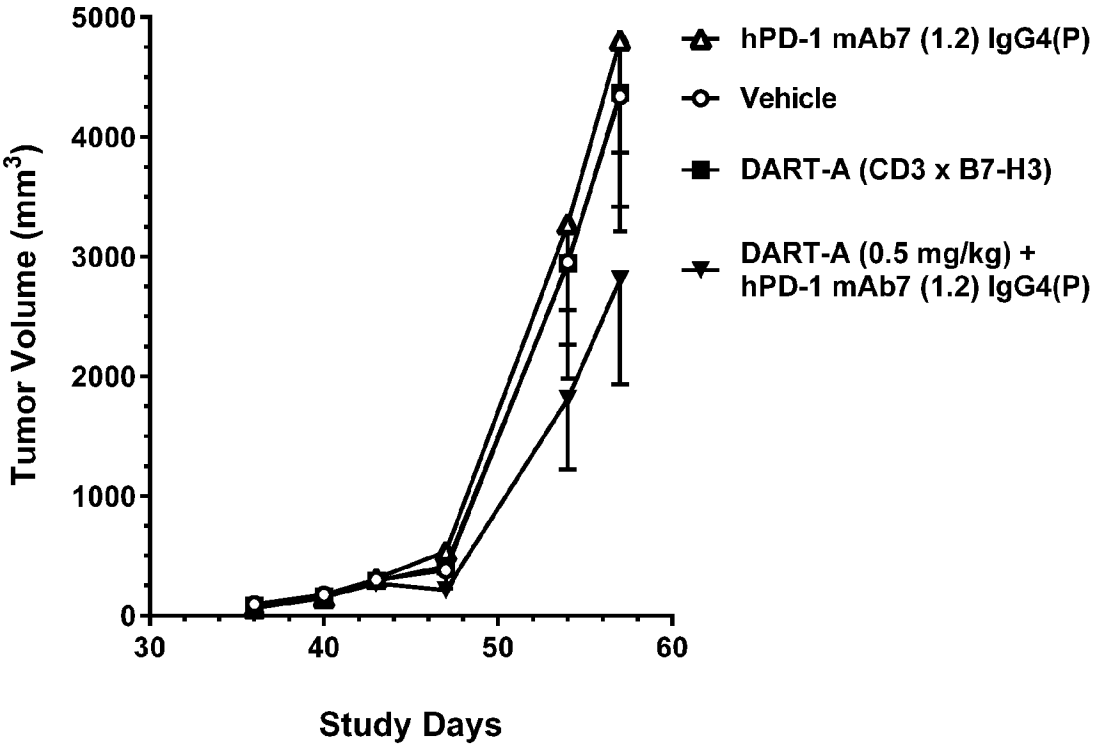
5000
4000
3000
2000
1000
0
Tumor Volume (mm3)
30
40
50
60
Study Days
hPD-1 mAb7 (1.2) IgG4(P)
Vehicle
DART-A (CD3 x B7-H3)
DART-A (0.5 mg/kg) +
hPD-1 mAb7 (1.2) IgG4(P)

Figure 7

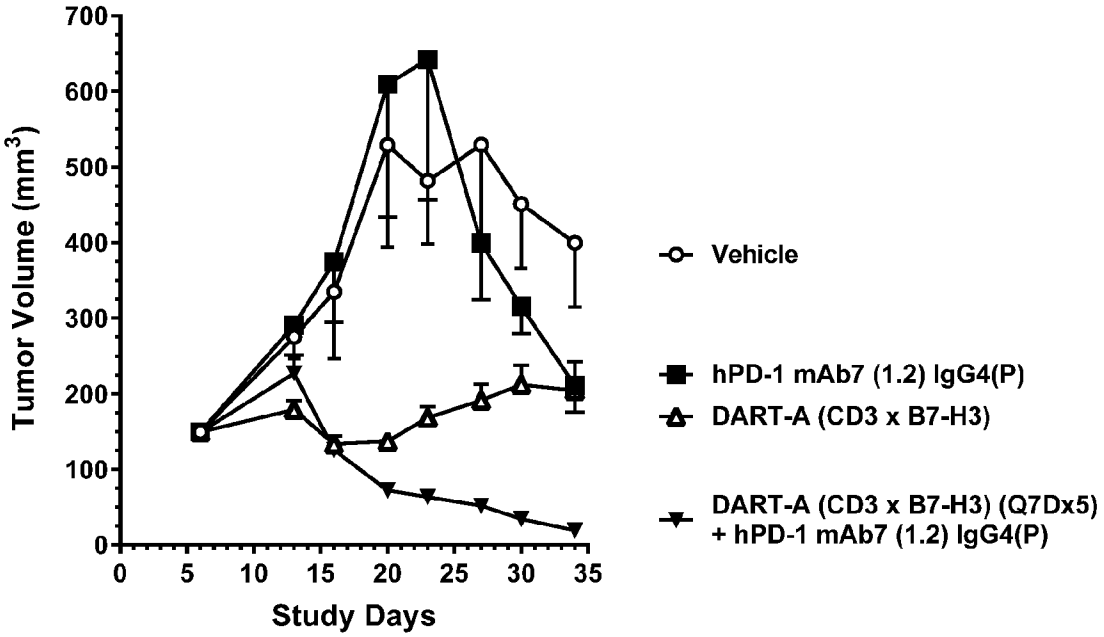
Tumor Volume (mm3)
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Study Days
Vehicle
hPD-1 mAb7 (1.2) lgG4(P)
DART-A (CD3 x B7-H3)
DART-A (CD3 x B7-H3) (Q7Dx5)
+ hPD-1 mAb7 (1.2) lgG4(P)

Figure 8A

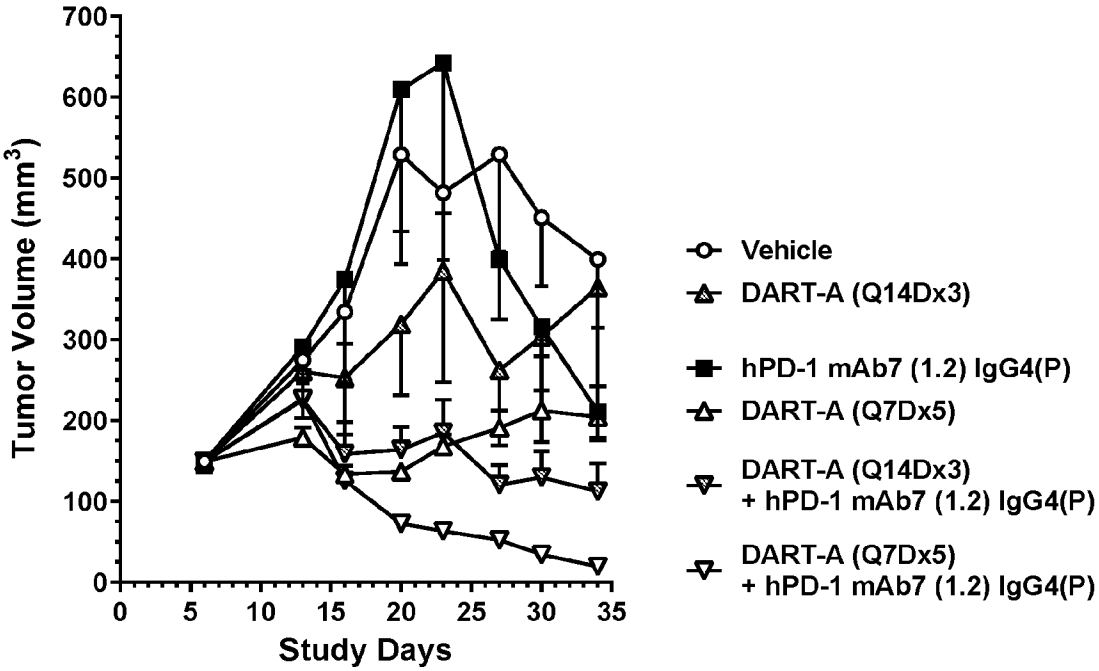
Tumor Volume (mm3)
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Study Days
Vehicle
DART-A (Q14Dx3)
hPD-1 mAb7 (1.2) IgG4(P)
DART-A (Q7Dx5)
DART-A (Q14Dx3)
+ hPD-1 mAb7 (1.2) IgG4(P)
DART-A (Q7Dx5)
+ hPD-1 mAb7 (1.2) IgG4(P)

Figure 8B

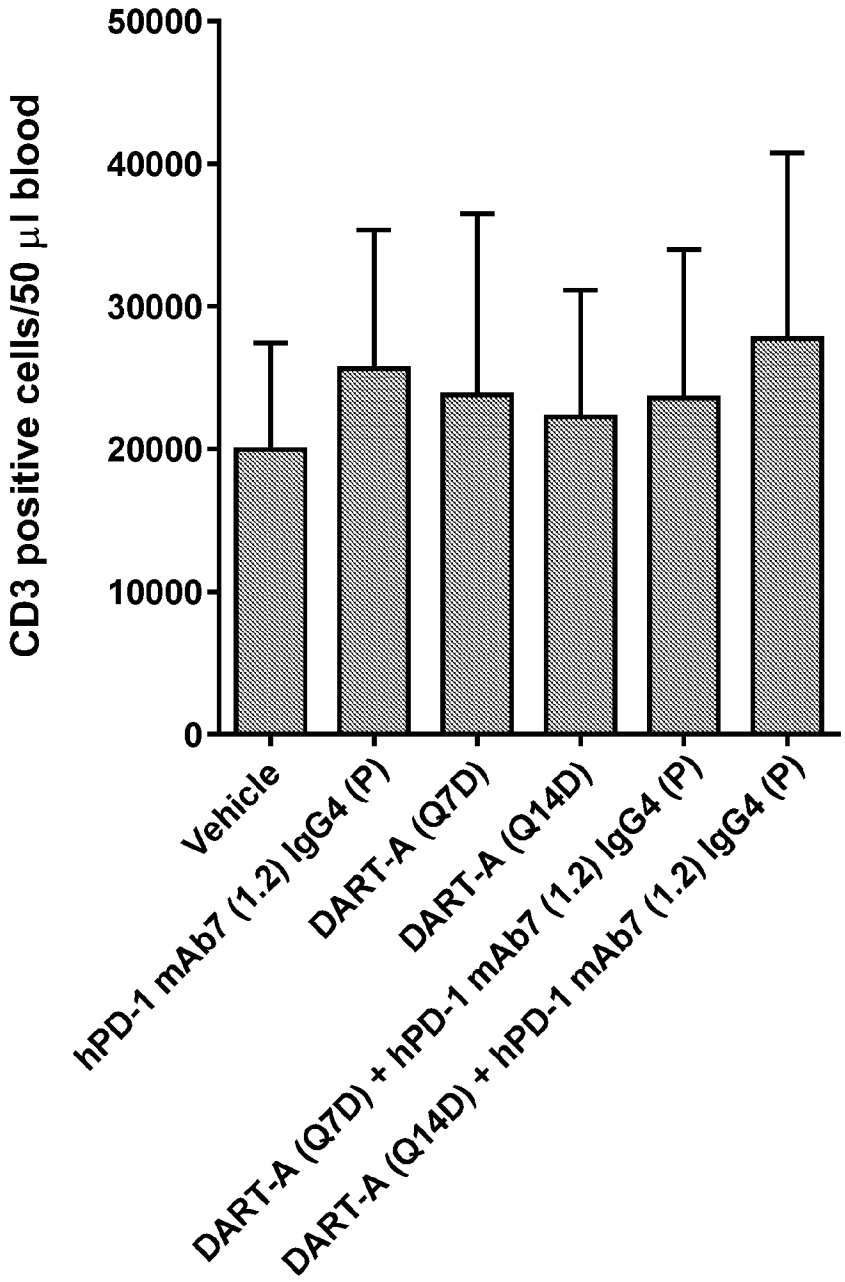
CD3 positive cells/50 µl blood
50000
40000
30000
20000
10000
0
Vehicle
hPD-1 mAb7 (1.2) IgG4 (P)
DART-A (Q7D)
DART-A (Q14D)
DART-A (Q7D) + hPD-1 mAb7 (1.2) IgG4 (P)
DART-A (Q14D) + hPD-1 mAb7 (1.2) IgG4 (P)

Figure 9

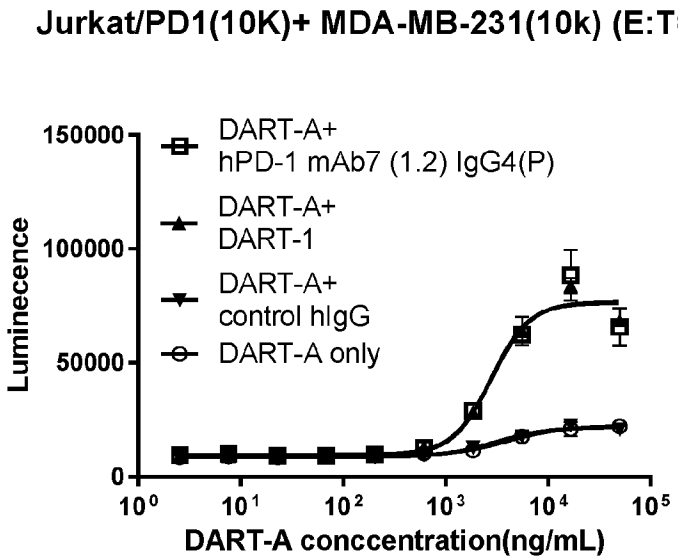

Figure 10A

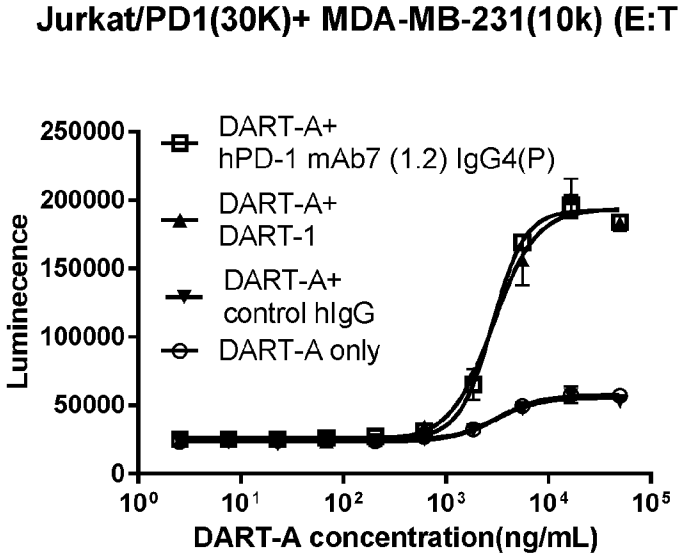

Figure 10B

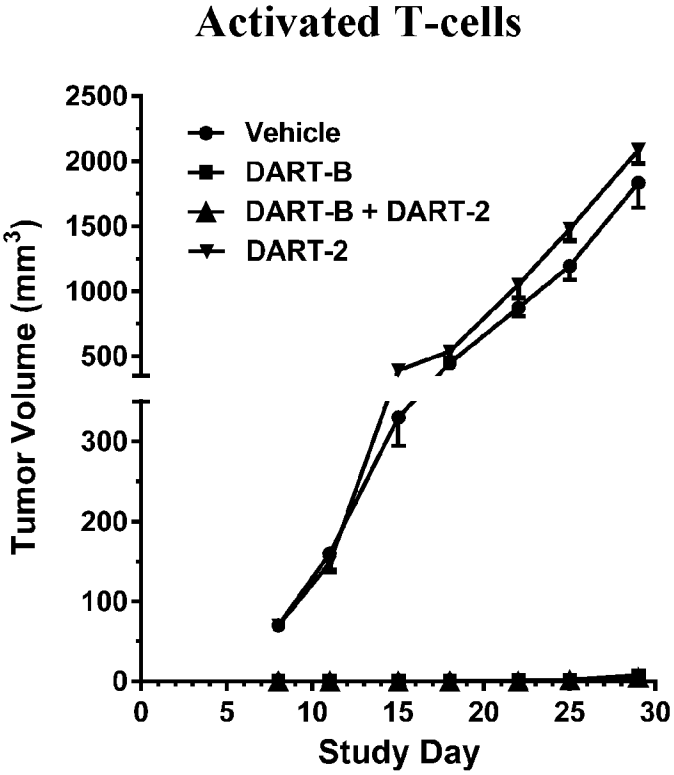

Figure 11A

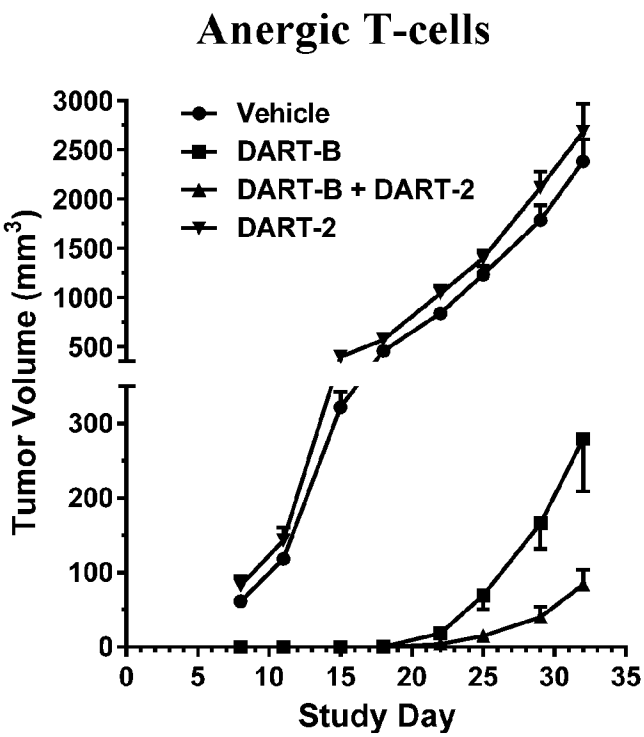

Figure 11B

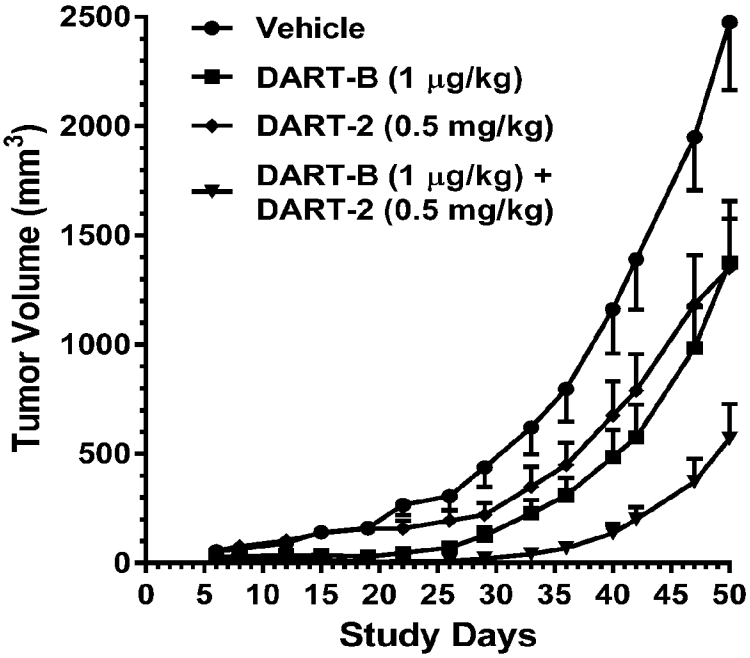

Figure 12A

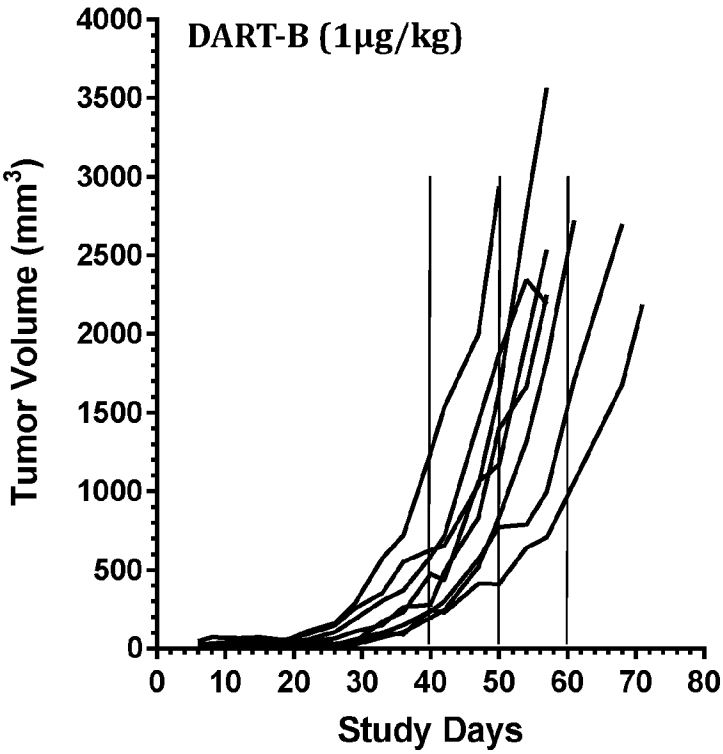

Figure 12B

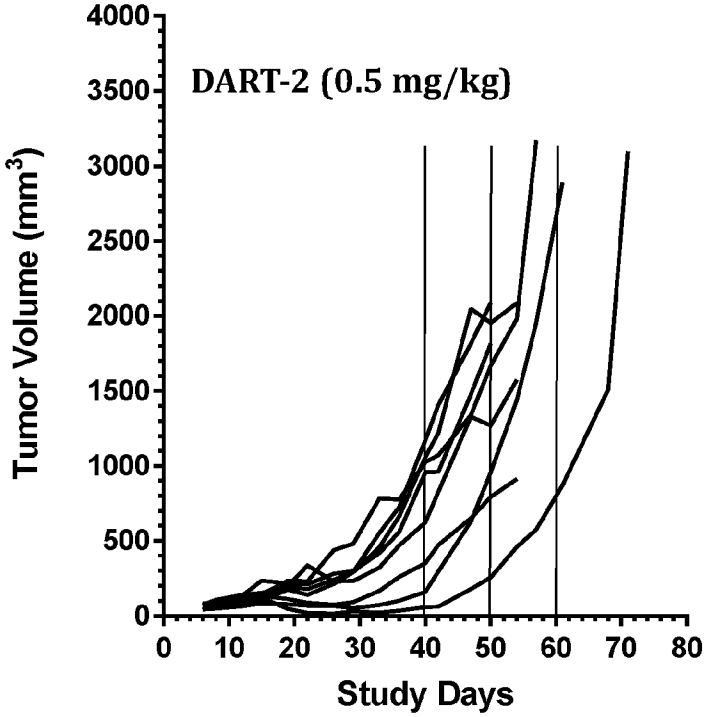

Figure 12C

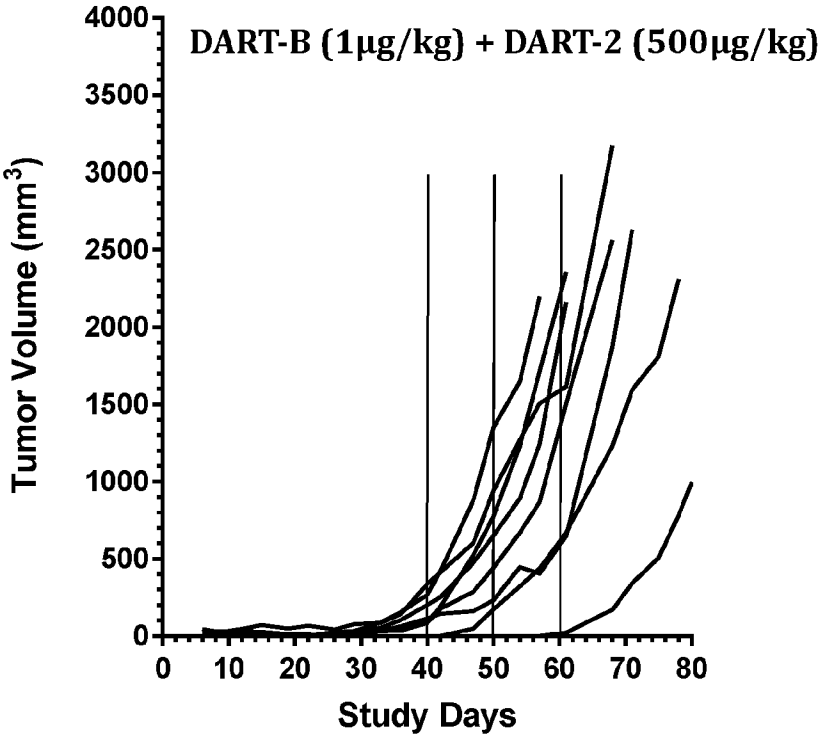

Figure 12D

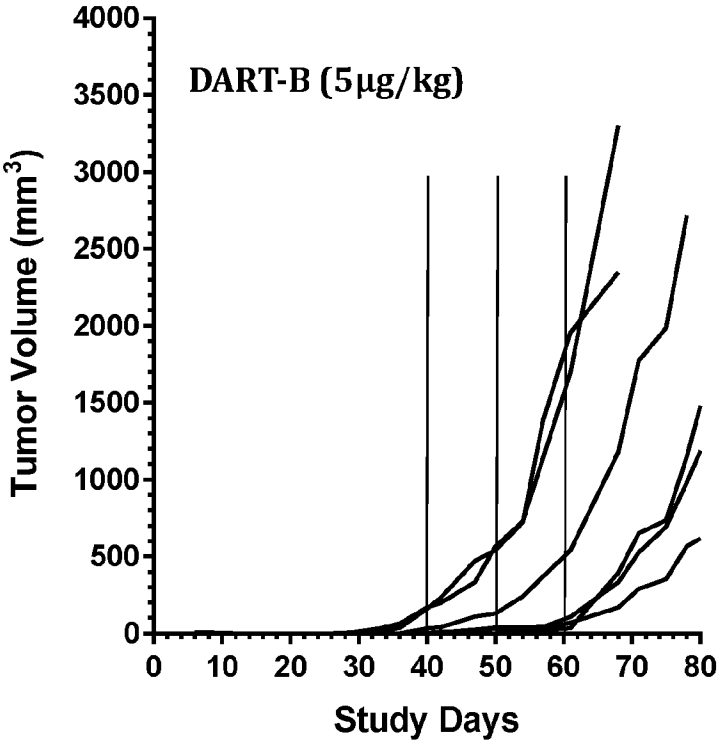

Figure 12E

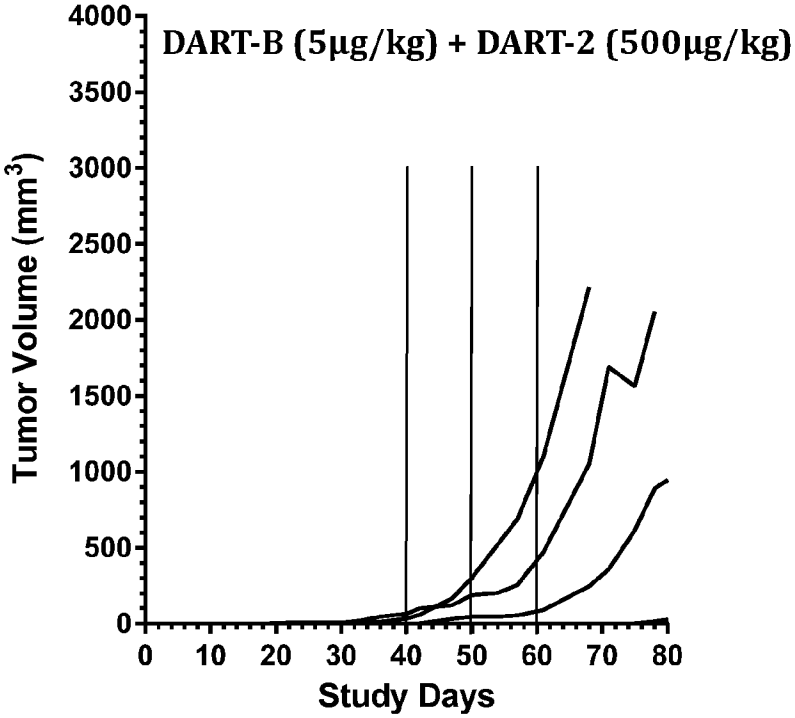

Figure 12F

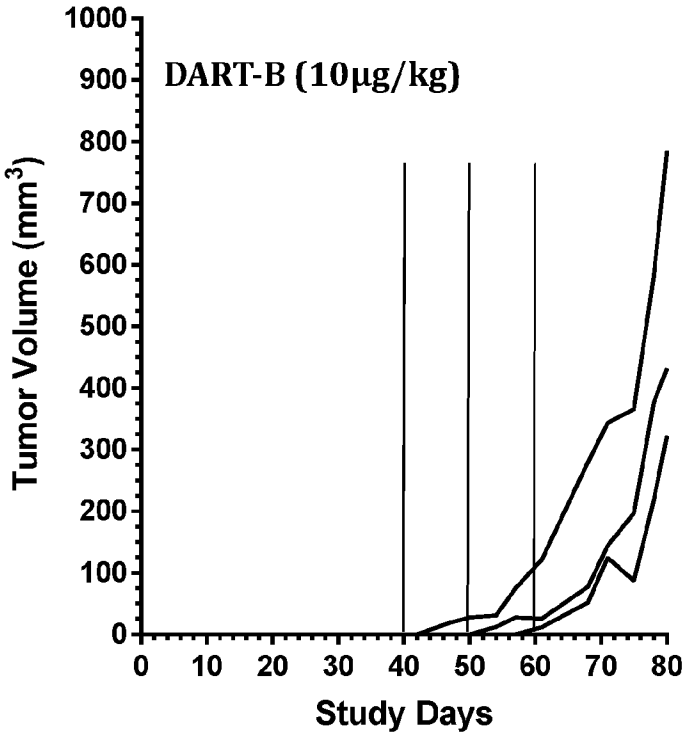

Figure 12G

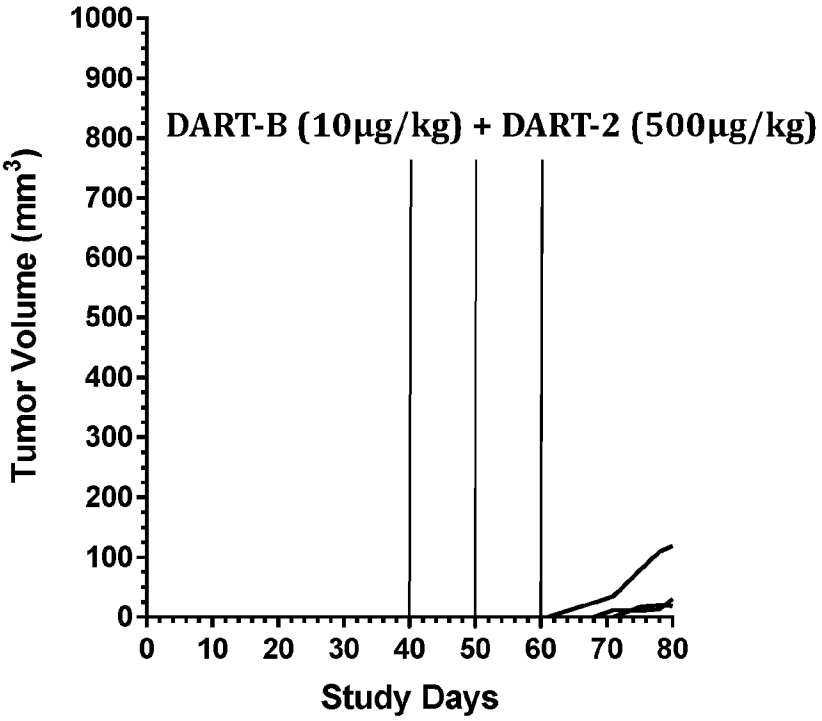

Figure 12H

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/36075

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395, C07K 16/28, A61P 35/00, A61P 31/00 (2017.01)
CPC - C07K 2317/50, A61K 2039/507, C07K 16/28, C07K 2317/73, A61K 39/39558

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/112800 A1 (REGENERON PHARMACEUTICALS, INC) 30 July 2015 (30.07.2015); abstract; para [032], [039], [048], [093], [0140], [0153], claim 45	1-10 and 20-28
Y	US 2014/0170149 A1 (NEIJSSEN et al.) 19 June 2014 (19.06.2014); abstract; para [0009]-[0010], [0046], [0047]	1-10 and 20-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
08 August 2017

Date of mailing of the international search report
01 SEP 2017

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/36075

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☒ Claims Nos.: 11-19 and 29-38
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.