

TITLE OF THE INVENTION**Bispecific Binding Molecules That Are Capable of Binding CD137 and Tumor Antigens, and Uses Thereof****CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Patent Application Serial Nos. 62/463,353 (filed on February 24, 2017; pending) and 62/597,594 (filed on December 12, 2017; pending), which applications are 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_0149PCT_ST25.txt, created on February 11, 2018, and having a size of 309,094 bytes), which file is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention is directed to binding molecules that possess one or more epitope-binding sites specific for an epitope of CD137 and one or more epitope-binding sites specific for an epitope of a tumor antigen (“TA”) (*e.g.*, a “**CD137 x TA Binding Molecule**”). In one embodiment, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific tetravalent diabodies, that are composed of two, three, four or more than four polypeptide chains and possessing two epitope-binding sites each specific for an epitope of CD137 and two epitope-binding sites each specific for an epitope of a **TA**. Alternatively, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific trivalent binding molecules composed of three or more polypeptide chains and possessing one or two epitope-binding sites each specific for an epitope of CD137 and one or two epitope-binding sites each specific for an epitope of a **TA**. The **CD137 x TA Binding Molecules** of the invention are capable of simultaneous binding to CD137, and a **TA**. The invention is directed to pharmaceutical compositions that contain any such **CD137 x TA Binding Molecules**. The invention is additionally directed to methods for the use of such molecules in the treatment of cancer and other diseases and conditions. The invention also provides novel CD137-binding molecules, and HER2/neu-binding molecules, as well as derivatives thereof and uses thereof.

BACKGROUND OF THE INVENTION

[0004] CD137 (also known as 4-1BB and “TNF receptor superfamily member 9” (“TNFRSF9”)) is a costimulatory receptor member of the tumor necrosis factor receptor superfamily, mediating CD28-dependent and independent T-cell costimulation (Vinay, D.S. and Kwon, B.S. (1998) “*Role of 4-1BB in immune responses*,” *Semin Immunol.* 10:481–489; Bartkowiak, T. *et al.* (2015) “*4-1BB Agonists: Multi-Potent Potentiators Of Tumor Immunity*,” *Frontiers Oncol.* 5:117; pp. 1-16; So, T., *et al.* (2008) “*Immune Regulation And Control Of Regulatory T Cells By OX40 And 4-1BB*,” *Cytokine & Growth Factor Rev.* 19:253-262; Croft, M. (2009) “*The Role Of TNF Superfamily Members In T-Cell Function And Diseases*,” *Nat. Rev. Immunol.* 9:271-285; Yonezawa, A. *et al.* (2015) “*Boosting cancer immunotherapy with Anti-CD137 antibody therapy*,” *Clin. Cancer Res.* 21(14):3113-3120; Li, S.Y. *et al.* (2013) “*Immunotherapy Of Melanoma With The Immunecostimulatory Monoclonal Antibodies Targeting CD137*,” *Clin. Pharmacol.* 5:47-53; Vinay, D.S. *et al.* (2012) “*Immunotherapy Of Cancer With 4-1BB*,” *Mol. Cancer Ther.* 11:1062-1070; Houot, R. *et al.* (2012) “*Boosting Antibody-Dependent Cellular Cytotoxicity Against Tumor Cells With A CD137 Stimulatory Antibody*,” *Oncoimmunology.* 1:957-958; Kwon, B.S. *et al.* (1989) “*cDNA Sequences Of Two Inducible T-Cell Genes*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1963-1967; Chen, L. *et al.* (2013) “*Molecular Mechanisms Of T Cell Co-Stimulation And Coinhibition*,” *Nat. Rev. Immunol.* 13:227-242; Yao S. *et al.* “*Advances In Targeting Cell Surface Signaling Molecules For Immune Modulation*,” *Nat. Rev. Drug Discov.* 12:130-146).

[0005] CD137 is inducibly expressed by T cells, natural killer (NK) cells, dendritic cells (DC), B cells, and other cells of the immune system (Vinay, D.S. *et al.* (2015) “*Therapeutic Potential Of Anti-CD137 (4-1BB) Monoclonal Antibodies, Expert Opinion On Therapeutic Targets*,” DOI:10.1517/14728222.2016.1091448; pp.1-14; Wang, C. *et al.* (2009) “*Immune Regulation By 4-1BB And 4-1BBL: Complexities And Challenges*,” *Immunol. Rev.* 229:192-215; Sallin, M.A. *et al.* (2014) “*The Anti-Lymphoma Activities Of Anti-CD137 Monoclonal Antibodies Are Enhanced In FcγRIII^{-/-} Mice*,” *Cancer Immunol. Immunother.* 63:947-958; Melero, I. *et al.* (2008) “*Multilayered Action Mechanisms Of CD137 (4-1BB)-Targeted Immunotherapies*,” *Trends Pharmacol Sci.* 29:383-390; Ramakrishna, V. *et al.* (2015) “*Characterization Of The Human T Cell Response To In Vitro CD27 Costimulation With Varlilumab*,” *J. Immunother. Canc.* 2:37; pp.1-13). The protein is composed of a 255-amino acid protein having a short N-terminal cytoplasmic portion, a transmembrane region, and an extracellular domain that possesses 3 cysteine-rich motifs (Schwarz, H. *et al.* (1993) “A

Receptor Induced By Lymphocyte Activation (ILA): A New Member Of The Human Nerve-Growth-Factor/Tumor-Necrosis-Factor Receptor Family,” Gene 134:295-298).

[0006] Ligation of CD137 by its ligand CD137L (4-1BBL; TNFSF9), which is mainly, though not exclusively, expressed on Antigen-Presenting Cells (APCs), evokes various T cell responses such as cell expansion, increased cytokine secretion and the prevention of activation-induced cell death (Qian, Y. *et al.* (2015) “*CD137 Ligand-Mediated Reverse Signaling Inhibits Proliferation And Induces Apoptosis In Non-Small Cell Lung Cancer,*” Med. Oncol. 32:44; pp.1-10); Sallin, M.A. *et al.* (2014) “*The Anti-Lymphoma Activities Of Anti-CD137 Monoclonal Antibodies Are Enhanced In FcγRIII^{-/-} Mice,*” Cancer Immunol. Immunother. 63:947-958; Lee, S.W. *et al.* (2009) “*4-1BB As A Therapeutic Target For Human Disease,*” Adv. Exp. Med. Biol. 647:120-129; Thum, E. *et al.* (2009) “*CD137, Implications In Immunity And Potential For Therapy,*” Front. Biosci. (Landmark Ed). 14:4173-4188; Wang, C. *et al.* (2009) “*Immune Regulation By 4-1BB And 4-1BBL: Complexities And Challenges,*” Immunol. Rev. 229(1):192-215; Long, A.H. *et al.* (2015) “*4-1BB Costimulation Ameliorates T Cell Exhaustion Induced By Tonic Signaling Of Chimeric Antigen Receptors,*” Nature Med. 21(6):581; pp. 1-13). Thus, such ligation serves to activate the immune system. However, cis-interactions between CD137 and CD137L also potently downregulate the expression of CD137L (Kwon, B. (2015) “*Is CD137 Ligand (CD137L) Signaling a Fine Tuner of Immune Responses?*,” Immune Network. 15(3): 121-124). The CD137 ligand thus functions to control the extent and kinetics of CD137-mediated immune system activation (Kwon, B. (2015) “*Is CD137 Ligand (CD137L) Signaling a Fine Tuner of Immune Responses?*,” Immune Network. 15(3): 121-124; Shuford WW *et al.* (1997) “*4-1BB Costimulatory Signals Preferentially Induce CD8⁺ T Cell Proliferation And Lead To The Amplification In Vivo Of Cytotoxic T Cell Responses,*” J. Exp. Med. 186:47-55).

[0007] Significantly, CD137 expressed on human NK cells becomes upregulated upon binding to anti-tumor antibodies (*i.e.*, antibodies that bind a tumor antigen (“TA”)) that have become bound to tumor cells (Houot, R. *et al.* (2012) “*Boosting Antibody-Dependent Cellular Cytotoxicity Against Tumor Cells With A CD137 Stimulatory Antibody,*” Oncoimmunology. 1:957-958; Kohrt, H.E. *et al.* (2014) “*Targeting CD137 Enhances The Efficacy Of Cetuximab,*” J. Clin. Invest. 124(4):2668-2682; Lin, W. *et al.* (2008) “*Fc-Dependent Expression Of CD137 On Human NK Cells: Insights Into “Agonistic” Effects Of Anti-CD137 Monoclonal Antibodies,*” Blood 112(3):699-707; Mittal, P. *et al.* (2015) “*Tumor-Unrelated CD4 T Cell*

Help Augments CD134 Plus CD137 Dual Costimulation Tumor Therapy,” J. Immunol. Nov 11. pii: 1502032; pp.1-14; Sánchez-Paulete, A.R. *et al.* (2015) “*Cancer Immunotherapy With Immunomodulatory Anti-CD137 And Anti-PD-1 Monoclonal Antibodies Requires Batf3-Dependent Dendritic Cells*,” Cancer Discov. Oct 22. pii: CD-15-0510; pp. 1-28; Wei, H. *et al.* (2014) “*Dual Targeting Of CD137 Co-Stimulatory And PD-1 Co-Inhibitory Molecules For Ovarian Cancer Immunotherapy*,” OncoImmunology 3:e28248; pp. 1-3; Seo, S.K. *et al.* (2004) “*4-1BB-Mediated Immunotherapy Of Rheumatoid Arthritis*,” Nat. Med. 10:1088-1094).

[0008] Such recognitions have led to the proposal that antibodies that are immunospecific for CD137 could be used to activate the immune system and thereby provide a therapy for cancer (Melero I. *et al.* (1997) “*Monoclonal Antibodies Against The 4-1BB T-Cell Activation Molecule Eradicate Established Tumors*,” Nat Med. 3:682-385; Sun, Y. *et al.* (2002) “*Costimulatory Molecule-Targeted Antibody Therapy Of A Spontaneous Autoimmune Disease*,” Nature Med. 8:1405-1413; Kammer, G.M. *et al.* (2002) “*Immunotherapy Tackles Lupus*,” Nat. Med. 8(12):1356-1358; Foell, J. *et al.* (2003) “*CD137 Costimulatory T Cell Receptor Engagement Reverses Acute Disease In Lupus-Prone NZB x NZW F1 Mice*,” J. Clin. Invest. 111(10):1505-1518; Mittler, R.S. *et al.* (2004) “*Anti-CD137 Antibodies In The Treatment Of Autoimmune Disease And Cancer*,” Immunol. Res. 29(1-3):197-208; Foell, J.L. *et al.* (2004) “*Engagement of The CD137 (4-1BB) Costimulatory Molecule Inhibits And Reverses The Autoimmune Process In Collagen-Induced Arthritis And Establishes Lasting Disease Resistance*,” Immunology 113(1):89-98; Sytwu, H.K. *et al.* (2003) “*Anti-4-1BB-Based Immunotherapy For Autoimmune Diabetes: Lessons From A Transgenic Non-Obese Diabetic (NOD) Model*,” J. Autoimmun. 21(3):247-254; Hernandez-Chacon JA *et al.* (2011) “*Costimulation Through The CD137/4-1BB Pathway Protects Human Melanoma Tumor-Infiltrating Lymphocytes From Activation Induced Cell Death And Enhances Antitumor Effector Function*,” J. Immunother. 34:236-250; Morales-Kastresana, A. *et al.* (2014) “*Combinations Of Immunostimulatory Antibodies With Synergistic Effects Against Spontaneous Cancer*,” OncoImmunology 3:2, e27812, pp.1-4; Sanmamed, M.F. *et al.* (2015) “*Agonists of Co-stimulation in Cancer Immunotherapy Directed Against CD137, OX40, GITR, CD27, CD28, and ICOS*,” Seminars Oncol. 42(4):640-655; Tongu, M. *et al.* (2015) “*Intermittent Chemotherapy Can Retain The Therapeutic Potential Of Anti-CD137 Antibody During The Late Tumor-Bearing State*,” Cancer Sci. 106(1):9-17; Takeda, K. *et al.* (2007) “*Combination Antibody-Based Cancer Immunotherapy*,” Cancer Sci. 98(9):1297-1302). Anti-CD137 antibodies are disclosed in US Patent Nos. 2014/0274909; 2013/0280265;

2013/0273078; 2013/0071403; 2012/0058047; 2011/0104049; 2011/0097313; 2008/0166336; 2008/0019905; 2006/0188439; 2006/0182744; 2006/0121030; and 2003/0223989.

[0009] However, despite all such prior advances, a need remains for improved compositions capable of more vigorously directing the body's immune system to attack cancer cells or pathogen-infected cells, especially at lower therapeutic concentrations. For although the adaptive immune system can be a potent defense mechanism against cancer and disease, it is often hampered by immune suppressive/evasion mechanisms in the tumor microenvironment, mediated by the reduced/absent co-stimulatory activity of CD137. Furthermore, co-inhibitory molecules expressed by tumor cells, immune cells, and stromal cells in the tumor milieu can dominantly attenuate T-cell responses against cancer cells.

[0010] As described in detail below, the present invention addresses this need by providing **CD137 x TA Binding Molecules**. Such bispecific molecules are capable of binding to tumor antigens that are expressed on the surfaces of tumor cells, and of co-localizing CD137-expressing NK cells to such tumor cells. Such co-localization upregulates the NK cells so as to promote the activation or continued activation of the immune system (*e.g.*, stimulating a cytotoxic T cell response, against tumor cells). These attributes permit such bispecific molecules to have utility in stimulating the immune system and particularly in the treatment of cancer and pathogen-associated diseases and conditions. The present invention is directed to these and other goals.

SUMMARY OF THE INVENTION

[0011] The present invention is directed to binding molecules that possess one or more epitope-binding sites specific for an epitope of CD137 and one or more epitope-binding sites specific for an epitope of a tumor antigen ("**TA**") (*e.g.*, a "**CD137 x TA Binding Molecule**"). In one embodiment, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific tetravalent diabodies, that are composed of two, three, four or more than four polypeptide chains and possessing two epitope-binding sites each specific for an epitope of CD137 and two epitope-binding sites each specific for an epitope of a **TA**. Alternatively, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific trivalent binding molecules composed of three or more polypeptide chains and possessing one or two epitope-binding sites each specific for an epitope of CD137 and one or two epitope-binding sites each specific for an epitope of a **TA**. The **CD137 x TA Binding Molecules** of the invention are capable of simultaneous binding to CD137, and a **TA**. The invention is

directed to pharmaceutical compositions that contain any such **CD137 x TA Binding Molecules**. The invention is additionally directed to methods for the use of such molecules in the treatment of cancer and other diseases and conditions. The invention also provides novel CD137-binding molecules, and HER2/neu-binding molecules, as well as derivatives thereof and uses thereof.

[0012] The present invention provides **CD137 x TA Binding Molecules** that are monovalent in that they are capable of binding to only one copy of an epitope of CD137 and to only one copy of an epitope of a **TA**, but are bispecific in that a single such diabody is able to bind simultaneously to the epitope of CD137 and to the epitope of a **TA**. The present invention is, however, particularly directed to **CD137 x TA Binding Molecules** that are composed of polypeptide chains that associate with one another in a heterodimeric manner to form two binding sites each specific for an epitope of CD137 and two binding sites each specific for an epitope of a **TA**. Such preferred **CD137 x TA Binding Molecules** of the invention are termed “**bispecific tetravalent**.” The present invention is also particularly directed to **CD137 x TA Binding Molecules** that are composed of polypeptide chains that associate with one another in a heterodimeric manner to form two binding sites each specific for an epitope of CD137 and one binding sites each specific for an epitope of a **TA**. Such preferred **CD137 x TA Binding Molecules** of the invention are termed “**bispecific trivalent**.”

[0013] The present invention provides **CD137 x TA Binding Molecules** that comprise three polypeptide chains (a “first,” “second” and “third” polypeptide chain), wherein the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are covalently bonded to one another. The preferred **CD137 x TA Binding Molecules** of the invention comprise four polypeptide chains (a “first,” “second,” “third,” and “fourth” polypeptide chain), wherein the first and second polypeptide chains are covalently bonded to one another, the third and fourth polypeptide chains are covalent bonded to one another, and the first and third polypeptide chains are covalently bonded to one another. Also preferred are **CD137 x TA Binding Molecules** of the invention comprising five polypeptide chains (a “first,” “second,” “third,” “fourth,” and “fifth” polypeptide chain), wherein the first and second polypeptide chains are covalently bonded to one another, the third and fourth polypeptide chain are covalent bonded to one another, the third and fifth polypeptide chains are covalent bonded to one another, and the first and third polypeptide chains are covalently bonded to one another.

[0014] In detail, the invention provides a **CD137 x TA** Binding Molecule, wherein said Binding Molecule is capable of specific binding to an epitope of CD137 and an epitope of a tumor antigen (**TA**), and wherein said **CD137 x TA** Binding Molecule comprises a first Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a first Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; and wherein:

- (A) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL15 (SEQ ID NO:222)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (B) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL14 (SEQ ID NO:221)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (C) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL11 (SEQ ID NO:218)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (D) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL10 (SEQ ID NO:217)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (E) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL6 (SEQ ID NO:213)**; and

- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (F) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL4 (SEQ ID NO:211)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (G) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH (SEQ ID NO:74)**;
- (H) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-4 VL (SEQ ID NO:91)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-4 VH (SEQ ID NO:90)**;
- (I) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-5 VL (SEQ ID NO:97)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-5 VH (SEQ ID NO:96)**;
- (J) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1A (SEQ ID NO:83)**;
- (K) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (L) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1C (SEQ ID NO:85)**;

or

- (M) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1D (SEQ ID NO:86)**.

[0015] The invention further concerns such a **CD137 x TA Binding Molecule**, wherein the first Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 (SEQ ID NO:77)**; or
- (B) **hCD137 MAB-4 (SEQ ID NO:92)**;

and/or wherein the first Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 (SEQ ID NO:82)**; or
- (B) **hCD137 MAB-4 (SEQ ID NO:93)**.

[0016] The invention further concerns such a **CD137 x TA Binding Molecule**, wherein the first Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 VH1E (SEQ ID NO:208)**;
- (B) **hCD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (C) **hCD137 MAB-3 VH1A (SEQ ID NO:83)**;
- (D) **hCD137 MAB-3 VH1 (SEQ ID NO:76)**;
- (E) **hCD137 MAB-3 VH1C (SEQ ID NO:85)**;
- (F) **hCD137 MAB-3 VH1D (SEQ ID NO:86)**;
- (G) **hCD137 MAB-3 VH1F (SEQ ID NO:209)**;
- (H) **hCD137 MAB-3 VH1G (SEQ ID NO:210)**; or
- (I) **hCD137 MAB-4 VH1 (SEQ ID NO:92)**.

[0017] The invention further concerns such **CD137 x TA Binding Molecules**, wherein the first Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 VL15 (SEQ ID NO:222)**;
- (B) **hCD137 MAB-3 VL14 (SEQ ID NO:221)**;
- (C) **hCD137 MAB-3 VL1 (SEQ ID NO:87)**;
- (D) **hCD137 MAB-3 VL2 (SEQ ID NO:88)**;
- (E) **hCD137 MAB-3 VL3 (SEQ ID NO:89)**;
- (F) **hCD137 MAB-3 VL4 (SEQ ID NO:211)**;
- (G) **hCD137 MAB-3 VL5 (SEQ ID NO:212)**;
- (H) **hCD137 MAB-3 VL6 (SEQ ID NO:213)**;

- (I) hCD137 MAB-3 VL7 (SEQ ID NO:214);
- (J) hCD137 MAB-3 VL8 (SEQ ID NO:215);
- (K) hCD137 MAB-3 VL9 (SEQ ID NO:216);
- (L) hCD137 MAB-3 VL10 (SEQ ID NO:217);
- (M) hCD137 MAB-3 VL11 (SEQ ID NO:218);
- (N) hCD137 MAB-3 VL12 (SEQ ID NO:219);
- (O) hCD137 MAB-3 VL13 (SEQ ID NO:220);
- (P) hCD137 MAB-4 VL1 (SEQ ID NO:94); or
- (Q) hCD137 MAB-4 VL2 (SEQ ID NO:95).

[0018] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (**TA**) is selected from the group of tumor antigens consisting of: 19.9; oncofetal protein 5T4; antigen 4.2; A33; AFP; ALCAM; BAGE; beta-catenin; CA125; Carboxypeptidase M; B1;CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD30; CD33; CD36; CD46; CD52; CD79a/CD79b; CD123; CD317; CEA; CEACAM5; CEACAM6; CO-43; CO-514; CTLA-1; CTLA-4; Cytokeratin 8; E1 series; EGF-R; an Ephrin receptor; Erb; F3; FC10.2; a GAGE GD2; GD3; GD49; GM2; GM3; GICA 19-9; gp37; gp75; gp100; HER-2/neu; human B-lymphoma antigen-CD20; human milk fat globule antigen; human papillomavirus-E6/human papillomavirus-E7; HMW-MAA; I antigen; ITGB6; IL13R α 2; JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; KS 1/4; KSA; L6; L20; LEA; LUCA-2; M1:22:25:8; M18; M39; a MAGE; MART; Myl; MUC-1; MUM-1; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; PSA; PSMA; PEMA; PIPA; prostatic acid phosphate; R24; ROR1; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; TAG-72; TL5; TNF- α receptor; TNF- β receptor; TNF- γ receptor; TRA-1-85; Transferrin Receptor; TSTA; and VEGF-R.

[0019] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (**TA**) is selected from the tumor antigens of **Table 1**, and particularly wherein the tumor antigen (**TA**) is: HER2/neu, EphA2 or 5T4.

[0020] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (**TA**) is HER2/neu and wherein the **CD137 x TA** Binding Molecule comprises a second Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a first Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; and wherein

- (A) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **HER2 MAB-1 VL (SEQ ID NO:63)**; and
- (B) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **HER2 MAB-1 VH (SEQ ID NO:62)**.

[0021] The invention further concerns such a **CD137 x TA Binding Molecule**, wherein:

- (A)
 - (1) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
 - (2) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL2 (SEQ ID NO:68)**;
 - or
 - (3) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL3 (SEQ ID NO:69)**;
- and
- (B)
 - (1) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
 - (2) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH2 (SEQ ID NO:65)**;
 - or
 - (3) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

[0022] The invention further concerns such a **CD137 x TA Binding Molecule**, wherein the second Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
- (B) **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
- (C) **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

[0023] The invention further concerns such a **CD137 x TA Binding Molecule**, wherein the second Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
- (B) **hHER2 MAB-1 VL2 (SEQ ID NO:68)**; or
- (C) **hHER2 MAB-1 VL3 (SEQ ID NO:69)**.

[0024] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (TA) is 5T4 and wherein the CD137 x TA Binding Molecule comprises a second Light Chain Variable Domain that comprises a CDRL1, CDRL2 and CDRL3, and a first Heavy Chain Variable Domain that comprises a CDRH1, CDRH2 and CDRH3; and wherein:

- (I) (A) the second Light Chain Variable Domain CDR_L1, CDR_L2, and CDR_L3 are the Light Chain CDRs of **5T4 MAB-1 VL (SEQ ID NO:135)**; and
- (B) the second Heavy Chain Variable Domain CDR_H1, CDR_H2, and CDR_H3 are the Heavy Chain CDRs of **5T4 MAB-1 VH (SEQ ID NO:134)**; or
- (II) (A) the second Light Chain Variable Domain CDR_L1, CDR_L2, and CDR_L3 are the Light Chain CDRs of **5T4 MAB-2 VL (SEQ ID NO:137)**; and
- (B) the second Heavy Chain Variable Domain CDR_H1, CDR_H2, and CDR_H3 are the Heavy Chain CDRs of **5T4 MAB-2 VH (SEQ ID NO:136)**.

[0025] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the second Heavy Chain Variable Domain comprises the amino acid sequence of: **MAB-1 VH1 (SEQ ID NO:135)**.

[0026] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the second Light Chain Variable Domain comprises the amino acid sequence of: **MAB-1 VH1 (SEQ ID NO:136)**.

[0027] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the molecule is a bispecific tetravalent Fc-bearing diabody comprising a first, a second, a third, and a fourth polypeptide chain, wherein the polypeptide chains form a covalently bonded complex.

[0028] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (TA) is HER2/neu and wherein:

- (I) (A) the first and the third polypeptide chain have the amino acid sequence of **SEQ ID NO:100**; and
 - (B) the second and the fourth polypeptide chain have the amino acid sequence of **SEQ ID NO:101**;
- or
- (II) (A) the first and the third polypeptide chain have the amino acid sequence of **SEQ ID NO:102**; and

- (B) the second and the fourth polypeptide chain have the amino acid sequence of **SEQ ID NO:103**.

[0029] The invention further concerns such **CD137 x TA** Binding Molecules, wherein the molecule is bispecific and tetravalent, and comprises a first, a second, a third, a fourth, and a fifth polypeptide chain, wherein the polypeptide chains form a covalently bonded complex.

[0030] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein the tumor antigen (**TA**) is HER2/neu and wherein:

- (I) (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:104**;
- (B) the second and the fifth polypeptide chain have the amino acid sequence of **SEQ ID NO:105**;
- (C) the third polypeptide chain has the amino acid sequence of **SEQ ID NO:106**; and
- (D) the fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:107**;

or

- (II) (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:104**;
- (B) the second and the fifth polypeptide chain have the amino acid sequence of **SEQ ID NO:105**;
- (C) the third polypeptide chain has the amino acid sequence of **SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, or SEQ ID NO:118**; and
- (D) the fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, or SEQ ID NO:123**.

[0031] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein said molecule is bispecific and trivalent, and comprises a first, a second, a third, and a fourth, polypeptide chain, wherein said polypeptide chains form a covalently bonded complex.

[0032] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein said tumor antigen (TA) is HER2/neu and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, or SEQ ID NO:196**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, or SEQ ID NO:201**;
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:104**; and
- (D) said fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:105**.

[0033] The invention further concerns such a **CD137 x TA** Binding Molecule, wherein said tumor antigen (TA) is 5T4 and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, or SEQ ID NO:229**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, or SEQ ID NO:230**;
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:231**; and
- (D) said fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:232**.

[0034] The invention further concerns a pharmaceutical composition comprising any of the above-described **CD137 x TA** Binding Molecules and a physiologically acceptable carrier.

[0035] The invention further concerns the use of any of the above-described **CD137 x TA** Binding Molecules or such pharmaceutical composition in the treatment of a disease or condition associated with or characterized by the expression of the tumor antigen (TA), and particularly wherein the disease or condition associated with or characterized by the expression of the tumor antigen (TA) is cancer.

[0036] The invention further concerns a CD137 binding molecule that comprises a Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; wherein:

- (A) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL15 (SEQ ID NO:222)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (B) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL14 (SEQ ID NO:221)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (C) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL11 (SEQ ID NO:218)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (D) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL10 (SEQ ID NO:217)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (E) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL6 (SEQ ID NO:213)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (F) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL4 (SEQ ID NO:211)**; and
- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (G) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
- (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH (SEQ ID NO:74)**;

- (H) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-4 VL (SEQ ID NO:91)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-4 VH (SEQ ID NO:90)**;
 - (I) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-5 VL (SEQ ID NO:97)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-5 VH (SEQ ID NO:96)**;
 - (J) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1A (SEQ ID NO:83)**;
 - (K) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
 - (L) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1C (SEQ ID NO:85)**;
- or
- (M) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1D (SEQ ID NO:86)**.

[0037] The invention further concerns the embodiment of such a CD137 binding molecule, wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 (SEQ ID NO:77)**; or
- (B) **hCD137 MAB-4 (SEQ ID NO:92)**.

[0038] The invention further concerns the embodiment of such a CD137 binding molecule, wherein the Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 (SEQ ID NO:82);** or
- (B) **hCD137 MAB-4 (SEQ ID NO:93).**

[0039] The invention further concerns the embodiment of such CD137 binding molecules, wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 VH1 (SEQ ID NO:76);**
- (B) **hCD137 MAB-3 VH1A (SEQ ID NO:83);**
- (C) **hCD137 MAB-3 VH1B (SEQ ID NO:84);**
- (D) **hCD137 MAB-3 VH1C (SEQ ID NO:85);**
- (E) **hCD137 MAB-3 VH1D (SEQ ID NO:86);**
- (F) **hCD137 MAB-3 VH1E (SEQ ID NO:208);**
- (G) **hCD137 MAB-3 VH1F (SEQ ID NO:209);**
- (H) **hCD137 MAB-3 VH1G (SEQ ID NO:210);** or
- (I) **hCD137 MAB-4 VH1 (SEQ ID NO:92).**

[0040] The invention further concerns the embodiment of such CD137 binding molecules, wherein the Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hCD137 MAB-3 VL15 (SEQ ID NO:222);**
- (B) **hCD137 MAB-3 VL14 (SEQ ID NO:221);**
- (C) **hCD137 MAB-3 VL1 (SEQ ID NO:87);**
- (D) **hCD137 MAB-3 VL2 (SEQ ID NO:88);**
- (E) **hCD137 MAB-3 VL3 (SEQ ID NO:89);**
- (F) **hCD137 MAB-3 VL4 (SEQ ID NO:211);**
- (G) **hCD137 MAB-3 VL5 (SEQ ID NO:212);**
- (H) **hCD137 MAB-3 VL6 (SEQ ID NO:213);**
- (I) **hCD137 MAB-3 VL7 (SEQ ID NO:214);**
- (J) **hCD137 MAB-3 VL8 (SEQ ID NO:215);**
- (K) **hCD137 MAB-3 VL9 (SEQ ID NO:216);**
- (L) **hCD137 MAB-3 VL10 (SEQ ID NO:217);**
- (M) **hCD137 MAB-3 VL11 (SEQ ID NO:218);**
- (N) **hCD137 MAB-3 VL12 (SEQ ID NO:219);**
- (O) **hCD137 MAB-3 VL13 (SEQ ID NO:220);**

- (P) **hCD137 MAB-4 VL1 (SEQ ID NO:94)**; or
(Q) **hCD137 MAB-4 VL2 (SEQ ID NO:95)**.

[0041] The invention further concerns the embodiment of such CD137 binding molecules, wherein the molecule is an antibody or an antigen binding fragment thereof

[0042] The invention further concerns a pharmaceutical composition comprising any of the above-described CD137 Binding Molecules, and a physiologically acceptable carrier.

[0043] The invention further concerns the use of any of the above-described CD137 Binding Molecules, or such pharmaceutical composition, in the treatment of a disease or condition associated with a suppressed immune system or characterized by the expression of a tumor antigen (**TA**).

[0044] The invention further concerns such use wherein the condition associated with a suppressed immune system or characterized by the expression of the tumor antigen (**TA**) is cancer.

[0045] The invention further concerns a HER2/neu Binding Molecule that comprises a Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; wherein:

- (A) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **HER2 MAB-1 VL (SEQ ID NO:63)**; and
(B) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **HER2 MAB-1 VH (SEQ ID NO:62)**.

[0046] The invention further concerns the embodiment of such HER2/neu Binding Molecules wherein:

- (A) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
(2) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL2 (SEQ ID NO:68)**; or
(3) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL3 (SEQ ID NO:69)**;

and

- (B) (1) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
- (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
- (3) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

[0047] The invention further concerns the embodiment of such HER2/neu Binding Molecules wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:

- (A) **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
- (B) **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
- (C) **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

[0048] The invention further concerns the embodiment of such HER2/neu Binding Molecules wherein the Light Chain Variable Domain comprises the amino acid sequence of:

- (A) **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
- (B) **hHER2 MAB-1 VL2 (SEQ ID NO:68)**; or
- (C) **hHER2 MAB-1 VL3 (SEQ ID NO:69)**.

[0049] The invention further concerns the embodiment of such HER2/neu Binding Molecules wherein the molecule is an antibody or an antigen binding fragment thereof.

[0050] The invention further concerns a pharmaceutical composition comprising any of the above-described HER2/neu Binding Molecules and a physiologically acceptable carrier

[0051] The invention further concerns the use of any of the above-described HER2/neu Binding Molecule, or such pharmaceutical composition, in the treatment of a disease or condition associated with or characterized by the expression of HER2/neu, and particularly wherein the condition associated with or characterized by the expression of HER2/neu is cancer.

[0052] The invention further concerns methods of enhancing the activity of a tumor targeting agent comprising administering such tumor target agent in combination with the any of the above-described **CD137 x TA** Binding Molecules, or a pharmaceutical composition comprising the same. The invention further concerns such methods further comprising administering a PD-1/PD-L1 checkpoint inhibitor and particularly wherein such checkpoint

inhibitor is an anti-PD-1 antibody or an anti-PD-L1 antibody. The invention particularly concerns such methods wherein the tumor target agent is an antibody, an epitope binding fragment of an antibody, or an agent that mediates T-cell redirected killing of a target cell.

[0053] The invention further concerns methods method of treating a disease or condition associated with a suppressed immune system or characterized by the expression of a tumor antigen (**TA**) comprising administering to a subject in need thereof any of the above-described **CD137 x TA** Binding Molecules, or a pharmaceutical composition comprising the same. The invention particularly, concerns such methods wherein the condition associated with a suppressed immune system or characterized by the expression of the tumor antigen (**TA**) is cancer. The invention also concerns such methods further comprising administering a tumor targeting agent, and particularly wherein the tumor target agent is an antibody, an epitope binding fragment of an antibody, or an agent that mediates T-cell redirected killing of a target cell. The invention further concerns such methods further comprising administering a PD-1/PD-L1 checkpoint inhibitor, and particularly wherein such checkpoint inhibitor is an anti-PD-1 antibody or an anti-PD-L1 antibody.

[0054] The invention further concerns the above uses and methods wherein the cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, 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, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary

tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0055] The invention particularly concerns the above uses and methods wherein the cancer is selected from the group consisting: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).

BRIEF DESCRIPTION OF THE DRAWINGS

[0056] **Figures 1A-1B** provide schematics of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a linker (**Figure 1A**) and/or in the Heterodimer-Promoting Domain (**Figure 1B**). VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The wavy line (WWW) in this and all of the Figures providing schematic presentations of binding molecule domains represents one or more optional Heterodimer-Promoting Domains, that is/are preferably present.

[0057] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0058] **Figures 3A-3E** provide schematics showing representative covalently bonded tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide chain of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. 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 sites 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 sites 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 sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc Region-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc Region-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-Region-Containing diabody, which contains antibody CH1 and CL domains. **Figures 3D-3E** illustrate how selection of the binding domains shown in **Figure 3B** can result in a **CD137 x TA** Binding Molecule having two binding sites specific for an epitope of CD137 and two binding sites specific for an epitope of a TA. **Figures 3D-3E** illustrate how domains may be selected to yield **CD137 x TA** Binding Molecules having differing orientations (*i.e.*, **Figure 3D** employs, a VL CD137 Domain as the VL1 Domain of the Binding Molecule, a VH CD137 Domain as the VH1 Domain of the Binding Molecule, a VL TA Domain as the VL2 Domain of the Binding Molecule, and a VH TA Domain as the VH2 Domain of the Binding Molecule. In contrast, **Figure 3E** employs, a VL TA Domain as the VL1 Domain of the Binding Molecule, a VH TA Domain as the VH1 Domain of the Binding Molecule, a VL CD137 Domain as the VL2 Domain of the Binding Molecule, and a VH CD137 Domain as the VH2 Domain of the Binding Molecule). As provided below, 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.

[0059] **Figures 4A and 4B** provide schematics of a representative covalently bonded diabody molecule having two epitope-binding sites 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 Region. 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.

[0060] **Figures 5A-5D** provide schematics of a representative covalently bonded Binding Molecule having four epitope-binding sites composed of five polypeptide chains. **Figure 5A** shows the general structure of such a **CD137 x TA** Binding Molecule. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of an Fc Region. 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. **Figure 5B** shows the structure of an alternative preferred **CD137 x TA** Binding Molecule in which the variable domains shown in **Figure 5A** have been selected to yield a resultant **CD137 x TA** Binding Molecule that possesses two non-diabody type binding domains specific for an illustrative **TA**, HER2/neu, and two diabody-type binding domains specific for CD137. **Figure 5C** shows the structure of an alternative preferred **CD137 x TA** Binding Molecule in which the variable domains shown in **Figure 5A** have been selected to yield a resultant **CD137 x TA** Binding Molecule that possesses two non-diabody type binding domains specific for CD137 and two diabody-type binding domains specific for HER2/neu. **Figure 5D** shows the structure of an alternative preferred **CD137 x TA** Binding Molecule in which the variable domains shown in **Figure 5A** have been selected to yield a resultant **CD137 x TA** Binding Molecule that possesses two non-diabody type binding domains specific for an epitope of CD137, one diabody-type binding domains specific for an epitope of HER2/neu and a second diabody-type binding domain specific for an epitope of CD137. Such CD137 epitopes may be the same or different. As will be appreciated, by proper selection of the binding domains shown in **Figure 5A**, any three of the binding domains could have been selected to bind an epitope of CD137. Likewise, any three of the binding domains could have been selected to bind an epitope of HER2/neu. As provided below, 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.

[0061] **Figures 6A-6F** provide schematics of representative Fc Region-containing trivalent binding molecules having three epitope-binding sites. **Figures 6A** illustrates 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 C-terminal to an Fc Region. **Figures 6B-6C** show the structure of illustrative preferred **CD137 x TA** Binding Molecule in which the variable domains shown in **Figures 6A** have been selected to yield a resultant **CD137 x TA** Binding Molecule that possesses a non-diabody type binding domains specific for CD137, a diabody-type binding domain that is specific for an illustrative **TA**, HER2/neu, and a second diabody-type binding domain that is specific for CD137. **Figure 6D** illustrates 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 C-terminal to an Fc Region. The molecules in **Figures 6A-6D** comprise four chains. **Figures 6E** and **6F**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains N-terminal to an Fc Region, 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 6G** and **6H**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains C-terminal to an Fc Region, 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-6H** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0062] **Figure 7** shows the binding curves of the anti-CD137 antibodies urelumab and utomilumab (**CD137 MAB-1** and **CD137 MAB-2**, respectively) and several novel chimeric CD137 mAbs (**chCD137 MAB-3**; **chCD137 MAB-4** and **chCD137 MAB-5**) to activated CD8⁺ T cells.

[0063] **Figure 8** shows the induction after 72 hours of IL-2 secretion by pan T cells upon stimulation with dry-coated anti-CD3 antibody (3 µg/mL-50 µg/mL) in the presence of the anti-CD137 antibodies urelumab or utomilumab (**CD137 MAB-1** or **CD137 MAB-2**, respectively) or the novel chimeric anti-CD137 antibodies (**chCD137 MAB-3**, **chCD137 MAB-4** or **chCD137 MAB-5**) that had been crosslinked with 4x hFc F(ab)[']₂. The cross-linked antibodies (Ab + αhFc) were used at 0.1, 1.0 or 10 µg/mL. The following controls are also plotted: stimulated pan T cells treated with isotype control antibody, hFc F(ab)[']₂ alone, or untreated cells.

[0064] **Figure 9** shows the induction after 72 hours of IFN- γ secretion by pan T cells upon stimulation with anti-CD3 beads in the presence of cross-linked chimeric anti-CD137 antibodies (**chCD137 MAB-3** or **chCD137 MAB-5**) (1 μ g/mL CD-137 antibody + 4 μ g/mL hFc F(ab) $'_2$) $\pm$ JIMT-1(HER2/neu $^{++}$) target cells, in the presence or absence of ligand (1 μ g/mL CD-137L-His). The following control samples are also plotted: stimulated pan T cells $\pm$ JIMT-1 cells, $\pm$ hFc F(ab) $'_2$, and untreated/unstimulated pan T cells and JIMT-1 cells.

[0065] **Figures 10A-10B** show the ability of **CD137 x TA Binding Molecules** to bind to CD137 of activated CD8 $^+$ T cells as measured by FACS using FITC-labeled CD8 and α hFc APC. **Figure 10A: DART-B** and **DART-C** (comprising **hCD137 MAB-3** and **hHER2 MAB-1** domains), **DART-D** and **DART-E** (comprising **CD137 MAB-3** and **hHER2 MAB-1** domains), and control molecules, **DART-3** (comprising **hHER2 MAB-1** and variant palivizumab domains) and **DART-6** (comprising variant palivizumab and **CD137 MAB-3** domains). **Figure 10B: DART-D** (comprising **CD137 MAB-3** and **hHER2 MAB-1** domains), **DART-F** (comprising **CD137 MAB-4** and **hHER2 MAB-1** domains), **DART-1**, and **DART-4** (comprising **CD137 MAB-1** and **hHER2 MAB-1** domains), **DART-2** and **DART-5** (comprising **CD137 MAB-2** and **hHER2 MAB-1** domains) and control binding molecule **DART-6** (comprising **CD137 MAB-3** and variant palivizumab domains).

[0066] **Figures 11A-11B** show the ability of **CD137 x TA Binding Molecules** to bind to an illustrative TA, HER2/neu, on the surface of target N87 (HER2 $^{+++}$) gastric cancer cells as measured by mean fluorescence intensity (MFI). **Figure 11A: DART-B, DART-C, DART-D, DART-E**, and the control binding molecules **DART-3** and **DART-6**. **Figure 10B: DART-D, DART-F, DART-1, DART-2, DART-4, DART-5**, and **DART-6**.

[0067] **Figures 12A-12B** show the ability of **CD137 x TA Binding Molecules DART-B, DART-D, and DART-G**, and the control molecules **DART-3**, and **DART-6** to bind to CD137 expressed by engineered CHO cells (**Figure 12A**) and CD137 expressed by activated CD8 $^+$ T cells (**Figure 12B**).

[0068] **Figures 13A-13B** show the ability of **CD137 x TA Binding Molecules DART-B, DART-D, and DART-G**, and the control molecules **DART-3**, and **DART-6** to bind to an illustrative TA, HER2/neu, expressed by target N87 (HER2 $^{+++}$) gastric cancer cells (**Figure 13A**) and target JIMT-1 (HER2 $^{++}$) cells (**Figure 13B**).

[0069] **Figures 14A-14B** provide the results of a first representative assay of the ability of **CD137 x TA Binding Molecules** to mediate a co-stimulatory activity in a T cell cytokine release assay (exemplified by release of IFN- γ). The Figures show results for **DART-1, DART-2, DART-4, DART-5, DART-A, DART-D, DART-E** and **DART-F**, and the control molecules, **DART-3** and **DART-6**, tested in the presence of HER2/neu-expressing N87 (HER2⁺⁺⁺) (**Figure 14A**) or JIMT-1 (HER2⁺⁺) target cells (**Figure 14B**), no co-stimulatory activity was seen using HER2/neu-negative Hs700T target cells, or no target cells.

[0070] **Figures 15A-15B** provide the results of a second representative assay of the ability of **CD137 x TA Binding Molecules** to mediate a co-stimulatory activity in a T cell cytokine release assay (exemplified by the release of IFN- γ). The Figures show results for **DART-1, DART-2, DART-4, DART-5, DART-B, DART-D** and **DART-G**, and the control molecules **DART-3** and **DART-6**, tested in the presence of HER2/neu-expressing N87 (HER2⁺⁺⁺) (**Figure 15A**) or JIMT-1 (HER2⁺⁺) target cells (**Figure 15B**), no co-stimulatory activity was seen using HER2/neu-negative Hs700T target cells, or in the absence of target cells.

[0071] **Figures 16A** and **16B** show the ability of **CD137 x TA Binding Molecules** to mediate dose and target dependent signal transduction of NF/ κ B pathway in CD137 expressing reporter cell line (Jurkat-NF- κ B-Luc) as demonstrated by increased luciferase expression. The Figures show results for **DART-G**, and the control binding molecules **DART-3** and **DART-6** on CD137 expressing Jurkat-NF- κ B-Luc cells co-cultured with either HER2/neu expressing JIMT-1 cells (**Figure 16A**) or HER2/neu-negative KG-1 cells (**Figure 16B**).

[0072] **Figure 17, Panels A-O** show the ability of **CD137 x TA Binding Molecules** to enhance T cell proliferation in co-culture with **TA** expressing target cells. CFSE-labeled human T cells $\pm$ sub-optimal α CD3/ α CD28 stimulation co-cultured with HER2/neu-high N87 target cells (**Figure 17, Panels A-C and J-K**), HER2/neu-low MCF-7 target cells (**Figure 17, Panels D-F and L-M**), or no target cells (**Figure 17, Panels G-I and N-O**) in the presence of **DART-A** (**Figure 17, Panels A, D and G**), **DART-3** (**Figure 17, Panels B, E and H**), **DART-6** (**Figure 17, Panels C, F and I**), or no molecule (**Figure 17, Panels J, L and N**) and monitored for T cell proliferation.

[0073] **Figures 18A-18B** show the ability of **CD137 x TA Binding Molecule DART-1** to enhance the anti-TA antibody margetuximab-mediated ADCC killing of N87 target cells as

measured by cell-associated luciferase activity (**Figure 18A**) and to enhance margituximab-mediated NK cell activation as measured by CD69 expression (**Figure 18B**).

[0074] **Figures 19A-19B** show the results of a representative assay of the ability of the **CD137 x TA** Binding Molecule **DART-7**, and the control molecule **DART-8** to mediate co-stimulatory activity in a T cell cytokine release assay (exemplified by the release of IFN- γ). Cytokine release was measured in the presence of EphA2-expressing Hs700T target cells (**Figure 19A**), or EphA2-neative Hs700T (EphA2.KO) target cells (**Figure 19B**), or with no target cells present. The following control samples are also plotted: stimulated pan T cells + target cells, and untreated/unstimulated pan T cells.

[0075] **Figures 20A-20B** show the ability of **DART-G** derivatives having optimized CDR_H3s to bind to CD137 expressed on the surface of engineered CHO cells (**Figure 20A**) or CD8⁺ T cells (**Figure 20B**).

[0076] **Figures 21A-21C** show the results of a representative assay of the ability of **CD137 x TA** Binding Molecules **DART-B**, **DART-D**, **DART-G**, **DART-G2**, **DART-G3** and **DART-G4**, and the control molecules **DART-3**, **DART-6**, to mediate co-stimulatory activity in a T cell cytokine release assay (exemplified by the release of IFN- γ). Cytokine release was measured in the presence of HER2/neu-expressing N87 target cells (**Figure 21A**), JIMT-1 target cells (**Figure 21B**), or MDA-231 (**Figure 21C**) target cells, or with no target cells present.

[0077] **Figure 22** shows the results of a representative assay of the ability of **CD137 x TA** Binding Molecules **DART-B**, **DART-G**, **DART-G1**, **DART-G2**, **DART-G3** and **DART-G4**, and the control molecules **DART-3**, **DART-6**, to mediate redirected cell killing of N87 (HER2⁺⁺⁺) target cells as measured by cell-associated luciferase activity.

[0078] **Figures 23A-23C** show the ability of **CD137 x TA** Binding Molecules to bind different cells expressing the illustrative **TA**, HER2/neu, as measured by mean fluorescence intensity (MFI). **Figure 23A**: binding to N87 (HER2⁺⁺⁺) gastric cancer cells. **Figure 23B**: binding to JIMT-1 (HER2⁺⁺) breast carcinoma cells. **Figure 23C**: binding to MCF-7 (HER2⁺) breast cancer cells. Binding for **TRIDENT-A**, **TRIDENT-A2**, **TRIDENT-A3**, **TRIDENT-A4**, **DART-G**, **DART-G2**, **DART-G3**, **DART-G4**, and the control binding molecules **TRIDENT-1** and **TRIDENT-2** is shown in both panels.

[0079] **Figures 24A-24B** show the ability of **CD137 x TA Binding Molecules** to bind to CD137 expressed by activated CD8⁺ T cells. **Figure 12A: TRIDENT-A, TRIDENT-A2, TRIDENT-A3, TRIDENT-A4**, and the control binding molecules **TRIDENT-1** and **TRIDENT-2**. **Figure 12B: DART-G, DART-G2, DART-G3, DART-G4**, and the control binding molecules **TRIDENT-1** and **TRIDENT-2**.

[0080] **Figures 25A-25C** show the ability of the **CD137 x TA Binding Molecules** to mediate cell-cell conjugation between CHO cells expressing CD137 and cells expressing the illustrative **TA**, HER2/neu, FACS. **Figure 25A: Cell-cell conjugation with N87 (HER2⁺⁺⁺) gastric cancer cells. Figure 25B: Cell-cell conjugation with JIMT-1 (HER2⁺⁺) breast carcinoma cells. Figure 25C: Cell-cell conjugation with MCF-7 (HER2⁺) breast cancer cells.** The activity of **TRIDENT-A, TRIDENT-A2, TRIDENT-A3, TRIDENT-A4, DART-G4**, and the **TRIDENT-2** control binding molecule is shown in both panels.

[0081] **Figures 26A-26C** show the results of a representative assay of the ability of **CD137 x TA Binding Molecules TRIDENT-A, TRIDENT-A2, TRIDENT-A3, TRIDENT-A4, DART-G, DART-G2, DART-G3, DART-G4**, and the control binding molecules **TRIDENT-1** and **TRIDENT-2**, to mediate co-stimulatory activity in a T cell cytokine release assay (exemplified by the release of IFN- γ). Cytokine release was measured in the presence of HER2/neu-expressing N87 target cells (**Figure 26A**), JIMT-1 target cells (**Figure 26B**), or with no target cells present (**Figure 26C**).

[0082] **Figure 27** shows the ability of an exemplary **CD137 x TA Binding Molecule (TRIDENT-A2)** to enhance the **TA x CD3** diabody-mediated redirected T-cell killing of Colo205/Luc target cells as measured by cell-associated luciferase as compared to the control molecule (**TRIDENT-1**)

[0083] **Figures 28A-28C** show the expression of T-cell markers present in 1 mg of tumor sampled post-treatment with vehicle control (●); **TRIDENT-A2** (▼); **TA x CD3** diabody (■); combination of **TRIDENT-A2** and **TA x CD3** diabody (◆); anti-PD-1 mAb (▽); or the combination of **TRIDENT-A2, TA x CD3** diabody, and **anti-PD-1 mAb**. **Figure 28A: CD4** expression, **Figure 28B: CD8** expression, **Figure 28C: CD69** expression, **Figure 28D: PD-1** expression.

[0084] **Figure 29** shows the ability of vehicle control (●); **TRIDENT-A2** (▼); **TA x CD3** diabody (■); the combination of **TRIDENT-A2** and **TA x CD3** diabody (◆); anti-PD-1 mAb

(▼); and the combination of **TRIDENT-A2**, **TA x CD3** diabody, and **anti-PD-1 mAb**, to inhibit tumor growth or development of ovarian carcinoma cells in a PBMC-reconstituted murine xenograph model.

[0085] **Figure 30** shows the ability of **CD137 x TA** Binding Molecules **TRIDENT-A2**, **TRIDENT-B2**, and the control binding molecules **TRIDENT-1** and **TRIDENT-2**, to mediate co-stimulatory activity in a cynomolgus monkey T cell cytokine release assay (exemplified by the release of IFN- γ). Cytokine release was measured in the presence of JIMT-1 target cells expressing 5T4 and HER2/neu.

[0086] **Figures 31A-31B** depict the amino acid sequences of **hCD137 MAB-3 VH1B** (**Figure 30A**, SEQ ID NO:84) and **hCD137 MAB-3 VL3** (**Figure 30B**, SEQ ID NO:89). underlining indicate CDR residues. Positions of substitution are boxed and the Kabat numbers are indicated with arrows; sequential amino acid residue numbering is indicated above the sequences.

[0087] **Figures 32A-32B** show the ability anti-CD137 antibodies comprising deimmunized VH and VL Domains to bind to CD137 of activated CD4⁺ T cells or CD8⁺ T cells as measured by FACS using V510-labeled CD4⁺ and α hFc APC (**Figure 32B**) or FITC-labeled CD8 and α hFc APC (**Figure 32B**).

[0088] **Figures 33A-33B** show induction after 72 hours of IFN- γ by pan T cells upon stimulation with dry-coated anti-CD3 antibody (3 μ g/mL-50 μ g/mL) in the presence of the optimized/deimmunized anti-CD137 antibodies **CD137 MAB-3 (1b.3)**, **CD137 MAB-3 (1E.15)** and **CD137 MAB-3 (1G.15)** in the absence of cross-linking (**Figure 33A**) or cross-linked with 4x hFc F(ab)₂ (**Figure 33B**).

[0089] **Figures 34A-34C** show the ability of **CD137 x TA** Binding Molecules **TRIDENT-B2**, **TRIDENT-B5**, and the control molecules **TRIDENT-1**, **TRIDENT-3**, and **TRIDENT-4** to bind to CD137 on the surface of activated CD8⁺ T cells as measured by FACS using FITC-labeled CD8 and α hFc APC (**Figure 34A**), and the illustrative **TA**, 5T4 on the surface of JIMT-1 (5T4^{hi}) (**Figure 34B**) or SKOV-3 (5T4^{lo}) target cells (**Figure 34C**).

[0090] **Figures 35A-35C** show the ability of **CD137 x TA** Binding Molecules to mediate a co-stimulatory activity in a T cell cytokine release assay (exemplified by release of IFN- γ). The **Figures** show results for **TRIDENT-B2**, **TRIDENT-B5**, and the control molecules

TRIDENT-1, **TRIDENT-3**, and **TRIDENT-4**, tested in the presence of 5T4-expressing JIMT-1 (5T4^{hi}) (**Figure 35A**) or SKOV-3 (5T4^{lo}) (**Figure 35B**) target cells no co-stimulatory activity was seen in the absence of target cells (**Figure 35C**).

DETAILED DESCRIPTION OF THE INVENTION:

[0091] The present invention is directed to binding molecules that possess one or more epitope-binding sites specific for an epitope of CD137 and one or more epitope-binding sites specific for an epitope of a tumor antigen (“**TA**”) (*e.g.*, a “**CD137 x TA Binding Molecule**”). In one embodiment, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific tetravalent diabodies, that are composed of two, three, four or more than four polypeptide chains and possessing two epitope-binding sites each specific for an epitope of CD137 and two epitope-binding sites each specific for an epitope of a **TA**. Alternatively, such **CD137 x TA Binding Molecules** will be bispecific molecules, especially bispecific trivalent binding molecules composed of three or more polypeptide chains and possessing one or two epitope-binding sites each specific for an epitope of CD137 and one or two epitope-binding sites each specific for an epitope of a **TA**. The **CD137 x TA Binding Molecules** of the invention are capable of simultaneous binding to CD137, and a **TA**. The invention is directed to pharmaceutical compositions that contain any such **CD137 x TA Binding Molecules**. The invention is additionally directed to methods for the use of such molecules in the treatment of cancer and other diseases and conditions. The invention also provides novel CD137-binding molecules, and HER2/neu-binding molecules, as well as derivatives thereof and uses thereof.

I. Antibodies and Other Binding Molecules

[0092] **Antibodies** are immunoglobulin molecules capable of specific binding to a target region (“epitope”) of a molecule, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.* (“antigen”), through at least one “**epitope-binding site**” located in the Variable Region 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.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass. Antibodies are capable of “**immunospecifically binding**” to a polypeptide or protein or a non-protein molecule due to the presence on such molecule of a particular domain or moiety or conformation (an “**epitope**”). As used herein, an “**epitope-binding fragment of an antibody**” is intended to denote a portion of an antibody capable of immunospecifically binding to an epitope. As used herein, such term encompasses fragments (such as Fab, Fab', F(ab')₂ Fv), and single chain (scFv), as well as the epitope-binding domain of a diabody. As used herein, an antibody or an epitope-binding fragment thereof 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 or avidity with that epitope relative to alternative epitopes. It is also understood by reading this definition that, for example, an antibody or an epitope-binding fragment thereof that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. An epitope-containing molecule may have immunogenic activity, such that it elicits an antibody production response in an animal; such molecules are termed “**antigens**”. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are “**monospecific**”), although they can bind multiple copies of that species (*i.e.*, exhibiting “**bivalency**” or “**multivalency**”).

[0093] The term “**monoclonal antibody**” refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring or non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term “monoclonal antibody” encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂ Fv), single-chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of “antibody.” Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) “*Continuous Cultures Of*

Fused Cells Secreting Antibody Of Predefined Specificity,” Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) “*Review of Selected Adjuvants Used in Antibody Production*,” ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freund’s adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced, and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the monospecific or multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized, a chimeric antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody. 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/or predicted amino acid sequence of the light and heavy chain Variable Domains of the starting antibody; (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region(s) to use during the humanizing or caninizing process; (3) application of the actual humanizing or caninizing methodologies/techniques; and (4) the transfection and expression of the humanized or caninized antibody (see, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415).

[0094] 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.

A. General Structural Attributes of Antibodies

[0095] The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is a tetramer composed of two shorter “**Light Chains**” complexed with two longer “**Heavy Chains**” and is usually expressed as a glycoprotein of about 150,000 Da. Each chain is composed of an amino-terminal (“**N-terminal**”) portion that comprises a “**Variable Domain**” and a carboxy-terminal (“**C-terminal**”) portion that comprises at least one “**Constant Domain**.” An IgG Light Chain is composed of a single “**Light Chain Variable Domain**” (“**VL**”) and a single “**Light Chain Constant Domain**” (“**CL**”). Thus, the structure of the light chains of an IgG molecule is **n-VL-CL-c** (where n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). An IgG Heavy Chain is composed of a single “**Heavy Chain Variable Domain**” (“**VH**”), three “**Heavy Chain Constant Domains**” (“**CH1**,” “**CH2**” and “**CH3**”), and a “**Hinge**” Region (“**H**”), located between the **CH1** and **CH2** Domains. Thus, the structure of an IgG heavy chain is **n-VH-CH1-H-CH2-CH3-c** (where n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). The ability of an intact, unmodified antibody (*e.g.*, an IgG antibody) to bind an epitope of an antigen depends upon the presence and sequences of the Variable Domains.

1. Constant Domains

(a) Light Chain Constant Domain

[0096] 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:1**):

```
RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC
```

[0097] 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:2**):

```
QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS
```

(b) Heavy Chain CH1 Domains

[0098] 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:3)**:

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHNKPS NTKVDKRV

[0099] 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:4)**:

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVNHNKPS NTKVDKTV

[00100] An exemplary CH1 Domain is a human IgG3 CH1 Domain. The amino acid sequence of an exemplary human IgG3 CH1 Domain is **(SEQ ID NO:5)**:

ASTKGPSVFP LAPCSRSTSE GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YTCNVNHNKPS NTKVDKRV

[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:6)**:

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTTK YTCNVNHNKPS NTKVDKRV

(c) Heavy Chain Hinge Regions

[00102] An exemplary Hinge Region is a human IgG1 Hinge Region. The amino acid sequence of an exemplary human IgG1 Hinge Region is **(SEQ ID NO:7)**:

EPKSCDKTHT CPPCP

[00103] Another exemplary Hinge Region is a human IgG2 Hinge Region. The amino acid sequence of an exemplary human IgG2 Hinge Region is **(SEQ ID NO:8)**:

ERKCCVECPP CP

[00104] Another exemplary Hinge Region is a human IgG3 Hinge Region. The amino acid sequence of an exemplary human IgG3 Hinge Region is **(SEQ ID NO:9)**:

ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
SCDTPPPCPR CP

[00105] Another exemplary Hinge Region is a human IgG4 Hinge Region. The amino acid sequence of an exemplary human IgG4 Hinge Region is **(SEQ ID NO:10)**:

ESKYGPPCPS CP

[00106] As described herein, an IgG4 Hinge Region may comprise a stabilizing mutation such as the S228P substitution (as numbered by the EU index as set forth in Kabat). The amino acid sequence of an exemplary stabilized IgG4 Hinge Region is (SEQ ID NO:11):

ESKYGPPCPP CP

(d) Heavy Chain CH2 and CH3 Domains

[00107] The CH2 and CH3 Domains of the two heavy chains interact to form the “Fc Region” of IgG antibodies that is recognized by cellular **Fc Receptors**, including but not limited to Fc gamma Receptors (**FcγRs**). As used herein, the term “Fc Region” is used to define a C-terminal region of an IgG heavy chain. A portion of an Fc Region (including a portion that encompasses an entire Fc Region) is referred to herein as an “Fc Domain.” An Fc Region 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.

[00108] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (SEQ ID NO:12):

```

231      240      250      260      270      280
APELLGGPSV FLFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD

      290      300      310      320      330
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

      340      350      360      370      380
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

      390      400      410      420      430
WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

      440      447
ALHNHYTQKS LSLSPGX

```

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

[00109] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (SEQ ID NO:13):

```

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

      290      300      310      320      330
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA

      340      350      360      370      380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

      390      400      410      420      430
WESNGQPENN YKTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

      440      447
ALHNHYTQKS LSLSPGX
as numbered by the EU index as set forth in Kabat, wherein X is a lysine (K) or is
absent.
```

[00110] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (SEQ ID NO:14):

```

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 a lysine (K) or is
absent.
```

[00111] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (SEQ ID NO:15):

```

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 YKTPPVLDSDGSFFLYSRL TVDKSRWQEG NVFSCSVME
 440 447
 ALHNHYTQKS LSLSLG**X**

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

[00112] 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.

[00113] 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 **CD137 x TA** Binding Molecules of the invention. Specifically encompassed by the instant invention are **CD137 x TA** 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. Variable Domains

[00114] The Variable Domains of an IgG molecule consist of three “**complementarity determining regions**” (“**CDRs**”), which contain the amino acid residues of the antibody that will be in contact with the epitope, as well as intervening non-CDR segments, referred to as “**framework regions**” (“**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 the epitope). Thus, the VL and VH Domains have the structure **n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c**. The amino acid sequences of the CDRs determine whether an antibody will be able to bind to a particular epitope. Interaction of an antibody light chain with an antibody heavy chain and, in particular, interaction of their VL and VH Domains, forms an epitope-binding site of the antibody.

[00115] 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 (SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NH1, MD (1991)) 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 and FRs 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.

[00116] 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 to 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 to 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 to such epitope, may exhibit an immunospecificity, affinity or selectivity toward 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 Fab₂ fragment, *etc.*). Unless specifically noted, the order of domains of the protein molecules described herein is in the “**N-Terminal to C-Terminal**” direction.

[00117] 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 sites 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.

B. Humanization of Antibodies

[00118] The invention particularly encompasses binding molecules (including antibodies and diabodies) 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. Patent Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[00119] 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. Patent Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

[00120] Notwithstanding such successes, the production of stable, functional heterodimeric, non-monospecific diabodies optimized for therapeutic use can be further improved by the careful consideration and placement of the domains employed in the polypeptide chains. The present invention is thus directed to the provision of specific polypeptides that are particularly designed to form, via covalent bonding, stable and therapeutically useful heterodimeric diabodies and heterodimeric Fc diabodies that are capable of simultaneously binding CD137 and a TA.

C. Bispecific Antibodies, Multi-Specific Diabodies and DART® Diabodies

[00121] As indicated above, natural antibodies are capable of binding to only one epitope species, although they can bind multiple copies of that species. The art has recognized the desirability of producing bispecific antibodies, and a wide variety of recombinant bispecific antibody formats have been developed to produce such bispecific antibodies (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 such approaches use linker peptides to fuse a further binding domain (*e.g.* an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple antibody binding portions to one another (*e.g.* two Fab fragments or scFv). Alternative formats use linker peptides to fuse a binding protein (*e.g.*, an scFv, VL, VH, *etc.*) to a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). Typically, such approaches involve compromises and trade-offs. For example, PCT Publication Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose that the use of linkers may cause problems in therapeutic settings, and teaches a tri-specific 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 to more than one antigen. Thus, the molecules disclosed in these documents trade binding specificity for the ability to bind additional antigen species. PCT Publication Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. The document notes that the CH2 Domain likely plays only a minimal role in mediating effector function. PCT Publication Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Regions have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publication Nos. WO 2003/025018

and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv domains. PCT Publication No. WO 2013/006544 discloses multi-valent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. Thus, the molecules disclosed in these documents trade all or some of the capability of mediating effector function for the ability to bind additional antigen species. PCT Publication Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional Binding Domains or functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

[00122] The art has additionally noted the capability of producing **diabodies** that differ from natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bi-valency or multi-valency) (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 (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).

[00123] The provision of non-monospecific “**diabodies**” provides a significant advantage over antibodies: the capacity to co-ligate and co-localize cells that express different epitopes. Bispecific 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 bivalency, 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). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T cells to tumor cells (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) to thereby co-localize T cells to the sites of tumor cells.

[00124] Alternatively to targeting such diabodies to bind to T cells, diabody epitope binding domains may be directed to a surface determinant of a B cell, such as CD19, CD20, CD22, CD30, CD37, CD40, and CD74 (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; Cheson, B.D. *et al.* (2008) “*Monoclonal Antibody Therapy For B-Cell Non-Hodgkin’s Lymphoma*,” N. Engl. J. Med. 359(6):613-626; Castillo, J. *et al.* (2008) “*Newer Monoclonal Antibodies For Hematological Malignancies*,” Exp. Hematol. 36(7):755-768). In many studies, diabody binding to effector cell determinants, *e.g.*, Fc γ receptors (Fc γ R), was also found to activate the effector cell (Holliger *et al.* (1996) “*Specific Killing Of Lymphoma Cells By Cytotoxic T cells Mediated By A Bispecific Diabody*,” Protein Eng. 9:299-305; Holliger *et al.* (1999) “*Carcinoembryonic Antigen (CEA)-Specific T cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-CEA Bispecific Diabodies And B7 x Anti-CEA Bispecific Fusion Proteins*,” Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen-bound antibody to an effector cell via an Fc Domain - Fc γ R interaction; thus, in this regard, diabody molecules may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.*, as assayed in any effector function

assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of a tumor cell but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) “*Bispecific Antibody Conjugates In Therapeutics*,” Adv. Drug. Deliv. Rev. 55:171-197).

[00125] However, the above advantages come at a salient cost. The formation of such non-mono-specific 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-mono-specific 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 address covalent bonding between polypeptides of the same species (*i.e.*, so as to minimize their 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).

[00126] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional single polypeptide chain 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).

[00127] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-mono-specific diabodies, termed **DART®** diabodies, see, *e.g.*, 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; 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. Molec. Biol.* 399(3):436-449; Veri, M.C. *et al.* (2010) “Therapeutic Control Of B Cell Activation Via Recruitment Of Fcγ Receptor IIB (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold,” *Arthritis Rheum.* 62(7):1933-1943; 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; US Patent Nos. 8,044,180; 8,133,982; 8,187,593; 8,193,318; 8,530,627; 8,669,349; 8,778,339; 8,784,808; 8,795,667; 8,802,091; 8,802,093; 8,946,387; 8,968,730; and 8,993,730; US Patent Publication Nos. 2009/0060910; 2010/0174053; 2011/0081347; 2011/0097323; 2011/0117089; 2012/0009186; 2012/0034221; 2012/0141476; 2012/0294796; 2013/0149236; 2013/0295121; 2014/0017237; and 2014/0099318; European Patent Documents No. EP 1868650; EP 2158221; EP 2247304; EP 2252631; EP 2282770; EP 2328934; EP 2376109; EP 2542256; EP 2601216; EP 2714079; EP 2714733; EP 2786762; EP 2839842; EP 2840091; and PCT Publication Nos. WO 2006/113665; WO 2008/157379; WO 2010/027797; WO 2010/033279; WO 2010/080538; WO 2011/109400; WO 2012/018687; WO 2012/162067; WO 2012/162068; WO 2014/159940; WO 2015/021089; WO 2015/026892; and WO 2015/026894). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[00128] The simplest **DART®** diabody comprises two polypeptide chains each comprising three Domains (**Figure 1**). The first polypeptide chain comprises: (i) a Domain that comprises a binding region of a light chain variable Domain of the a first immunoglobulin (VL1), (ii) a

second Domain that comprises a binding region of a heavy chain variable Domain of a second immunoglobulin (VH2), and (iii) a third Domain that serves to promote heterodimerization (a “**Heterodimer-Promoting Domain**”) with the second polypeptide chain and to covalently bond the first polypeptide to the second polypeptide chain of the diabody. The second polypeptide chain contains a complementary first Domain (a VL2 Domain), a complementary second Domain (a VH1 Domain) and a third Domain that complexes with the third Domain of the first polypeptide chain in order to promote heterodimerization (a “**Heterodimer-Promoting Domain**”) and covalent bonding with the first polypeptide chain. Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. In one embodiment, the third Domains of the first and second polypeptide chains each contain a cysteine (“©”) residue, which serves to bind the polypeptides together via a disulfide bond. The third Domain of one or both of the polypeptide chains may additionally possess the sequence of a CH2-CH3 Domain, such that complexing of the diabody polypeptides forms an Fc Domain that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). Many variations of such molecules have been described (see, *e.g.*, United States Patent Publication Nos. 2013-0295121; 2010-0174053; 2007-0004909; 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; WO 2006/113665) and are provided herein.

II. Components of the Preferred CD137 x TA Binding Molecules Of The Present Invention

[00129] The **CD137 x TA** Binding Molecules of the present invention are composed of polypeptides, and may be composed of two, three, four or more than four polypeptide chains. As used herein, the term “**composed of**” is intended to be open-ended, such that a **CD137 x TA** Binding Molecules of the present invention that is composed of two polypeptide chains may possess additional polypeptide chains. Such chains may have the same sequence as another polypeptide chain of the Binding Molecule, or may be different in sequence from any other polypeptide chain of the Binding Molecule.

A. Preferred “Linker” Peptides

[00130] The polypeptides of the **CD137 x TA** Binding Molecules of the present invention comprise domains that are preceded by, followed by, and/or linked to one another by “linker”

peptides, such as **Linker 1**, **Linker 2**, **Linker 3**, *etc.* Although the invention utilizes certain preferred “linker” peptides, in light of the teachings provided herein, alternative linkers could readily be identified and employed to achieve **CD137 x TA Binding Molecules**.

[00131] Most preferably, the length of **Linker 1**, which separates such VL and VH domains of a polypeptide chain is selected to substantially or completely prevent such VL and VH domains from binding to one another (*e.g.*, 12 or less amino acid residues in length). Thus, the **VL1** and **VH2** domains of the first polypeptide chain are substantially or completely incapable of binding to one another, and do not form an epitope-binding site that is capable of substantially binding to either the first or second antigen. Likewise, the **VL2** and **VH1** domains of the second polypeptide chain are substantially or completely incapable of binding to one another, and do not form an epitope-binding site that is capable of substantially binding to either the first or second antigen. A preferred intervening spacer peptide (**Linker 1**) has the sequence (**SEQ ID NO:16**): GGGSGGGG, which is too short to allow the VL and VH Domains of the same polypeptide chain to complex together (in contrast to the longer intervening spacer peptide that is employed to produce scFv molecules (*e.g.*, GGGGSGGGGSGGGGS (**SEQ ID NO:17**)).

[00132] The purpose of **Linker 2** is to separate the VH Domain of a polypeptide chain from the optionally present Heterodimer-Promoting Domain of that polypeptide chain. Any of a variety of linkers can be used for the purpose of **Linker 2**. A preferred sequence for such **Linker 2** has the amino acid sequence: GGCGGG (**SEQ ID NO:18**), which possesses a cysteine residue that may be used to covalently bond the first and second polypeptide chains to one another via a disulfide bond, or ASTKG (**SEQ ID NO:19**), which is derived from the IgG CH1 domain. Since the **Linker 2**, ASTKG (**SEQ ID NO:19**) does not possess such a cysteine, the use of such **Linker 2** is preferably associated with the use of a cysteine-containing Heterodimer-Promoting Domain, such as the E-coil of **SEQ ID NO:38** or the K-coil of **SEQ ID NO:39** (see below).

[00133] One purpose of **Linker 3** is to separate the Heterodimer-Promoting Domain of a polypeptide chain from the Fc Domain of that polypeptide chain. A second purpose is to provide a cysteine-containing polypeptide domain. Any of a variety of linkers can be used for the purpose of **Linker 3**. A preferred sequence for such **Linker 3** has the amino acid sequence:

DKTHTCPPCP (SEQ ID NO:20). Another preferred sequence for **Linker 3** has the amino acid sequence: GGGDKTHTCPPCP (SEQ ID NO:21).

[00134] The purpose of **Linker 4** is to separate the C-terminus of the CH2-CH3 domains of an Fc Region (“**Fc Domain**”) from the N-terminus of a VL Domain. Any of a variety of linkers can be used for the purpose of **Linker 4**. A preferred sequence for such **Linker 4** has the amino acid sequence: APSSS (SEQ ID NO:22) or the amino acid sequence APSSSPME (SEQ ID NO:23) or the amino acid sequence GGGSGGGSGGG (SEQ ID NO:24),

[00135] The Fc Region-containing 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 Region (preferably a cysteine-containing portion of an IgG Hinge Region). Linkers that may be employed in the bispecific Fc Region-containing diabody molecules of the present invention include: GGC, GGG, ASTKG (SEQ ID NO:19), DKTHTCPPCP (SEQ ID NO:20), APSSS (SEQ ID NO:22), APSSSPME (SEQ ID NO:23), GGGSGGGSGGG (SEQ ID NO:24), LGGGSG (SEQ ID NO:25), GGGS (SEQ ID NO:26), LEPKSS (SEQ ID NO:27), VEPKSADKTHTCPPCP (SEQ ID NO:28), LEPKSADKTHTCPPCP (SEQ ID NO:29). LEPKSS (SEQ ID NO:27) may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or LEPKSS (SEQ ID NO:27) may be immediately followed by DKTHTCPPCP (SEQ ID NO:20) to form the alternate linkers: GGGDKTHTCPPCP (SEQ ID NO:21); and LEPKSSDKTHTCPPCP (SEQ ID NO:30). Bispecific Fc Region-containing molecules of the present invention may incorporate an IgG Hinge Region, such as the IgG Hinge Region of a human IgG1, IgG2, IgG3 or IgG4 antibody, or a portion thereof.

B. Preferred Heterodimer-Promoting Domains

[00136] As indicated above, the formation of the **CD137 x TA Binding Molecules** of the present invention involves the assembly of two or more different polypeptide chains (*i.e.*, heterodimerization). The formation of heterodimers of the first and second polypeptide chains can be driven by the inclusion of “**Heterodimer-Promoting Domains**.” The Heterodimer-Promoting Domains may be a domain of a Hinge Region of an IgG (or a polypeptide derived

from a Hinge Region, such as, for example, GVEPKSC (SEQ ID NO:31), VEPKSC (SEQ ID NO:32)) or AEPKSC (SEQ ID NO:33)) on one polypeptide chain, and a CL Domain (or a polypeptide derived from the CL Domain, such as, for example, GFNRGEC (SEQ ID NO:34) or FNRGEC (SEQ ID NO:35)) on the other polypeptide chain (US2007/0004909).

[00137] More preferably, however, the Heterodimer-Promoting Domains of the present invention will comprise tandemly repeated coil domains of opposing charge, for example “E-coil” helical domains (SEQ ID NO:36: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem “K-coil” domains (SEQ ID NO:37: 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 heterodimerization. In another preferred embodiment, a Heterodimer-Promoting Domain in which one of the four tandem “E-coil” helical domains of SEQ ID NO:36 has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:38) is utilized. Likewise, in another preferred embodiment, a Heterodimer-Promoting Domain in which one of the four tandem “K-coil” helical domains of SEQ ID NO:37 has been modified to contain a cysteine residue: KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:39) is utilized.

C. Covalent Bonding of the Polypeptide Chains

[00138] The CD137 x TA Binding Molecules of the present invention are engineered so that pairs of their polypeptide chains covalently bond to one another via one or more cysteine residues positioned along their length to produce a covalently associated molecular complex. Such cysteine residues may be introduced into the intervening linker that separates the VL and VH domains of the polypeptides. Alternatively, Linker 2 or Linker 3, or an alternative linker may contain a cysteine residue. Most preferably, one or more coil domains of a coil-containing Heterodimer-Promoting Domain will comprise an amino acid substitution that incorporates a cysteine residue as in SEQ ID NO:38 or SEQ ID NO:39.

D. Preferred Fc Domains

[00139] The Fc Domain of an Fc-bearing CD137 x TA Binding Molecule of the present invention may comprise a complete Fc region (e.g., a complete IgG Fc region) or only a fragment of a complete Fc region. The Fc Domain of the Fc-bearing CD137 x TA Binding

Molecules of the present invention may thus include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc region, 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 region). The Fc Domain of the bispecific Fc diabodies of the present invention may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc regions, 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.*).

[00140] Although the Fc Domain of an Fc-bearing **CD137 x TA** Binding Molecule of the present invention may comprise the amino acid sequence of a naturally occurring FC Domain, it is preferred for the CH2-CH3 Domains that form such Fc Domain to comprise one or more substitutions such that the resultant FC Domain exhibits decreased (*e.g.*, less than 50%, less than 40%, less than 30%, less than 20%, or less than 10%, of the binding exhibited by such molecule if having an Fc Domain having the amino acid sequence of a naturally-occurring Fc Region), or substantially no detectable, binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region). Fc variants and mutant forms capable of mediating such altered binding are well known in the art and include amino acid substitutions at one or more positions elected from the group consisting of: 234, 235, 265, and 297, wherein said numbering is that of the EU index as in Kabat (see, for example, US Patent No. 5,624,821, herein incorporated by reference). In one embodiment, the CH2-CH3 Domain of the first and/or third polypeptide chains of the Fc-bearing molecules of the invention include any 1, 2, 3, or 4 of the substitutions: L234A, L235A, D265A, N297Q, and N297G. Alternatively, a CH2-CH3 Domain of a naturally occurring Fc region that inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding and effector function exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:12**)) is utilized. In a specific embodiment, the Fc-bearing molecules of the present invention comprise an IgG2 Fc Region (**SEQ ID NO:13**) or an IgG4 Fc Region (**SEQ ID:NO:15**). When an IgG4 Fc Region 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:11**).

[00141] In a preferred embodiment, the employed CH2-CH3 Domain of Fc-bearing **CD137 x TA** Binding Molecules of the present invention include a substitution at position 234 with alanine and 235 with alanine, wherein said numbering is that of the EU index as in Kabat (**SEQ ID NO:40**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVME
 ALHNHYTQKS LSLSPGX

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

[00142] The serum half-life of proteins comprising Fc Regions may be increased by increasing the binding affinity of the Fc Region 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.

[00143] In some embodiments, the Fc-bearing **CD137 x TA** Binding Molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an increased half-life (relative to a molecule comprising a wild-type Fc Region). In some embodiments, the Fc-bearing **CD137 x TA** Binding Molecules of the present invention comprise a variant IgG Fc Region, wherein said variant Fc Region comprises a half-live extending amino acid substitution. Numerous amino acid substitutions capable of increasing the half-life of an Fc-bearing molecule are known in the art see for example the amino acid substitutions described in U.S. Patent Nos. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and PCT Publication Nos. WO 98/23289; WO 2009/058492; and WO 2010/033279, which are herein incorporated by reference in their entireties. A Fc-bearing **CD137 x TA** Binding Molecule having enhanced half-life may comprise two or more substitutions selected from: T250Q, M252Y, S254T, T256E, K288D, T307Q, V308P, A378V, M428L, N434A, H435K, and Y436I, wherein said numbering is that of the EU index as in Kabat.

[00144] In particular, the employed CH2-CH3 Domain may comprise the substitutions:

- (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,

wherein said numbering is that of the EU index as in Kabat.

[00145] A preferred sequence for the CH2 and CH3 Domains comprises the triple amino acid substitution: M252Y/S254T/T256E (YTE), which significantly enhances serum-half life (Dall'Acqua, W.F. *et al.* (2006) “*Properties of Human IgGs Engineered for Enhanced Binding to the Neonatal Fc Receptor (FcRn)*,” J. Biol. Chem. 281(33):23514-23524), as in **SEQ ID NO:41** or **SEQ ID NO:42**, which are variants of the IgG1 CH2-CH3 domain, or as in **SEQ ID NO:43**, which is a variant of the IgG4 CH2-CH3 Domain:

SEQ ID NO:41:

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

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

SEQ ID NO:42:

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

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

SEQ ID NO:43:

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

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

[00146] The invention also encompasses Fc-bearing **CD137 x TA** Binding Molecules comprising variant Fc Domains that exhibit altered effector function, altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay, *etc.* Fc Domain modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) “*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcγgamma Receptors*,” *Cancer Res.* 57(18):8882-8890). Exemplary variants of human IgG1 Fc Domains with reduced binding to CD32B and/or increased binding to 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 human IgG1 Fc Domain variant contains a F243L, R292P and Y300L substitution, wherein said numbering is that of the EU index as in Kabat. In another embodiment, the human IgG1 Fc Domain variant contains a F243L, R292P, Y300L, V305I and P296L substitution, wherein said numbering is that of the EU index as in Kabat.

[00147] The CH2 and/or CH3 Domains of the **CD137 x TA** Binding Molecules of the present invention need not be identical in sequence, and advantageously are modified to promote heterodimerization between the two CH2-CH3-bearing 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.*, a “**hole**” (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the bispecific Fc-bearing diabody molecule, and further, engineered into any portion of the polypeptides chains of said pair. 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 documents is hereby incorporated herein by reference in its entirety). In one embodiment, the knob is engineered into the CH2-CH3 Domains of the first polypeptide chain and the hole is engineered into the CH2-CH3 Domains of the third polypeptide chain. Thus, the knob will help in preventing two molecules of the first polypeptide chain from homodimerizing via their CH2 and/or CH3 Domains. As the third polypeptide chain of this embodiment preferably contains the hole substitution it will have the ability to heterodimerize with the first polypeptide chain as well as homodimerize with itself (however, such homodimerization does not form a molecule possessing epitope-binding sites). A preferred knob is created by modifying a native IgG Fc Domain to contain the modification T366W. A preferred hole is created by modifying a native IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying the third polypeptide chain homodimer from the final bispecific Fc-bearing diabody comprising heterodimers of the first and third polypeptide chains, the protein A binding site of the CH2 and CH3 Domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the third polypeptide chain homodimer will not bind to protein A, whereas the properly assembled bispecific Fc-bearing diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[00148] SEQ ID NO:44, SEQ ID NO:45 and SEQ ID NO:46 provide exemplary preferred sequences for “knob-bearing” CH2 and CH3 Domains that may be used in the CD137 x TA Binding Molecules of the present invention:

SEQ ID NO:44:

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
 ALHNH~~Y~~YTQKS LSLSPGX

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

SEQ ID NO:45:

APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
 GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
 SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLWCLVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLYSRL TVDKSRWQEG NVFSCSVMHE
 ALHNH~~Y~~YTQKS LSLSLGX

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

SEQ ID NO:46:

APEAAGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
 ALHNHYTQKS LSLSPGX

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

[00149] **SEQ ID NO:47, SEQ ID NO:48 and SEQ ID NO:49** provide exemplary preferred sequences for “hole-bearing” CH2 and CH3 Domains that may be used in the **CD137 x TA** Binding Molecules of the present invention:

SEQ ID NO:47:

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLVSKL TVDKSRWQQG NVFSCSVMHE
 ALHNRYTQKS LSLSPGX

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

SEQ ID NO:48:

APEFLGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSQED PEVQFNWYVD
 GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS
 SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLSCAVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLVSRL TVDKSRWQEG NVFSCSVMHE
 ALHNRYTQKS LSLSLGX

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

SEQ ID NO:49:

APEAAGGPSV FLFPPKPKDT LYITREPEVT CVVVDVSHED PEVKFNWYVD
 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
 WESNGQPENN YKTPPVLDSDGSFFLVSKL TVDKSRWQQG NVFSCSVMHE
 ALHNRYTQKS LSLSPGX

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

[00150] As will be noted, the CH2-CH3 Domains of **SEQ ID NOs:45 and 48** are IgG4 Domains, while the CH2-CH3 Domains of **SEQ ID NOs:44, 46 47 and 49** are IgG1 Domains. **SEQ ID NOs:44, 46 47 and 49** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain that exhibits decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region (**SEQ ID NO:12**)). Furthermore, specifically encompassed by the instant invention are **CD137 x TA** Binding Molecule constructs lacking the above-indicated C-terminal lysine residue.

[00151] In the embodiment described above, the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of **SEQ ID NO:44**. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:47**) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:44**) would be employed in the third polypeptide chain.

E. Albumin-Binding Domain

[00152] 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. Such considerations are also applicable to the Tri-Specific Binding Molecules of the present invention. Most preferably, when a polypeptide portion of a serum-binding protein is desired to be incorporated into the Tri-Specific Binding Molecules of the present invention, such polypeptide portion will be installed at the C-terminus of one of the polypeptide chains of the Tri-Specific Binding Molecule.

[00153] 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 to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) “*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Modules*,” J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:50**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00154] As disclosed in WO 2012/162068 (herein incorporated by reference), “**deimmunized**” variants of **SEQ ID NO:50** 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 Albumin-Binding Domain: 66S/70S +71A; 66S/70S +79A; 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 of **SEQ ID NOs:51, 52 or 53** are particularly preferred as such deimmunized Albumin-Binding Domains exhibit substantially wild-type binding while providing attenuated MHC class II binding:

SEQ ID NO: NO:51:

LAEAKVLANR ELDKYGVSDY YKNLID₆₆NAKS₇₀ A₇₁EGVKALIDE ILAALP

SEQ ID NO: NO:52:

LAEAKVLANR ELDKYGVSDY YKNA₆₄A₆₅NNAKT VEGVKALIAA₇₉E ILAALP

SEQ ID NO: NO:53:

LAEAKVLANR ELDKYGVSDY YKNLIS₆₆NAKS₇₀ VEGVKALIAA₇₉E ILAALP

[00155] Although such Albumin-Binding Domains may be incorporated into any of the polypeptide chains of the Tri-Specific Binding Molecules of the present invention, it is preferred to position such Domain C-terminally to the E-coil (or K-coil) Domain of the first or third polypeptide chain (via a linker that intervenes between the E-coil (or K-coil) Domain and the Albumin-Binding Domain (which is preferably a deimmunized Albumin-Binding Domain)). A preferred sequence for such a linker is **SEQ ID NO:26**: GGGGS.

F. Preferred Tumor Antigens (TA) and Exemplary Variable Domains

[00156] The **CD137 x TA** Binding Molecules of the present invention comprise at least one epitope-binding site specific for an epitope of a tumor antigen. Exemplary Tumor Antigens (“**TAs**”), which may be bound by the **CD137 x TA** Binding Molecules of the present invention include, but are not limited to: colon cancer antigen **19.9**; oncofetal protein **5T4**; gastric cancer mucin **antigen 4.2**; colorectal carcinoma antigen **A33** (Almqvist, Y. 2006, *Nucl Med Biol.* Nov;33(8):991-998); **ADAM-9** (United States Patent Publication No. 2006/0172350; PCT Publication No. WO 06/084075; **AFP** oncofetal antigen-alpha-fetoprotein (Malaguarnera, G. *et al.* (2010) “Serum markers of hepatocellular carcinoma,” *Dig. Dis. Sci.* 55(10):2744-2755); **ALCAM** (PCT Publication No. WO 03/093443); **BAGE** (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **beta-catenin** (Prange W. *et al.* 2003 *J Pathol.* 201(2):250-9); **CA125** (Bast, R.C. Jr. *et al.* 2005 *Int J Gynecol Cancer* 15 Suppl 3:274-81); **Carboxypeptidase M** (United States Patent Publication No. 2006/0166291); **B1** (Egloff, A.M. *et al.* 2006, *Cancer Res.* 66(1):6-9); **CD5** (Calin, G.A. *et al.* 2006 *Semin Oncol.* 33(2):167-73; **CD19** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CD20** (Thomas, D.A. *et al.* 2006 *Hematol*

Oncol Clin North Am. 20(5):1125-36); **CD20** (Cang, S. *et al.* (2012) “*Novel CD20 Monoclonal Antibodies For Lymphoma Therapy*,” J. Hematol. Oncol. 5:64 pp.1-9); **CD22** (Kreitman, R.J. 2006 AAPS J. 18;8(3):E532-51); **CD23** (Rosati, S. *et al.* 2005 *Curr Top Microbiol Immunol.* 5;294:91-107); **CD25** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CD27** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD28** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD30** (Muta, H. *et al.* (2013) “*CD30: From Basic Research To Cancer Therapy*,” Immunol. Res. 57(1-3):151-158); **CD33** (Walter, R.B. *et al.* (2012) “Acute myeloid leukemia stem cells and CD33-targeted immunotherapy,” Blood 119(26):6198-6208); **CD36** (Ge, Y. 2005 *Lab Hematol.* 11(1):31-7); **CD40/CD154** (Messmer, D. *et al.* 2005 *Ann N Y Acad Sci.* 1062:51-60); **CD45** (Jurcic, J.G. 2005 *Curr Oncol Rep.* 7(5):339-46); **CD56** (Bataille, R. 2006 *Haematologica* 91(9):1234-40); **CD46** (United States Patent No. 7,148,038; PCT Publication No. WO 03/032814; Russell, S. *et al.* (2004) “*CD46: A Complement Regulator And Pathogen Receptor That Mediates Links Between Innate And Acquired Immune Function*,” Tissue Antigens 64(2):111-118); **CD52** (Hoelzer, D. *et al.* (2013) “Targeted therapy with monoclonal antibodies in acute lymphoblastic leukemia,” Curr. Opin. Oncol. 25(6):701-706); **CD79a/CD79b** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48; Chu, P.G. *et al.* 2001 *Appl Immunohistochem Mol Morphol.* 9(2):97-106); **CD103** (Troussard, X. *et al.* 1998 *Hematol Cell Ther.* 40(4):139-48); **CD123** (Taussig, D. *et al.* (2005) “*Hematopoietic Stem Cells Express Multiple Myeloid Markers: Implications For The Origin And Targeted Therapy Of Acute Myeloid Leukemia*,” Blood 106:4086-4092); **CD317** (Palma, G. *et al.* (2012) “*Plasmacytoids Dendritic Cells Are A Therapeutic Target In Anticancer Immunity*,” Biochim. Biophys. Acta. 1826(2):407-414; **CDK4** (Lee, Y.M. *et al.* 2006 *Cell Cycle* 5(18):2110-4); **CEA** (carcinoembryonic antigen; Mathelin, C. 2006 *Gynecol Obstet Fertil.* 34(7-8):638-46; Tellez-Avila, F.I. *et al.* 2005 *Rev Invest Clin.* 57(6):814-9); **CEACAM5** and **CEACAM6** (PCT Publication No. WO 2011/034660; 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** (Adkins, J.C. *et al.* (1998) “*Edrecolomab (Monoclonal Antibody 17-1A)*,” Drugs 56(4):619-626; **CO-43** (blood group Leb) and **CO-514** (blood group Lea) (Garratty, G. (1995) “*Blood Group Antigens As Tumor Markers, Parasitic/Bacterial/Viral Receptors, And Their Association With Immunologically Important Proteins*,” Immunol. Invest. 24(1-2):213-232; **CTLA-1** and **CTLA-4** (Peggs, K.S. *et al.* 2006 *Curr Opin Immunol.* 18(2):206-13); **Cytokeratin 8** (PCT Publication No. WO 03/024191); antigen D1.1 (Dao, T. *et al.* (2009) “*Identification Of A Human Cyclin D1-Derived Peptide That Induces Human Cytotoxic CD4 T Cells*,” PLoS One.

4(8):e6730); DR5 (Abdulghani, J. *et al.* (2010) “*TRAIL Receptor Signaling And Therapeutics*,” Expert Opin. Ther. Targets 14(10):1091-1108; Andera, L. (2009) “*Signaling Activated By The Death Receptors Of The TNFR Family*,” Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub. 153(3):173-180; Carlo-Stella, C. *et al.* (2007) “*Targeting TRAIL Agonistic Receptors for Cancer Therapy*,” Clin. Cancer 13(8):2313-2317; Chaudhari, B.R. *et al.* (2006) “*Following the TRAIL to Apoptosis*,” Immunologic Res. 35(3):249-262); **E1 series** (blood group B); **EGF-R** (epidermal growth factor receptor; Adenis, A. *et al.* 2003 *Bull Cancer*. 90 Spec No:S228-32); **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 *Oncogene* 21(57):8732-40; Rimón, E. *et al.* 2004 *Int J Oncol*. 24(5):1325-38); lung adenocarcinoma antigen **F3** (Greulich, H. *et al.* (2012) “*Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2*,” Proc. Natl. Acad. Sci. (U.S.A.) 109(36):14476-14481); antigen **FC10.2** (Loveless, W. *et al.* (1990) “*Developmental Patterning Of The Carbohydrate Antigen FC10.2 During Early Embryogenesis In The Chick*,” Development 108(1):97-106); **GAGE** (GAGE-1; GAGE-2; Akcakanat, A. *et al.* 2006 *Int J Cancer*. 118(1):123-8); **GD2/GD3/GD49/GM2/GM3** (Livingston, P.O. *et al.* 2005 *Cancer Immunol Immunother*. 54(10):1018-25); **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); **gp37** (human leukemia T cell antigen ((Bhattacharya-Chatterjee *et al.* (1988) “*Idiotypic Vaccines Against Human T Cell Leukemia. II. Generation And Characterization Of A Monoclonal Idiotypic Cascade (Ab1, Ab2, and Ab3)*,” J. Immunol. 141:1398-1403); **gp75** (melanoma antigen) (Vijayasardahl *et al.* (1990) “*The Melanoma Antigen Gp75 Is The Human Homologue Of The Mouse B (Brown) Locus Gene Product*,” J. Exp. Med. 171(4):1375-1380); **gp100** (Lotem, M. *et al.* 2006 *J Immunother*. 29(6):616-27); **HER-2/neu** (Kumar, Pal S *et al.* 2006 *Semin Oncol*. 33(4):386-91); **human B-lymphoma antigen-CD20** (Reff *et al.* (1994) “*Depletion Of B Cells In Vivo By A Chimeric Mouse Human Monoclonal Antibody To CD20*,” Blood 83:435-445); **human milk fat globule antigen**; **human papillomavirus-E6/human papillomavirus-E7** (DiMaio, D. *et al.* 2006 *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) such as **I(Ma)** as found in gastric adenocarcinomas; Integrin Alpha-V-Beta-6 Integrin β 6 (**ITGB6**) (PCT Publication No. WO 03/087340); JAM-3 (PCT Publication No. WO 06/084078); Interleukin-13 Receptor α 2 (**IL13R α 2**) (Bodhinayake, I. *et al.* (2014) “*Targeting A Heterogeneous Tumor: The Promise Of The Interleukin-13 Receptor α 2*,” Neurosurgery 75(2):N18-9); **JAM-3** (PCT Publication No. WO 06/084078); **KID3** (PCT Publication No. WO 05/028498); **KID31** (PCT Publication No. WO 06/076584); **KS 1/4 pan-carcinoma antigen** (Perez *et al.* (1989) “*Isolation And Characterization Of A cDNA Encoding The Ks1/4 Epithelial Carcinoma Marker*,” J. Immunol. 142:3662-3667; Möller *et al.* (1991) “*Bispecific-Monoclonal-Antibody-Directed Lysis Of Ovarian Carcinoma Cells By Activated Human T Lymphocytes*,” Cancer Immunol. Immunother. 33(4):210-216; Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); **KS 1/4 pan-carcinoma antigen** (Perez *et al.* (1989) “*Isolation And Characterization Of A cDNA Encoding The Ks1/4 Epithelial Carcinoma Marker*,” J. Immunol. 142:3662-3667; Möller *et al.* (1991) “*Bispecific-Monoclonal-Antibody-Directed Lysis Of Ovarian Carcinoma Cells By Activated Human T Lymphocytes*,” Cancer Immunol. Immunother. 33(4):210-216; Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); **KSA** (17-1A) (Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); human lung carcinoma antigens **L6** and **L20** (Hellström *et al.* (1986) “*Monoclonal Mouse Antibodies Raised Against Human Lung Carcinoma*,” Cancer Res. 46:3917-3923); **LEA** (Velázquez-Márquez, N. *et al.* (2012) “*Sialyl Lewis x expression in cervical scrapes of premalignant lesions*,” J. Biosci. 37(6):999-1004); **LUCA-2** (United States Patent Publication No. 2006/0172349; PCT Publication No. WO 06/083852); **M1:22:25:8**, **M18**, **M39** (Cambier, L. *et al.* (2012) “*M19 Modulates Skeletal Muscle Differentiation And Insulin Secretion In Pancreatic B-Cells Through Modulation Of Respiratory Chain Activity*,” PLoS One 7(2):e31815; Pui, C.H. *et al.* (1991) “*Characterization of childhood acute leukemia with multiple myeloid and lymphoid markers at diagnosis and at relapse*,” Blood 78(5):1327-1337); **MAGE** (MAGE-1; MAGE-3; (Bodey, B. 2002 *Expert Opin Biol Ther.* 2(6):577-84); **MART** (Kounalakis, N. *et al.* 2005 *Curr Oncol Rep.* 7(5):377-82; **MyI**, **MUC-1** (Mathelin, C. 2006 *Gynecol Obstet Fertil.* 34(7-8):638-46); **MUM-1** (Castelli, C. *et al.* 2000 *J Cell Physiol.* 182(3):323-31); **N-acetylglucosaminyltransferase** (Dennis, J.W. 1999 *Biochim Biophys Acta.* 6;1473(1):21-34); **neoglycoprotein** (Legendre, H. *et al.* (2004) “*Prognostic Stratification Of Dukes B Colon Cancer By A Neoglycoprotein*,” Int. J. Oncol. 25(2):269-276); **NS-10**; **OFA-1**

and **OFA-2** (Takahashi, M. (1984) "A Study On Clinical Significance Of Oncofetal Antigen-1 In Gynecologic Tumors," *Nihon Sanka Fujinka Gakkai Zasshi*. 36(12):2613-2618); **Oncostatin M** (Oncostatin Receptor Beta) (United States Patent No. 7,572,896; PCT Publication No. WO 06/084092); **p15** (Gil, J. *et al.* 2006 *Nat Rev Mol Cell Biol*. 7(9):667-77); **PSA** (prostate specific antigen; Cracco, C.M. *et al.* 2005 *Minerva Urol Nefrol*. 57(4):301-11); **PSMA** (Ragupathi, G. 2005 *Cancer Treat Res*. 123:157-80); **PEMA (polymorphic epithelial mucin antigen)** (Chu, N.J. *et al.* (2015) "Nonviral Oncogenic Antigens and the Inflammatory Signals Driving Early Cancer Development as Targets for Cancer Immunoprevention," *Clin. Cancer Res*. 21(7):1549-1557); **PIPA** (United States Patent No. 7,405,061; PCT Publication No. WO 04/043239); **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); **R24** (Zhou, M. *et al.* (2008) "Constitutive Overexpression Of A Novel 21 Kda Protein By Hodgkin Lymphoma And Aggressive Non-Hodgkin Lymphomas," *Mol. Cancer* 7:12); **ROR1** (United States Patent No. 5,843,749); Rabbani, H. *et al.* (2010) "Expression Of ROR1 In Patients With Renal Cancer--A Potential Diagnostic Marker," *Iran Biomed. J*. 14(3):77-82); sphingolipids (Hakomori, S. (1998) "Cancer-Associated Glycosphingolipid Antigens: Their Structure, Organization, And Function," *Acta Anat. (Basel)* 161(1-4):79-90; **SSEA-1, SSEA-3 and SSEA-4** (Muramatsu, T. *et al.* (2004) "Carbohydrate Antigens Expressed On Stem Cells And Early Embryonic Cells," *Glycoconj. J*. 21(1-2):41-45); **sTn** (Holmberg, L.A. 2001 *Expert Opin Biol Ther*. 1(5):881-91); **T cell receptor derived peptide** (Edelson (1998) "Cutaneous T-Cell Lymphoma: A Model For Selective Immunotherapy," *Cancer J Sci Am*. 4:62-71); T₅A₇ (Hogg, R.J. *et al.* (1991) "A monoclonal antibody exhibiting reactivity with both X-hapten- and lactose-bearing glycolipids," *Tissue Antigens* 37(1):33-38); **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) (Gooi, H.C. *et al.* (1983) "Monoclonal antibody reactive with the human epidermal-growth-factor receptor recognizes the blood-group-A antigen," *Biosci. Rep*. 3(11):1045-1052); **TNF-receptor** (TNF- α receptor, TNF- β receptor; or **TNF- γ receptor** (van Horssen, R. *et al.* 2006 *Oncologist*. 11(4):397-408; Gardnerova, M. *et al.* 2000 *Curr Drug Targets*. 1(4):327-64); **TRA-1-85** (blood group H) (Williams, B.P. *et al.* (1988) "Biochemical and genetic analysis of the OKa blood group antigen," *Immunogenetics* 27(5):322-329); **Transferrin Receptor** (United States Patent No. 7,572,895; PCT Publication No. WO 05/121179); **TSTA** tumor-specific transplantation antigen (Hellström *et al.* (1985) "Monoclonal Antibodies To Cell Surface Antigens Shared By Chemically Induced Mouse

Bladder Carcinomas,” *Cancer. Res.* 45:2210-2188); **VEGF-R** (O’Dwyer. P.J. 2006 *Oncologist*. 11(9):992-8); and Y hapten, Le^y (Durrant, L.G. *et al.* (1989) “*Development Of An ELISA To Detect Early Local Relapse Of Colorectal Cancer*,” *Br. J. Cancer* 60(4):533-537).

[00157] Antibodies that recognize such Tumor Antigens are known in the art or can be generated using well-known methods, including those described in WO 2002/014870. Exemplary antibodies that comprise VL and VH Domains capable of binding to a Tumor Antigen, and whose sequences or polypeptide chains may thus be employed in the construction of the **CD137 x TA** Binding Molecules of the present invention, are listed in **Table 1**. Exemplary VH and VL Domains for antibodies binding to several Tumor Antigens are presented below.

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

Table 1		
Antibody Name	Tumor Antigen(s)	Therapeutic Target Application
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
Citatumumab	Epcam	Ovarian Cancer And Other Solid Tumors
Cixutumumab	IGF-1 Receptor	Solid Tumors
Clivatuzumab	MUC1	Pancreatic Cancer
Conatumumab	TRAIL-R2	Cancer
Dacetuzumab	CD40	Hematologic Cancers
Dalotuzumab	Insulin-Like Growth Factor I Receptor	Cancer
Daratumumab	CD38	Cancer
Dencizumab	DLL4	Cancer
Detumomab	B-Lymphoma Cell	Lymphoma
Drozitumab	DR5	Cancer
Duligotumab	HER3	Cancer
Dusigitumab	ILGF2	Cancer
Ecomeximab	GD3 Ganglioside	Malignant Melanoma
Eculizumab	C5	Paroxysmal Nocturnal Hemoglobinuria
Edrecolomab	Epcam	Colorectal Carcinoma
Elotuzumab	SLAMF7	Multiple Myeloma
Elsilimomab	IL-6	Cancer
Enavatuzumab	TWEAK Receptor	Cancer
Enlimomab	ICAM-1 (CD54)	Cancer
Enokizumab	IL9	Asthma
Enoticumab	DLL4	Cancer
Ensituximab	5AC	Cancer
Epitumomab Cituxetan	Episialin	Cancer
Epratuzumab	CD22	Cancer, SLE
Ertumaxomab	HER2/Neu, CD3	Breast Cancer
Etaracizumab	Integrin $\alpha_v\beta_3$	Melanoma, Prostate Cancer, Ovarian Cancer
Faralimomab	Interferon Receptor	Cancer
Farletuzumab	Folate Receptor 1	Ovarian Cancer
Fasimumab	HNGF	Cancer
Fbta05	CD20	Chronic Lymphocytic Leukemia
Ficlatuzumab	HGF	Cancer
Figitumumab	IGF-1 Receptor	Adrenocortical Carcinoma, Non-Small Cell Lung Carcinoma

Table 1		
Antibody Name	Tumor Antigen(s)	Therapeutic Target Application
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-1 β	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
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

Table 1		
Antibody Name	Tumor Antigen(s)	Therapeutic Target Application
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
Milatumumab	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
Nacolumab Tafenatox	C242 Antigen	Colorectal Cancer
Namilumab	CSF2	Cancer
Naptumomab Estafenatox	5T4	Non-Small Cell Lung Carcinoma, Renal Cell Carcinoma
Narnatumab	RON	Cancer
Nebacumab	Endotoxin	Sepsis
Necitumumab	EGFR	Non-Small Cell Lung Carcinoma
Nerelimomab	TNF-A	Cancer
Nesvacumab	Angiopoietin 2	Cancer
Nimotuzumab	EGFR	Squamous Cell Carcinoma, Head And Neck Cancer, Nasopharyngeal Cancer, Glioma
Nivolumab	PD-1	Cancer
Nofetumomab Merpentan	Undetermined	Cancer
Ocaratuzumab	CD20	Cancer
Ofatumumab	CD20	Chronic Lymphocytic Leukemia
Olaratumab	PDGF-R A	Cancer
Olokizumab	IL6	Cancer
Onartuzumab	Human Scatter Factor Receptor Kinase	Cancer
Ontuxizumab	TEM1	Cancer
Oportuzumab Monatox	Epcam	Cancer
Oregovomab	CA-125	Ovarian Cancer
Orticumab	Oxldl	Cancer
Otlertuzumab	CD37	Cancer
Panitumumab	EGFR	Colorectal Cancer

Table 1		
Antibody Name	Tumor Antigen(s)	Therapeutic Target Application
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	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Pritumumab	Vimentin	Brain Cancer
Quilizumab	IGHE	Cancer
Racotumomab	N-Glycolylneuraminic Acid	Cancer
Radretumab	Fibronectin Extra Domain-B	Cancer
Ramucirumab	VEGFR2	Solid Tumors
Rilotumumab	HGF	Solid Tumors
Rituximab	CD20	Lymphomas, Leukemias, Some Autoimmune Disorders
Robatumumab	IGF-1 Receptor	Cancer
Roledumab	RHD	Cancer
Samalizumab	CD200	Cancer
Satumomab Pendetide	TAG-72	Cancer
Seribantumab	ERBB3	Cancer
Setoxaximab	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Sgn-CD19a	CD19	Acute Lymphoblastic Leukemia And B Cell Non-Hodgkin Lymphoma
Sgn-CD33a	CD33	Acute Myeloid Leukemia
Sibrotuzumab	FAP	Cancer
Siltuximab	IL-6	Cancer
Solitumab	Epcam	Cancer
Sontuzumab	Episialin	Cancer

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

1. Antibodies that Bind to HER2/neu

[00158] 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) “*The Biology of erbB-2/neu/HER-2 and its Role in Cancer*,” Biochim. Biophys. Acta 1198:165-184; Dougall *et al.* (1994) “*The neu-Oncogene: Signal Transduction Pathways, Transformation Mechanisms and Evolving Therapies*,” Oncogene

9:2109-2123; Lee *et al.* (1995) “Requirement for Neuregulin Receptor *erbB2* in Neural and Cardiac Development,” Nature 378:394-398).

[00159] The epitope-binding site of any anti-HER2/neu antibody may be used in accordance with the present invention, and the principles of the present invention are illustrated with respect to the HER2/neu tumor antigen. Exemplary antibodies that bind human HER2/neu include “**Margetuximab**,” “**Trastuzumab**” and “**Pertuzumab**.” Margetuximab (also known as MGAH22; CAS Reg No. 1350624-75-7, see, for example, US Patent No. 8,802,093) 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. Additional anti-HER2/neu antibodies are also provided.

(a) Margetuximab

[00160] The amino acid sequence of the VH Domain of Margetuximab is (SEQ ID NO:54) (CDR_H residues are shown underlined):

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

[00161] The amino acid sequence of the VL Domain of Margetuximab is (SEQ ID NO:55) (CDR_L residues are shown underlined):

DIVMTQSHKF MSTSVGDRVS ITCKASQDVN TAVAWYQQKP GHSPKLLIYS
ASFRYTGVDPD RFTGSRSGTD FTFTISSVQA EDLAVYYCQQ HYTTPPTFGG
 GTKVEIK

[00162] 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

[00163] The amino acid sequence of the VH Domain of **Trastuzumab** is (SEQ ID NO:56)

(CDR_H residues are shown underlined):

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

[00164] The amino acid sequence of the VL Domain of **Trastuzumab** is (SEQ ID NO:57)

(CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS
ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
 GTKVEIK

(c) Pertuzumab

[00165] The amino acid sequence of the VH Domain of **Pertuzumab** is (SEQ ID NO:58)

(CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFNFT DYTMDWVRQA PGKGLEWVAD
VNPNSGGSIY NQRFKGRFTL SVDRSKNTLY LQMNSLRAED TAVYYCARNL
GPSFYFDYW QGTLTVTVSS

[00166] The amino acid sequence of the VL Domain of **Pertuzumab** is (SEQ ID NO:59)

(CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCKASQDVS IGVAWYQQKP GKAPKLLIYS
ASYRYTGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYIYPYTFGQ
 GTKVEIK

(d) HER2-MAB-1

[00167] Antibody **HER2-MAB-1** is a murine anti-HER2/neu monoclonal antibody that binds an epitope of HER2/neu that is distinct from the epitope recognized by Margetuximab, Trastuzumab and Pertuzumab. The amino acid sequence of the VH Domain of **HER2-MAB-1** (referred to herein as **HER2 MAB-1 VH**) is (SEQ ID NO:60) (CDR_H residues are shown underlined):

EVQLQESGPE LKKPGETVKI SCKASGYTFT NYGMNWVKQA PGKGLKWMGW
INTNIGEPTY TEEFKGRFAF SLGTSASTAF LQINNLKNEE TATYFCARDDD
GYGNRVSYWG QGTLTVTVSA

[00168] The amino acid sequences of the CDR_{HS} of **HER2 MAB-1 VH** are:

CDR_{H1} (SEQ ID NO:177): NYGMN

CDR_{H2} (SEQ ID NO:178): WINTNIGEPTYTEEFKG

CDR_{H3} (SEQ ID NO:179): DDGYGNRVSY

[00169] The amino acid sequence of the VL Domain of **HER2-MAB-1** (referred to herein as **HER2 MAB-1 VL**) is (SEQ ID NO:61) (CDRL residues are shown underlined):

DILMTQSPLS MYTSLGERVT ITCKASQDIN SYLSWFOQKP GKSPKTLIYR
ANRLVDGVPS RFGSGSGQD YSLTISSEY EDMGIYYCLQ HDEFPWTFGG
 GTKLEIK

[00170] The amino acid sequences of the CDRLs of **HER2 MAB-1 VL** are:

CDRL1 (SEQ ID NO:180): KASQDINSYLS

CDRL2 (SEQ ID NO:181): RANRLVD

CDRL3 (SEQ ID NO:182): LQHDEFWPT

(e) Humanized HER2-MAB-1

[00171] Antibody **HER2-MAB-1** was humanized to form antibody **hHER2-MAB-1**. The amino acid sequence of the VH Domain of such humanized antibody (**hHER2-MAB-1 VH**) is (SEQ ID NO:62) (CDRH residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARDX₁
X₂YGNRVSYWG QGTLVTVSS
 wherein: X₁ is D or E and X₂ is G or I

[00172] The amino acid sequence of the VL Domain of such humanized antibody (**hHER2 MAB-1 VL**) is (SEQ ID NO:63) (CDRL residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCKASQDIX₃ X₄YLSWFOQKP GKAPKTLIYR
ANRLX₅X₆GVPS RFGSGSGTD FTLTISLQP EDFATYYCLQ HDEFPWTFGQ
 GTKLEIK
 wherein: X₃ is N or S; X₄ is S, T or N; X₅ is V or Q and X₆ is D, E or S

[00173] Three variant **hHER2-MAB-1 VH** Domains were isolated: **hHER2 MAB-1 VH1**, **hHER2 MAB-1 VH2**, and **hHER2 MAB-1 VH3**. The amino acid sequences of such variant **hHER2 MAB-1 VH** Domains are presented below.

[00174] The amino acid sequence of **hHER2 MAB-1 VH1** is (SEQ ID NO:64) (CDRH residues are shown underlined; note that the second and third residues of **CDRH3** are D and G, respectively):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARDD
GYGNRVSYWG QGTLVTVSS

[00175] The amino acid sequence of **hHER2 MAB-1 VH2** is (SEQ ID NO:65) (CDRH residues are shown underlined; note that the second and third residues of **CDRH3** are E and G, respectively):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARDE
GYGNRVSYWG QGTLTVVSS

[00176] The amino acid sequence of **hHER2 MAB-1 VH3** is (SEQ ID NO:66) (CDR_H residues are shown underlined; note that the second and third residues of CDR_{H3} are D and I, respectively):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARDD
IYGNRVSYWG QGTLTVVSS

[00177] Thus, the amino acid sequence of CDR_{H1} of **hHER2 MAB-1 VH1**, **hHER2 MAB-1 VH2** and **hHER2 MAB-1 VH3** are the same (NYGMN; SEQ ID NO:177) and the amino acid sequence of CDR_{H2} of **hHER2 MAB-1 VH1**, **hHER2 MAB-1 VH2** and **hHER2 MAB-1 VH3** are the same (SEQ ID NO:178). However, the amino acid sequences of CDR_{H3} of **hHER2 MAB-1 VH1**, **hHER2 MAB-1 VH2** and **hHER2 MAB-1 VH3** differ:

hHER2 MAB-1 VH1 CDR_{H3} (SEQ ID NO:183): DDGYGNRVSY

hHER2 MAB-1 VH2 CDR_{H3} (SEQ ID NO:184): DEGYGNRVSY

hHER2 MAB-1 VH3 CDR_{H3} (SEQ ID NO:185): DDIYGNRVSY

[00178] Three variant **hHER2-MAB-1 VL** Domains were isolated: **hHER2 MAB-1 VL1**, **hHER2 MAB-1 VL2**, and **hHER2 MAB-1 VL3**. The amino acid sequences of such variant **hHER2 MAB-1 VH** Domains are presented below.

[00179] The amino acid sequence of **hHER2 MAB-1 VL1** is (SEQ ID NO:67) (CDR_L residues are shown underlined; note that the seventh and eighth residues of CDR_{L1} are N and S, respectively, and that the sixth and seventh residues of CDR_{L2} are V and D, respectively):

DIQMTQSPSS LSASVGDRVT ITCKKASQDIN SYLSWFQQKP GKAPKTLIYR
ANRLVDGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
 GTKLEIK

[00180] The amino acid sequence of **hHER2 MAB-1 VL2** is (SEQ ID NO:68) (CDR_L residues are shown underlined; note that the seventh and eighth residues of CDR_{L1} are N and T, respectively, and that the sixth and seventh residues of CDR_{L2} are V and E, respectively):

DIQMTQSPSS LSASVGDRVT ITCKKASQDIN TYLSWFQQKP GKAPKTLIYR
ANRLVEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
 GTKLEIK

[00181] The amino acid sequence of **hHER2 MAB-1 VL3** is (SEQ ID NO:69) (CDRL residues are shown underlined; note that the seventh and eighth residues of **CDRL1** are S and N, respectively, and that the sixth and seventh residues of **CDRL2** are Q and S, respectively):

DIQMTQSPSS LSASVGDRVT ITCKASQDIS NYLSWFQQKP GKAPKTLIYR
ANRLQSGVPS RFGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
 GTKLEIK

[00182] Thus, the amino acid sequence of CDR_{L3} of **hHER2 MAB-1 VL1**, **hHER2 MAB-1 VL2** and **hHER2 MAB-1 VL3** are the same (LQHDEFPTWT; SEQ ID NO:182). However, the amino acid sequences of CDR_{L1} and CDR_{L2} of **hHER2 MAB-1 VL1**, **hHER2 MAB-1 VL2** and **hHER2 MAB-1 VL3** differ:

hHER2 MAB-1 VL1 CDR_{L1} (SEQ ID NO:186): KASQDINSYLS

hHER2 MAB-1 VL2 CDR_{L1} (SEQ ID NO:187): KASQDINTYLS

hHER2 MAB-1 VL3 CDR_{L1} (SEQ ID NO:188): KASQDISNYLS

hHER2 MAB-1 VL1 CDR_{L2} (SEQ ID NO:189): RANRLVD

hHER2 MAB-1 VL2 CDR_{L2} (SEQ ID NO:190): RANRLVE

hHER2 MAB-1 VL3 CDR_{L2} (SEQ ID NO:191): RANRLQS

[00183] Any of such humanized VH and VL **hHER2-MAB-1** Domains, including any embraced within the generic sequence(s) of the **hHER2-MAB-1** VH and/or VL Domains presented above may be used to form an antibody, diabody or binding molecule capable of binding Her2/neu.

(f) Other Anti-HER2/neu Antibodies

[00184] In addition to the above-identified preferred anti-HER2/neu Binding Molecules, the invention contemplates the use 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 Patent Nos. 7,892,554; 8,173,424; 8,974,792; and PCT Patent Publication WO 99/55367); and also the anti-Her-2 Binding Molecules of US Patent Publication 2011/0097323, 2013/017114, 2014/0328836, 2016/0130360 and 2016/0257761, and PCT Patent Publication WO2011/147986), all of which publications are herein incorporated by reference for their disclosures of anti-HER2/neu Binding Molecules.

[00185] The present invention specifically includes and encompasses **CD137 x HER2/neu** Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDRLs

of the VL Region and/or 1, 2 or all 3 of the CDRHs of the VH Domain of any of Margetuximab, Trastuzumab, Pertuzumab, hHER2-MAB-1, or any of the other anti-HER2/neu antibodies provided herein; and more preferably possess 1, 2 or all 3 of the CDRLs of the VL Region and/or 1, 2 or all 3 of the CDRHs of the VH Domain of such anti-HER2/neu monoclonal antibodies.

2. Antibodies That Bind to EphA2

[00186] 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 tumor 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). The epitope-binding site of any anti-EphA2 antibody may be used in accordance with the present invention. Presented below are several exemplary murine anti-EphA2 antibodies, humanized derivatives of such antibodies are particularly preferred.

(a) EphA2 MAB-1

[00187] Antibody **EphA2 MAB-1** is a murine anti-EphA2 monoclonal antibody. The amino acid sequence of the VH Domain of **EphA2 MAB-1** is (SEQ ID NO:128) (CDR residues are shown underlined):

QVQLKESGPG LVAPSQSLSI TCTVSGFSL **RYSVHWVRQP** PGKGLEWLGM
IWGGGSTDYN **SALKS**RLSIS KDNSKSQVFL KMNSLQTD **DDT** AMYYCARK**KHG**
NYYTMDYWGQ GTSVTVSS

[00188] The amino acid sequence of the VL Domain of **EphA2 MAB-1** is (SEQ ID NO:129) (CDR residues are shown underlined):

DIQMTQTSS LSASLGDRIT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFGSGSGTD YSLTISNLEQ EDIATYFCQQ GYTLYTFGGG
 TKLEIK

(b) EphA2 MAB-2

[00189] Antibody **EphA2 MAB-2** is a murine anti-EphA2 monoclonal antibody. The amino acid sequence of the VH Domain of **EphA2 MAB-2** is (SEQ ID NO:130) (CDR residues are shown underlined):

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

[00190] The amino acid sequence of the VL Domain of **EphA2 MAB-2** is (SEQ ID NO:131) (CDR residues are shown underlined):

DVVMQTPLS LPVSLGDQAS ISCRSSQSLV HSSGNTYLHW YLQKPGQSPK
 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
TFGSGTKLEI K

(c) EphA2 MAB-3

[00191] Antibody **EphA2 MAB-3** is a murine anti-EphA2 monoclonal antibody. The amino acid sequence of the VH Domain of **EphA2 MAB-3** is (SEQ ID NO:132) (CDR residues are shown underlined):

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

[00192] The amino acid sequence of the VL Domain of **EphA2 MAB-3** is (SEQ ID NO:133) (CDR residues are shown underlined):

DIVLTQSHRS MSTSVGDRVN ITCKASQDVT TAVAWYQQKP GQSPKLLIFW
ASTRHAGVPD RFTGSGSGTD FTLTISSVQA GDLALYYCQQ HYSTPYTFGG
 GTKLEIK

(d) Other EphA2 Antibodies

[00193] In addition to the above-identified anti-EphA2 antibodies, the invention contemplates the use of any of the following anti-EphA2 antibodies: **SPL1**, **LUCA19**, **SG5**, or **LUCA40** (see, PCT Patent Publication WO 2006/084226); **B13** (see, US Patent No. 7,101,976); **D7** (see, US Patent No. 7,192,698); **B-233**, and **EA2** (see, PCT Patent Publication WO 2003/094859).

[00194] The present invention specifically includes and encompasses **CD137 x EphA2** Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDRLs

of the VL Region and/or 1, 2 or all 3 of the CDRHs of the VH Domain of anti-EphA2 monoclonal antibodies EphA2 MAB-1, EphA2 MAB-2 and EphA2 MAB-3.

3. Antibodies that Bind to 5T4

[00195] 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). The epitope-binding site of any anti-5T4 antibody may be used in accordance with the present invention. Presented below are two exemplary anti-5T4 antibodies, “**5T4 MAB-1**,” and “**5T4 MAB-2**”. Additional ant-5T4 antibodies are described in the art (see, *e.g.*, US Patent Nos: 8,084,249; 8,409,577; 8,759,495; 8,409,577; PCT Publication Nos: WO 2013/041687; WO 2014/137931; WO 2016/022939)

(a) 5T4 MAB-1

[00196] The amino acid sequence of the VH Domain of **5T4 MAB-1** is (SEQ ID NO:134) (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SFWMHWVRQA PGQGLEWMGR
IDPNRGGTEY NEKAKSRVTM TADKSTSTAY MELSSLRSED TAVYYCAGGN
PYYPMDYWGQ GTTVTVSS

[00197] The amino acid sequence of the VL Domain of **5T4 MAB-1** is (SEQ ID NO:135) (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
 GTKLEIK

(b) 5T4 MAB-2

[00198] The amino acid sequence of the VH Domain of **5T4 MAB-2** is (SEQ ID NO:136) (CDR residues are shown underlined):

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYWITWVKQR PGQGLEWIGD
IYPGSGRANY NEKFSKATL TVDTSSSTAY MQLSSLTSED SAVYNCARYYG
PLFTTVVDPN SYAMDYWGQ TSVTVSS

[00199] The amino acid sequence of the VL Domain of **5T4 MAB-2** is (SEQ ID NO:137) (CDR residues are shown underlined):

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

[00200] The present invention specifically includes and encompasses **CD137 x 5T4** Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDRLs of the VL Region and/or 1, 2 or all 3 of the CDRHs 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 2007/106744; WO 2013/041687 or WO 2015/184203.

4. Antibodies that Bind to B7-H3

[00201] B7-H3 is a Tumor 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 tumor cells (e.g., tumor 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).

[00202] The epitope-binding site of any anti-B7-H3 antibody may be used in accordance with the present invention. One exemplary antibody that bind human B7-H3 is "**Enoblituzumab**." Enoblituzumab (also known as MGA271; CAS Reg No. 1353485-38-7; see for example, US Patent No. 8,802,091) is an Fc-optimized monoclonal antibody that binds to B7-H3 and mediates enhanced ADCC activity. Additional exemplary anti-B7-H3 antibodies are also presented.

(a) Enoblituzumab

[00203] The amino acid sequence of the VH Domain of **Enoblituzumab** is (SEQ ID NO:138) (CDR_H residues are shown underlined):

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

[00204] The amino acid sequence of the VL Domain of **Enoblituzumab** is (SEQ ID NO:139) (CDR_L residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
 GTKLEIK

(b) BRCA69D

[00205] Antibody **BRCA69D** is a murine anti-B7-H3 monoclonal antibody. The amino acid sequence of the VH Domain of **BRCA69D** (SEQ ID NO:140) is shown below (CDR_H residues are shown underlined).

QVQLQQSGAE LARPGASVKL SCKASGYTFT SYWMQWVKQR PGQGLEWIGT
IYPGDGDTRY TQKFKGKATL TADKSSSTAY MQLSSLASED SAVYYCARRG
IPRLWYFDVW GAGTTVTVSS

[00206] The amino acid sequence of the VL Domain of **BRCA69D** (SEQ ID NO:141) is shown below (CDR_L residues are shown underlined).

DIQMTQTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTIDNLEQ EDIATYFCQQ GNTLPPTFGG
 GTKLEIK

(c) Humanized BRCA69D

[00207] Antibody **BRCA69D** was humanized yielding two variant VH Domains, **hBRCA69D VH1** and **hBRCA69D VH2**; and two variant VL Domains **hBRCA69D VL1** and **hBRCA69D VL2**, which may be used in any combination of VH/VL to yield a functional humanized binding domain. The amino sequences of such humanized variants are provided below.

[00208] The amino acid sequence of the VH Domain of **hBRCA69D VH1** is (SEQ ID NO:142) (CDR_H residues are shown underlined):

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

[00209] The amino acid sequence of the VH Domain of **hBRCA69D VH2** is (SEQ ID NO:143) (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWMQWVRQA PGQGLEWMGT
IYPGGDTRY TQKFQGRVTI TADKSTSTAY MELSSLRSED TAVYYCARRG
IPRLWYFDVW GQGTTVTVSS

[00210] The amino acid sequence of the VL Domain of **hBRCA69D VL1** is (SEQ ID NO:144) (CDR_L residues are shown underlined).

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY
TSRLHSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
 GTKLEIK

[00211] The amino acid sequence of the VL Domain of **hBRCA69D VL2** is (SEQ ID NO:145) (CDR_L residues are shown underlined).

DIQMTQSPSS LSASVGDRVT ITCRASQDIS SYLNWYQQKP GKAPKLLIYY
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
 GTKLEIK

(d) **PRCA157**

[00212] Antibody **PRCA157** is a murine anti-B7-H3 monoclonal antibody. The amino acid sequence of the VH Domain of **PRCA157** is (SEQ ID NO:146) (CDR_H residues are shown underlined).

EVQQVESGGD LVKPGGSLKL SCAASGFTFS SYGMSWVRQT PDKRLEWVAT
INSGGSNTYY PDSLKGRFTI SRDNAKNTLY LQMRSLKSED TAVYYCARHD
GGAMDYWGQG TSVTVSS

[00213] The amino acid sequence of the VL Domain of **PRCA157** is (SEQ ID NO:147) (CDR_H residues are shown underlined).

DIQMTQSPAS LSVSVGETVT ITCRASESIY SYLAWYQQKQ GKSPQLLVYN
TKTLPEGVPS RFSGSGSGTQ FSLKINSLQP EDFGRYYCQH HYGTPPWTFG
 GGTNLEIK

(e) **Humanized PRCA157**

[00214] Antibody **PRCA157** was humanized yielding one humanized VH Domain, **hPRCA157 VH1** and one humanized VL Domains **hPRCA157 VL1**, to yield a functional humanized binding domain. The amino sequences of humanized **PRCA157** are provided below.

[00215] The amino acid sequence of the VH Domain of **hPRCA157 VH1** is (SEQ ID NO:202) (CDR_H residues are shown underlined):

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

[00216] The amino acid sequence of the VL Domain of **hPRCA157 VL1** is (SEQ ID NO:203) (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASESIY SYLAWYQQKP GKAPKLLVYN
 TKTLPEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH HYGTPPWTFG
 QGTRLEIK

(f) Other anti-B7-H3 antibodies

[00217] In addition to the above-identified preferred anti-B7-H3 Binding Molecules, the invention contemplates the use of any of the following anti-B7-H3 Binding Molecules: **LUCA1; BLA8; PA20; or SKN2** (see, US Patent Nos. 7,527,969; 8,779,098 and PCT Patent Publication WO 2004/001381); **M30; cM30; M30-H1-L1; M30-H1-L2; M30-H1-L3; M30-H1-L4; M30-H1-L5; M30-H1-L6; M30-H1-L7; M30-H4-L1; M30-H4-L2; M30-H4-L3; and M30-H4-L4** (see, US Patent Publication 2013/0078234 and PCT Patent Publication WO 2012/147713; and **8H9** (see US Patent Nos. 7,666,424; 7,737,258; 7,740,845; 8,148,154; 8,414,892; 8,501,471; 9,062,110; US Patent Publication 2010/0143245 and PCT Patent Publication WO 2008/116219).

[00218] The present invention specifically includes and encompasses **CD137 x B7-H3** 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, **BRCA69D**, humanized **BRCA69D**, **PRCA157**, humanized **PRCA157**, or Enoblituzumab, or any of the other anti-B7-H3 antibodies provided herein; 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-B7-H3 monoclonal antibodies.

5. Antibodies that Bind to GpA33

[00219] 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). The epitope-binding site of any anti-gpA33 antibody may be used in accordance with the present invention. An exemplary anti-gpA33 antibody (“**gpA33 MAB-1**”) is presented below.

[00220] The amino acid sequence of the VH Domain of **gpA33 MAB-1** is (SEQ ID NO:148) (CDR residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT GSWMNWVRQA PGQGLEWIGR
IYPGDGETNY NGKFKDRVTI TADKSTSTAY MELSSLRSED TAVYYCARIY
GNNVYFDVWG QGTTVTVSS

[00221] The amino acid sequence of the VL Domain of **gpA33 MAB-1** is (SEQ ID NO:149) (CDR residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITCSARSSIS FMYWYQQKPG KAPKLLIYDT
SNLASGVPSR FSGSGSGTEF TLTISSEAE DAATYYCQQW SSYPLTFGQG
 TKLEIK

[00222] The present invention specifically includes and encompasses **CD137 x gpA33** Binding Molecules that comprise the VL and/or VH Domain, and/or 1, 2 or all 3 of the CDRLs of the VL Region and/or 1, 2 or all 3 of the CDRHs 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.

6. Antibodies that Bind to CEACAM5 and CEACAM6

[00223] Carcinoembryonic Antigen-Related Cell Adhesion Molecules 5 (CEACAM5) and 6 (CEACAM6) have been found to be associated with various types of cancers including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660), and particularly colorectal, gastrointestinal, pancreatic, non-small cell lung cancer (NSCL), breast, thyroid, stomach, ovarian and uterine carcinomas (Zheng, C. *et al.* (2011) “*A Novel Anti-CEACAM5 Monoclonal Antibody, CC4, Suppresses Colorectal Tumor Growth and Enhances NK Cells-Mediated Tumor Immunity*,” PLoS One 6(6):e21146, pp. 1-11). CEACAM5 has been found to be overexpressed in 90% of gastrointestinal, colorectal and pancreatic cancers, 70% of non-small cell lung cancer cells and 50% of breast cancers (Thompson, J.A. *et al.* (1991) “*Carcinoembryonic Antigen Gene Family:*

Molecular Biology And Clinical Perspectives,” J. Clin. Lab. Anal. 5:344-366). Overexpressed carcinoembryonic antigen-related cellular adhesion molecule 6 (CEACAM6) plays important roles in the invasion and metastasis of a variety of human cancers, including medullary thyroid cancer, colorectal cancer, pancreatic cancer, hepatocellular carcinoma, gastric cancer, lung cancer, head and neck cancers, urinary bladder cancer, prostate cancer, uterine cancer, endometrial cancer, breast cancer, hematopoietic cancer, leukemia and ovarian cancer (PCT Publication No. WO 2011/034660; Deng, X., *et al.* (2014) “*Expression Profiling Of CEACAM6 Associated With The Tumorigenesis And Progression In Gastric Adenocarcinoma*,” Genet. Mol. Res. 13(3):7686-7697; Cameron, S. *et al.* (2012) “*Focal Overexpression Of CEACAM6 Contributes To Enhanced Tumourigenesis In Head And Neck Cancer Via Suppression Of Apoptosis*,” Mol. Cancer 11:74, pp. 1-11; Chapin, C. *et al.* (2012) “*Distribution And Surfactant Association Of Carcinoembryonic Cell Adhesion Molecule 6 In Human Lung*,” Amer. J. Physiol. Lung Cell. Mol. Physiol. 302(2):L216-L25; Riley, C.J. *et al.* (2009) “*Design And Activity Of A Murine And Humanized Anti-CEACAM6 Single-Chain Variable Fragment In The Treatment Of Pancreatic Cancer*,” Cancer Res. 69(5):1933-1940; Lewis-Wambi, J.S. *et al.* (2008) “*Overexpression Of CEACAM6 Promotes Migration And Invasion Of Oestrogen-Deprived Breast Cancer Cells*,” Eur. J. Cancer 44(12):1770-1779; Blumenthal, R.D. *et al.* (2007) “*Expression Patterns Of CEACAM5 And CEACAM6 In Primary And Metastatic Cancers*,” BMC Cancer. 7:2, pp. 1-15). The epitope-binding site of any anti-CEACAM5 / CEACAM6 antibody may be used in accordance with the present invention. Exemplary anti-CEACAM5 / CEACAM6 antibodies are provided below.

(a) 16C3

[00224] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **16C3** (EP 2585476) is (**SEQ ID NO:150**) (CDR residues are shown underlined):

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

[00225] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **16C3** (EP 2585476) is (**SEQ ID NO:151**) (CDR residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCGASENIY GALNWYQRKP GKSPKLLIWG
ASNLADGMPS RFSGSGSGRQ YTLTISSLQP EDVATYYCQN VLSSPYTFGG
 GTKLEIK

(b) hMN15

[00226] The amino acid sequence of the VH Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) is (**SEQ ID NO:152**) (CDR residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCSSSGFALT DYYMSWVRQA PGKGLEWLGF
IANKANGHTT DYSPSVKGRF TISRDN SKNT LFLQMDSLRP EDTGVYFCAR
DMGIRWNFDV WGQGTPVTVS S

[00227] The amino acid sequence of the VL Domain of the humanized anti-CEACAM5 / CEACAM6 antibody **hMN15** (WO 2011/034660) is (**SEQ ID NO:153**) (CDR residues are shown underlined):

DIQLTQSPSS LSASVGDRVT MTCSASSRVS YIHWYQQKPG KAPKRWIYGT
STLASGVPAR FSGSGSGTDF TFTISSLQPE DIATYYCQOW SYNPPTFGQG
 TKVEIKR

[00228] The present invention specifically includes and encompasses **CD137 x CEACAM5/CEACAM6** 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**.

7. Antibodies that Bind to CD19

[00229] 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).

[00230] An exemplary antibody that binds to human CD19, and that may be employed in the present invention, is the anti-CD19 antibody disclosed in WO 2016/048938 (referred to herein as "**CD19 MAB-1**").

[00231] The amino acid sequence of the VH Domain of **CD19 MAB-1 (SEQ ID NO:204)** is shown below (CDR_H residues are shown underlined):

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMGVGVWIR QPPGKALEWL
AHIWDDDKR YNPALKSRILT ISKDTSKNQV FLTMTNMDPV DTATYYCARM
ELWSYYFDYW GQGTTVTVSS

[00232] The amino acid sequence of the VL Domain of **CD19 MAB-1 (SEQ ID NO:205)** is shown below (CDR_L residues are shown underlined):

ENVLTQSPAT LSVTPGEKAT ITCRASQSVS YMHWYQQKPG QAPRLLIYDA
SNRASGVPSR FSGSGSGTDH TLTISSEAE DAATYYCFQG SVYPFTFGQG
 TKLEIK

[00233] The present invention specifically includes and encompasses **CD137 x CD19** 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, or present in 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).

8. Antibodies that Bind to CD123

[00234] 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).

[00235] 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).

[00236] The amino acid sequence of the VH Domain of **CD123 MAB-1** (SEQ ID NO:206) is shown below (CDR_H residues are shown underlined):

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

[00237] The amino acid sequence of the VL Domain of **CD123 MAB-1** (SEQ ID NO:207) is shown below (CDR_L residues are shown underlined):

DFVMTQSPDS LAVSLGERVT MSCKSSQSLL NSGNQKNYLT WYQQKPGQPP
 KLLIYWASTR ESGVPDRFSG SGSGTDFTLT ISSLQAEDVA VYYCQNDYSY
PYTFGQGTKL EIK

[00238] The present invention specifically includes and encompasses **CD137 x CD123** 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**, or any of the anti-CD123 antibodies disclosed in US 2017/081424 and WO 2016/036937, or present in JNJ-63709178 (Johnson & Johnson, also see, WO 2016/036937) and XmAb14045 (Xencor, also see, US 2017/081424).

G. Preferred CD137 Variable Domains

[00239] The epitope-binding site of any anti-CD137 antibody may be used in accordance with the present invention. Exemplary antibodies that bind human CD137 include “**urelumab**,” and “**utomilumab**” currently being evaluated in human clinical trials. Urelumab (also known as BMS-663513, designated “**CD137 MAB-1**” herein) is a fully human monoclonal antibody having IgG4/kappa constant regions (see, US Patent No. 8,137,667). Utomilumab (also known as PF-05082566, designated “**CD137 MAB-2**” herein) is a fully human monoclonal antibody having IgG2/lambda constant regions (see, US Patent No. 8,337,850). Additional exemplary antibodies that are immunospecific for human CD137 (designated “**CD137 MAB-3**,” “**CD137 MAB-4**” and “**CD137 MAB-5**”) are provided below.

1. CD137 MAB-1

[00240] The amino acid sequence of the VH Domain of **CD137 MAB-1** (**CD137 MAB-1 VH**) is (**SEQ ID NO:70**) (CDR_H residues are shown underlined):

QVQLQQWGAG LLKPSETLSL TCAVYGGSF GYWSWIRQS PEKGLEWIGE
INHGGYVTYN PSLESRVTIS VDTSKNQFSL KLSSVTAADT AVYYCARDDYG
PGNYDWYFDL WGRGTLTVS S

[00241] The amino acid sequence of the VL Domain of **CD137 MAB-1** (**CD137 MAB-1 VL**) is (**SEQ ID NO:71**) (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFSGSGSGTD FTLTISSELP EDFAVYYCQQ RSNWPPALTF
GGGTKVEIK

2. CD137 MAB-2

[00242] The amino acid sequence of the VH Domain of **CD137 MAB-2** (**CD137 MAB-2 VH**) is (**SEQ ID NO:72**) (CDR_H residues are shown underlined):

EVQLVQSGAE VKKPGESLRI SCKGSGYSFS TYWISWVRQM PGKGLEWMGK
IYPGDSYTNY SPSFQGQVTI SADKSISTAY LQWSSLKASD TAMYYCARGY
GIFDYWGQGT LTVSS

[00243] The amino acid sequence of the VL Domain of **CD137 MAB-2** (**CD137 MAB-2 VL**) is (**SEQ ID NO:73**) (CDR_L residues are shown underlined):

SYELTQPPSV SVSPGQTASI TCSGDNIGDQ YAHWYQQKPG QSPVLVIYQD
KNRPSGIPER FSGSNSGNTA TLTISGTQAM DEADYYCCATY TGFGSLAVFG
GGTKLTVL

3. CD137 MAB-3

[00244] **CD137 MAB-3** is a novel murine monoclonal antibody. The amino acid sequence of the VH Domain of **CD137 MAB-3** (**CD137 MAB-3 VH**) is (**SEQ ID NO:74**) (CDR_H residues are shown underlined):

QVQLQQPGAE LVRPGASVKL SCKASGYTFT SYWINWVKQR PGQGLEWIGN
IYPSDSYTNY NQKFKDKATL TVDKSSSTAY MQLSSPTSED SAVYYCTRDY
GSSYSFDYWG QGTTTLTVSS

[00245] The amino acid sequences of the CDR_Hs of **CD137 MAB-3 VH** are:

CDR_{H1} (**SEQ ID NO:154**): SYWIN

CDR_{H2} (**SEQ ID NO:155**): NIYPSDSYTNYNQKFKD

CDR_{H3} (**SEQ ID NO:156**): DYGSSYSFDY

[00246] The amino acid sequence of the VL Domain of **CD137 MAB-3** (**CD137 MAB-3 VL**) is (**SEQ ID NO:75**) (CDR_L residues are shown underlined):

DIQMTQTSS LSASLGDRVT ISCRPSQDIS NYLNWYQQKP DGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GDTLPYTFGG
 GTKLEIK

[00247] The amino acid sequences of the CDR_Ls of **CD137 MAB-3 VL** are:

CDR_{L1} (**SEQ ID NO:157**): RPSQDISNYLN

CDR_{L2} (**SEQ ID NO:158**): YTSRLRS

CDR_{L3} (**SEQ ID NO:159**): QQGDTLPYT

4. hCD137 MAB-3

[00248] Antibody **CD137 MAB-3** was humanized to form antibody **hCD137 MAB-3**. The amino acid sequence of the VH Domain of such humanized antibody (**hCD137 MAB-3 VH1**) is (**SEQ ID NO:76**) (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA PGQGLEWIGN
IYPSDSYTNY NQKFKDKATI TADKSTSTAY MELSSLRSED TAVYYCTRDY
GSSYSFDYWG QGTTVTVSS

[00249] The VH Domain of humanized antibody **hCD137 MAB-3** (**hCD137 MAB-3 VH1**) was optimized to yield a VH Domain having the amino acid sequence of **SEQ ID NO:77**:

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA
 PGQGLEWIGN IYPSDSYTNY NQKFKDKATI TADKSTSTAY
 MELSSLRSED TAVYYCTRDY GSX₁₀X₁₁X₁₂X₁₃X₁₄X₁₅WG QGTTVTVSS

wherein: X₁₀X₁₁X₁₂X₁₃X₁₄X₁₅ are:

AYSFHP (1A; **SEQ ID NO:78**), or

AYSMST (1B; **SEQ ID NO:79**), or

AYSYSL (1C; **SEQ ID NO:80**), or

SYSYNV (1D; **SEQ ID NO:81**).

[00250] As indicated above, the corresponding parental sequence (present in **SEQ ID NO:74**) is SYSFDY (**SEQ ID NO:160**).

[00251] The VL Domain of humanized antibody **hCD137 MAB-3** (**hCD137 MAB-3 VL**) was optimized to yield a VL Domain having the amino acid sequence of **SEQ ID NO:82**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP X₇X₈TVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

wherein: X₇ is D or G; X₈ is G or K.

[00252] Four variant **hCD137 MAB-3 VH1** Domains were isolated: **hCD137 MAB-3 VH1A**, **hCD137 MAB-3 VH1B**, **hCD137 MAB-3 VH1C** and **hCD137 MAB-3 VH1D**. The amino acid sequences of such variant **hCD137 MAB-3 VH1** Domains are presented below.

[00253] The amino acid sequence of **hCD137 MAB-3 VH1A** is (SEQ ID NO:83) (CDR_H residues are shown underlined; note that residues 5-10 of CDR_{3H} are AYSFHP (SEQ ID NO:78):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA PGQGLEWIGN
IYPSDSYTN NQKFKDKATI TADKSTSTAY MELSSLRSED TAVYYCTRDY
GSAYSFHPWG QGTTVTVSS

[00254] The amino acid sequence of **hCD137 MAB-3 VH1B** is (SEQ ID NO:84) (CDR_H residues are shown underlined; note that residues 5-10 of CDR_{3H} are AYSMST (SEQ ID NO:79):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA PGQGLEWIGN
IYPSDSYTN NQKFKDKATI TADKSTSTAY MELSSLRSED TAVYYCTRDY
GSAYSMSTWG QGTTVTVSS

[00255] The amino acid sequence of **hCD137 MAB-3 VH1C** is (SEQ ID NO:85) (CDR_H residues are shown underlined; note that residues 5-10 of CDR_{3H} are AYSYSL (SEQ ID NO:80):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA PGQGLEWIGN
IYPSDSYTN NQKFKDKATI TADKSTSTAY MELSSLRSED TAVYYCTRDY
GSAYSYSSLWG QGTTVTVSS

[00256] The amino acid sequence of **hCD137 MAB-3 VH1D** is (SEQ ID NO:86) (CDR_H residues are shown underlined; note that residues 5-10 of CDR_{3H} are SYSYNV (1D; SEQ ID NO:81):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVKQA PGQGLEWIGN
IYPSDSYTN NQKFKDKATI TADKSTSTAY MELSSLRSED TAVYYCTRDY
GSSYSYNVWG QGTTVTVSS

[00257] Thus, the amino acid sequence of CDR_{H1} of **hCD137 MAB-3 VH1A**, **hCD137 MAB-3 VH1B**, **hCD137 MAB-3 VH1C**, and **hCD137 MAB-3 VH1D** are the same (SEQ ID NO:154) and the amino acid sequence of CDR_{H2} of **hCD137 MAB-3 VH1A**, **hCD137 MAB-3 VH1B**, **hCD137 MAB-3 VH1C**, and **hCD137 MAB-3 VH1D** are the same (SEQ ID NO:155). However, the amino acid sequences of CDR_{H3} of **hCD137 MAB-3 VH1A**, **hCD137 MAB-3 VH1B**, **hCD137 MAB-3 VH1C**, and **hCD137 MAB-3 VH1D** differ:

hCD137 MAB-3 VH1A CDR_{H3} (SEQ ID NO:161):	DYGSAYSFHP
hCD137 MAB-3 VH1B CDR_{H3} (SEQ ID NO:162):	DYGSAYSMST
hCD137 MAB-3 VH1C CDR_{H3} (SEQ ID NO:163):	DYGSAYSYSL
hCD137 MAB-3 VH1D CDR_{H3} (SEQ ID NO:164):	DYGSSYSYNV

[00258] Three variant **hCD137 MAB-3 VL** Domains were isolated: **hCD137 MAB-3 VL1**, **hCD137 MAB-3 VL2** and **hCD137 MAB-3 VL3**. The amino acid sequences of such variant **hCD137 MAB-3 VL** Domains are presented below.

[00259] The amino acid sequence of **hCD137 MAB-3 VL1** is (**SEQ ID NO:87**) (CDRL residues are shown underlined; note that residues 41 and 42 are D and G, respectively):

DIQMTQSPSS LSASVGDRVT ITCCRPSQDIS NYLNWYQQKP DGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00260] The amino acid sequence of **hCD137 MAB-3 VL2** is (**SEQ ID NO:88**) (CDRL residues are shown underlined; note that that residues 41 and 42 are both G):

DIQMTQSPSS LSASVGDRVT ITCCRPSQDIS NYLNWYQQKP GGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00261] The amino acid sequence of **hCD137 MAB-3 VL3** is (**SEQ ID NO:89**) (CDRL residues are shown underlined; note that that residues 41 and 42 are D and K, respectively):

DIQMTQSPSS LSASVGDRVT ITCCRPSQDIS NYLNWYQQKP DKTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00262] The VH Domain **hCD137 MAB-3 VH1B** was deimmunized as described in the Examples below. The amino acid sequences of three of the resulting deimmunized VH Domains designated **hCD137 MAB-3 VH1E**, **hCD137 MAB-3 VH1F**, and **hCD137 MAB-3 VH1G**, having an amino acid substitution at Kabat residue 38 and/or 48 are provided below. It is specifically contemplated that the identified substitutions, R38K and/or I48A may be incorporated into any of the disclosed **hCD137 MAB-3 VH** Domains. In a specific embodiment, the K38R amino acid substitution is incorporated into **SEQ ID NO:76**, **SEQ ID NO:77**, **SEQ ID NO:83**, **SEQ ID NO:84**, **SEQ ID NO:85**, or **SEQ ID NO:86**.

[00263] The amino acid sequence of **hCD137 MAB-3 VH1E** comprising K38R is (SEQ ID NO:208) (CDR_H residues are shown underlined; substitution is doubled underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYWINWVRQA** PGQGLEWIGN
IYPSDSYTN **NQKFKD**KATI TADKSTSTAY MELSSLRSED TAVYYCTR**DY**
GSAYSMSTWG QGTTVTVSS

[00264] The amino acid sequence of **hCD137 MAB-3 VH1F** comprising I48A is (SEQ ID NO:209) (CDR_H residues are shown underlined; substitutions are doubled underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYWINWVKQA** PGQGLEW**AGN**
IYPSDSYTN **NQKFKD**KATI TADKSTSTAY MELSSLRSED TAVYYCTR**DY**
GSAYSMSTWG QGTTVTVSS

[00265] The amino acid sequence of **hCD137 MAB-3 VH1G** comprising K38R/I48A (SEQ ID NO:210) (CDR_H residues are shown underlined; substitutions are doubled underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT **SYWINWVRQA** PGQGLEW**AGN**
IYPSDSYTN **NQKFKD**KATI TADKSTSTAY MELSSLRSED TAVYYCTR**DY**
GSAYSMSTWG QGTTVTVSS

[00266] The VL Domain **hCD137 MAB-3 VL3** was deimmunized as described in the Examples below. The amino acid sequences of twelve of the resulting deimmunized VL Domains designated **hCD137 MAB-3 VL4-VL15**, having an amino acid substitution at Kabat residue 24, 25, 44, 48, 52, and/or 54 are provided below. It is specifically contemplated that the identified substitutions, R24Q, P25A, V44A, I48A, S52T, S52G, and/or L54A may be incorporated into any of the disclosed **hCD137 MAB-3** VL Domains provided above to yield a deimmunized VL Domain. In a specific embodiment, R24Q, P25A, I48A, S52G, and L54A amino acid substitutions are incorporated into **SEQ ID NO: 82**, **SEQ ID NO: 87**, **SEQ ID NO: 88** or **SEQ ID NO: 89**. In another specific embodiment, R24Q, P25A, I48A, S52T, and L54A amino acid substitutions are incorporated into **SEQ ID NO: 82**, **SEQ ID NO: 87**, **SEQ ID NO: 88** or **SEQ ID NO: 89**.

[00267] The amino acid sequence of **hCD137 MAB-3 VL4** comprising R24Q/P25A is (SEQ ID NO:211) (CDR_H residues are shown underlined; substitutions are (doubled underlined):

DIQMTQSPSS LSASVGDRVIT ITC**QASQDIS** **NYLNWYQQK**P DKTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFC**QQ** **GDTPYTFGQ**
 GTKLEIK

[00268] The amino acid sequence of **hCD137 MAB-3 VL5** comprising V44A is (SEQ ID NO:212) (CDR_H residues are shown underlined; substitution is doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTAKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00269] The amino acid sequence of **hCD137 MAB-3 VL6** comprising L54A is (SEQ ID NO:213) (CDR_H residues are shown underlined; substitution is doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTTVKLLIYY
TSRARSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00270] The amino acid sequence of **hCD137 MAB-3 VL7** comprising R24Q/P25A/V44A is (SEQ ID NO:214) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTAKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00271] The amino acid sequence of **hCD137 MAB-3 VL8** comprising R24Q/P25A/V44A/L54A is (SEQ ID NO:215) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTAKLLIYY
TSRARSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00272] The amino acid sequence of **hCD137 MAB-3 VL9** comprising R24Q/P25A/L54A is (SEQ ID NO:216) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTTVKLLIYY
TSRARSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00273] The amino acid sequence of **hCD137 MAB-3 VL10** comprising S52T is (SEQ ID NO:217) (CDR_H residues are shown underlined; substitution is doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTTVKLLIYY
TTRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIK

[00274] The amino acid sequence of **hCD137 MAB-3 VL11** comprising S52G is (SEQ ID NO:218) (CDR_H residues are shown underlined; substitution is doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
TGRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GD~~TL~~PYTFGQ
 GTKLEIK

[00275] The amino acid sequence of **hCD137 MAB-3 VL12** comprising I48A/S52T is (SEQ ID NO:219) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLAYY
TTRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GD~~TL~~PYTFGQ
 GTKLEIK

[00276] The amino acid sequence of **hCD137 MAB-3 VL13** comprising I48A/S52G is (SEQ ID NO:220) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLAYY
TGRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GD~~TL~~PYTFGQ
 GTKLEIK

[00277] The amino acid sequence of **hCD137 MAB-3 VL14** comprising R24Q/P25A/S52T/L54A is (SEQ ID NO:221) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTVKLLIYY
TTRARSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GD~~TL~~PYTFGQ
 GTKLEIK

[00278] The amino acid sequence of **hCD137 MAB-3 VL15** comprising R24Q/P25A/S52G/L54A is (SEQ ID NO:222) (CDR_H residues are shown underlined; substitutions are doubled underlined):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTVKLLIYY
TGRARSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GD~~TL~~PYTFGQ
 GTKLEIK

[00279] Thus, the amino acid sequence of CDR_{L3} of **hCD137 MAB-3 VL3-VL15** are the same (QQGD~~TL~~PYT; SEQ ID NO:159). However, the amino acid sequences of CDR_{L1} and/or CDR_{L2} of **hCD137 MAB-3 VL4**, and **hCD137 MAB-3 VL6-VL15** differ:

[00280] **hCD137 MAB-3 VL4, VL7, VL8, VL9, VL14, and VL15** CDR_{L1} (SEQ ID NO:223): QASQDISNYLN

hCD137 MAB-3 VL6, VL8, and VL9 CDR_{L2} (SEQ ID NO:224): YTSRARS

hCD137 MAB-3 VL10, and VL12 CDR_L2 (SEQ ID NO:225): YTTRLRS

hCD137 MAB-3 VL11, and VL13 CDR_L2 (SEQ ID NO:226): YTGRLRS

hCD137 MAB-3 VL14 CDR_L2 (SEQ ID NO:227): YTTRARS

hCD137 MAB-3 VL15 CDR_L2 (SEQ ID NO:228): YTGRARS

[00281] The CDRs, VL Domain, and/or VH Domain of any of such humanized, optimized, and/or deimmunized VH and VL **hCD137 MAB-3** Domains, including any embraced within the generic sequence(s) of the **hCD137 MAB-3** VH and/or VL Domains presented above may be used to form an antibody, diabody or binding molecule capable of binding CD137.

5. CD137 MAB-4

[00282] **CD137 MAB-4** is a novel murine monoclonal antibody. The amino acid sequence of the VH Domain of **CD137 MAB-4 (CD137 MAB-4 VH)** is (SEQ ID NO:90) (CDR_H residues are shown underlined):

QVQLQQPGAE LVRPGASVKL SCKASGYTFT SYWINWVKQR PGQGLEWIGN
IYPSDSYTNY DQKFKDKATL TVDKSSSTAY MQLSSPTSED SAVYYCTKSG
EYGKIGYYAM DYWGQGTSTV VSS

[00283] The amino acid sequences of the CDR_Hs of **CD137 MAB-4 VH** are:

CDR_H1 (SEQ ID NO:165): SYWIN

CDR_H2 (SEQ ID NO:166): NIYPSDSYTNVDQKFKD

CDR_H3 (SEQ ID NO:167): SGEYKGIGYYAMDY

[00284] The amino acid sequence of the VL Domain of **CD137 MAB-4 (CD137 MAB-4 VL)** is (SEQ ID NO:91) (CDR_L residues are shown underlined):

DIQMTQTTSS LSASLGDRVT ISCRRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPYTFGG
 GTKLEIK

[00285] The amino acid sequences of the CDR_Ls of **CD137 MAB-4 VL** are:

CDR_L1 (SEQ ID NO:168): RASQDISNYLN

CDR_L2 (SEQ ID NO:169): YTSRLHS

CDR_L3 (SEQ ID NO:170): QQGNTLPYT

6. hCD137 MAB-4

[00286] Antibody **CD137 MAB-4** was humanized to form antibody **hCD137 MAB-4**. The amino acid sequence of the VH Domain of humanized antibody **hCD137 MAB-4** (**hCD137 MAB-4 VH1**) is (**SEQ ID NO:92**) (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWINWVRQA PGQGLEWMGN
IYPSDSYTN DQKFKDRVTM TRDTSTSTVY MELSSLRSED TAVYYCTKSG
EYGKIGYYAM DYWGQGTTVT VSS

[00287] The VL Domain of humanized antibody **hCD137 MAB-4** was humanized and optimized to yield a VL Domain (**hCD137 MAB-3 VL**) having the amino acid sequence of **SEQ ID NO:93** (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP DKTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YTLTISSLQP EDIATYX₉COO GNTLPYTFGQ
 GTKLEIK
 wherein X₉ is F or Y

[00288] Two variant **hCD137 MAB-4 VL** Domains were isolated: **hCD137 MAB-4 VL1** and **hCD137 MAB-4 VL2**. The amino acid sequences of such variant **hCD137 MAB-4 VL** Domains are presented below.

[00289] The amino acid sequence of **hCD137 MAB-4 VL1** is (**SEQ ID NO:94**) (CDR_L residues are shown underlined; note that residue 87 is F):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP DKTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YTLTISSLQP EDIATYFCCOO GNTLPYTFGQ
 GTKLEIK

[00290] The amino acid sequence of **hCD137 MAB-4 VL2** is (**SEQ ID NO:95**) (CDR_L residues are shown underlined; note that residue 87 is Y):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP DKTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YTLTISSLQP EDIATYYCCOO GNTLPYTFGQ
 GTKLEIK

7. CD137 MAB-5

[00291] **CD137 MAB-5** is a novel murine monoclonal antibody. The amino acid sequence of the VH Domain of **CD137 MAB-5** (**CD137 MAB-5 VH**) is (**SEQ ID NO:96**) (CDR_H residues are shown underlined):

QVQLKESGPG LVAPSQSLSI TCTVSGFSLT SYDISWIRQP PGKGLEWLGV
VWTGGGTNYN SAFMSRLSIS KDNSKSQVFL KMNSLQTDDT AIYYCERVDY
 WGQGTSVTVS S

[00292] The amino acid sequences of the CDR_Hs of **CD137 MAB-5 VH** are:

CDR_{H1} (SEQ ID NO:171): SYDIS

CDR_{H2} (SEQ ID NO:172): VVWTGGGTNYNSAFMS

CDR_{H3} (SEQ ID NO:173): VDY

[00293] The amino acid sequence of the VL Domain of **CD137 MAB-5 (CD137 MAB-5 VL)** is (SEQ ID NO:97) (CDR_L residues are shown underlined):

DVVMQTPLS LPVSLGDQAS ISCRSSQSLV HSNGNTYLHW YLQKPGQSPK
 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
WTFGGGTKLE IK

[00294] The amino acid sequences of the CDR_Ls of **CD137 MAB-5 VL** are:

CDR_{L1} (SEQ ID NO:174): RSSQSLVHSNGNTYLH

CDR_{L2} (SEQ ID NO:175): KVSNRFS

CDR_{L3} (SEQ ID NO:176): SQSTHVPWT

III. CD137 x TA Binding Molecules of the Present Invention

[00295] The present invention is particularly directed to **Trivalent CD137 x TA Binding Molecules** that comprise an Fc Domain, Fc-bearing **CD137 x TA DART®** diabodies capable of simultaneous binding to CD137 and a **TA**, and other Fc-bearing **CD137 x TA Binding Molecules** capable of simultaneous binding to CD137 and a **TA**. The present invention is further directed to the use of such molecules in the treatment of cancer and other diseases and conditions. Although non-optimized **CD137 x TA Binding Molecules** are fully functional, analogous to the improvements obtained in gene expression through codon optimization (see, e.g., Grosjean, H. *et al.* (1982) “*Preferential Codon Usage In Prokaryotic Genes: The Optimal Codon-Anticodon Interaction Energy And The Selective Codon Usage In Efficiently Expressed Genes*” Gene 18(3):199-209), it is possible to further enhance the stability and/or function of the **CD137 x TA Binding Molecules** of the present invention by modifying or refining their sequences.

A. CD137 x TA Fc-Bearing DART® Diabodies

[00296] The present invention particularly encompasses a wide variety of Fc-bearing DART® diabodies capable of simultaneous binding to CD137 and a **TA**. Exemplary **CD137 x TA Fc-bearing DART® Diabodies** are described below.

[00297] In one embodiment, such Fc-bearing diabodies will comprise two polypeptide chains. As shown in **Figure 2**, the first of such polypeptide chains may contain, in the N-terminal to C-terminal direction, an N-terminus, a Light Chain Variable Domain (VL) capable of binding to an epitope of a “first” antigen (VL1) (either CD137 or TA), a Heavy Chain Variable Domain (VH) capable of binding to an epitope of a “second” antigen (VH2) (TA, if VL1 was selected to bind to an epitope of CD137; CD137 if VL1 was selected to bind to an epitope of TA), a cysteine-containing linker, a CH2-CH3 Domain, and a C-terminus. The second of such polypeptide chains may contain, in the N-terminal to C-terminal direction, an N-terminus, a Light Chain Variable Domain (VL) capable of binding to an epitope of the “second” antigen (VL2) (TA, if the first antigen was CD137; CD137, if the first antigen was TA), a Heavy Chain Variable Domain (VH) capable of binding to an epitope of the “second” antigen (VH2) (TA, if VL2 was selected to bind to an epitope of CD137; CD137 if VL2 was selected to bind to an epitope of TA), a cysteine-containing linker, a CH2-CH3 Domain, and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Light Chain Variable Domain (VL1 or VL2) from the Heavy Chain Variable Domain (VH1 or VH2).

[00298] In another embodiment, Fc-bearing diabodies of the present invention comprise three polypeptide chains, and are depicted in **Figures 4A-4B**. The first polypeptide chain of such a diabody contains four Domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Domain that promotes heterodimerization (a “**Heterodimer-Promoting Domain**”) and covalent bonding with the diabody’s second polypeptide chain, and (iv) a Domain containing a CH2-CH3 sequence. The second polypeptide chain of such Fc-bearing diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization (a “**Heterodimer-Promoting Domain**”) and covalent bonding with the diabody’s first polypeptide chain. The third polypeptide chain of such Fc-bearing diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such Fc-bearing diabodies associate together to form a VL1/VH1 binding site that is capable of binding to a first epitope (1), as well as a VL2/VH2 binding site that is capable of binding to a second epitope (2). The preferred Fc-bearing diabodies of the present invention are **CD137 x TA** bispecific diabodies that are capable of binding to the “first epitope,” which may be either CD137 or TA, and the “second epitope,” which is TA when the first epitope is CD137, and is CD137 when the first epitope is TA. The first and second polypeptides are bonded to one another through one or more disulfide bonds involving cysteine residues in their respective linkers and/or 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 diabodies have enhanced potency. Preferred Fc-bearing diabodies of the present invention may have either of two orientations (**Table 2**):

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

(– ◎ – denotes a cysteine-containing polypeptide domain that possesses one, two, or more than two cysteine residues. The representation is intended to be illustrative and non-limiting. Cysteine residues may be present in additional or alternative domains, such as within the Heterodimer-Promoting Domain (HPD))

[00299] As shown in **Figure 4A**, in a first embodiment, the first of such three polypeptide chains may contain, in the N-terminal to C-terminal direction, an N-terminus, a Light Chain Variable Domain (VL) capable of binding to an epitope of a “first” antigen (VL1) (either CD137 or TA), a Heavy Chain Variable Domain (VH) capable of binding to an epitope of a “second” antigen (VH2) (TA, if the second antigen was CD137; CD137, if the second antigen was TA), a cysteine-containing domain, a Heterodimer-Promoting Domain, a second cysteine-containing domain, a CH₂-CH₃ domain and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Light Chain Variable Domain (VL1) from the Heavy Chain Variable Domain (VH2). Preferably, the Heavy Chain Variable Domain (VH1) is linked to a Heterodimer-Promoting Domain by an intervening linker peptide (**Linker 2**). In a preferred **CD137 x TA** bispecific Fc-bearing diabody embodiment, the C-terminus of the Heterodimer-Promoting Domain is linked to CH₂-CH₃ domains of an Fc Region (“**Fc Domain**”) by an intervening linker peptide (**Linker 3**) or by an intervening spacer peptide (**Linker 3**). Most preferably, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL1 – Linker 1 – VH2 – Linker 2 – Heterodimer-Promoting Domain – Linker 3 – Fc Domain.

[00300] The second of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, a Light Chain Variable Domain (VL) capable of binding to the epitope of the “second” antigen (VL2), a Heavy Chain Variable Domain (VH) capable of binding to the epitope of the “first” antigen (VH1), a cysteine-containing domain, a

Heterodimer-Promoting Domain and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Light Chain Variable Domain (**VL2**) from the Heavy Chain Variable Domain (**VH1**). Preferably, the Heavy Chain Variable Domain (**VH1**) is linked to the Heterodimer-Promoting Domain by an intervening linker peptide (**Linker 2**). Most preferably, the second of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: VL2 – Linker 1 – VH1 – Linker 2 – Heterodimer-Promoting Domain.

[00301] The third of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, a cysteine-containing peptide (such as **Linker 3**), and the CH2-CH3 domains of an Fc region (“Fc Domain”). As the third polypeptide chain does not comprise a VL Domain or a VH Domain the third polypeptide chain may be identical between two or more different **CD137 x TA** bispecific Fc diabodies of the present invention.

[00302] Alternatively, as shown in **Figure 4B**, in a second embodiment, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, a cysteine-containing peptide (such as **Linker 3**), the CH2-CH3 domains of an Fc Region (“**Fc Domain**”), an intervening spacer peptide (**Linker 4**), a Light Chain Variable Domain (**VL**) capable of binding to an epitope of the “first” antigen (**VL1**) (either CD137 or **TA**), a Heavy Chain Variable Domain (**VH**) capable of binding to an epitope of the “second” antigen (**VH2**) (**TA**, if the first antigen was CD137; CD137, if the first antigen was **TA**), a cysteine-containing linker, a Heterodimer-Promoting Domain, and a C-terminus. An intervening linker peptide (**Linker 1**) separates the Light Chain Variable Domain (**VL1**) from the Heavy Chain Variable Domain (**VH2**). Preferably, the Heavy Chain Variable Domain (**VH1**) is linked to a Heterodimer-Promoting Domain by an intervening linker peptide (**Linker 2**). Most preferably, in such alternative orientation, the first of the three polypeptide chains will thus contain, in the N-terminal to C-terminal direction: Linker 3 – Fc Domain – Linker 4 – VL1 – Linker 1 – VH2 – Linker 2 – Heterodimer-Promoting Domain. The second and third polypeptide chains of such alternative diabody may be the same as the second and third polypeptide chains of the first embodiment.

[00303] In each of the above embodiments, the Light Chain Variable Domain of the first polypeptide chain (**VL1**) is coordinately selected so as to permit it to interact with the Heavy Chain Variable Domain of the second polypeptide chain (**VH1**) to thereby form a functional epitope-binding site that is capable of immunospecifically binding an epitope of the first antigen (*i.e.*, either **TA** or CD137). Likewise, the Light Chain Variable Domain of the second

polypeptide chain (**VL2**) is coordinately selected so as to permit it to interact with the Heavy Chain Variable Domain of the first polypeptide chain (**VH2**) to thereby form a functional epitope-binding site that is capable of immunospecifically binding an epitope of the second antigen (*i.e.*, either a **TA** or CD137). Thus, the selection of the Light Chain Variable Domains and the Heavy Chain Variable Domains are coordinated, such that the two polypeptide chains collectively comprise epitope-binding sites capable of binding to CD137 and a **TA**.

[00304] Preferred Fc-bearing diabodies of the present invention are covalently bonded tetravalent diabodies having four epitope-binding sites that comprise four polypeptide chains, and are depicted in **Figures 3A-3C**. The first and third polypeptide chains of such a diabody contain: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) 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 and covalent bonding of the first/third polypeptide chains with the second/fourth polypeptide chains. Preferably, the VH Domains are linked to the Heterodimer-Promoting Domains by intervening linker peptides (**Linker 2**). In a preferred **CD137 x TA** bispecific Fc-bearing diabody embodiment, the C-terminus of the Heterodimer-Promoting Domain of the first polypeptide chain is linked to CH2-CH3 domains by an intervening linker peptide (**Linker 3**) or by an intervening spacer peptide (**Linker 3**). 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 notations "**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 notations "**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 Region-containing diabodies of invention is provided in **Table 3**:

Table 3		
Bispecific	2 nd Chain	NH ₂ -VL2-VH1-⊙-HPD-COOH
	1 st Chain	NH ₂ -VL1-VH2-⊙-HPD-⊙-CH2-CH3-COOH
	1 st Chain	NH ₂ -VL1-VH2-⊙-HPD-⊙-CH2-CH3-COOH
	2 nd Chain	NH ₂ -VL2-VH1-⊙-HPD-COOH
Tetraspecific	2 nd Chain	NH ₂ -VL2-VH1-⊙-HPD-COOH
	1 st Chain	NH ₂ -VL1-VH2-⊙-HPD-⊙-CH2-CH3-COOH
	3 rd Chain	NH ₂ -VL3-VH4-⊙-HPD-⊙-CH2-CH3-COOH
	4 th Chain	NH ₂ -VL4-VH3-⊙-HPD-COOH

(– ⊙ – denotes a cysteine-containing polypeptide domain that possesses one, two, or more than two cysteine residues. The representation is intended to be illustrative and non-limiting. Cysteine residues may be present in additional or alternative domains, such as within the Heterodimer-Promoting Domain (HPD))

[00305] In preferred embodiments, **CD137 x TA** Binding Molecules of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-bearing diabodies that are composed of four total polypeptide chains (**Figures 3A-3C**). The **CD137 x TA** Binding Molecules of the invention are bispecific, tetravalent, Fc-bearing diabodies that comprise two epitope-binding sites immunospecific for CD137 (which may be capable of binding to the same epitope of CD137 or to different epitopes of CD137), and two epitope-binding sites immunospecific for a tumor antigen (which may be capable of binding to the same epitope of a **TA** or to different epitopes of a **TA** or different epitopes of different TAs).

[00306] Additional preferred Fc-bearing diabodies of the present invention comprise five polypeptide chains, and are depicted in **Figures 5A-5D**. 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. In a preferred embodiment, the second and fifth polypeptide chains have the same amino acid sequence. 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. Preferably, the C-terminus of the VH3- and VH2-containing domains of the third and fourth polypeptide chains are linked to a Heterodimer-Promoting Domain by an intervening linker peptide (**Linker 2**), and the C-terminus of the CH2-CH3 domains of the third polypeptide chain is linked to the VL2-containing Domain by an intervening linker peptide (**Linker 4**).

[00307] Thus, the first and second, and the third and fifth, polypeptide chains of such diabodies associate together to form two VL1/VH1 binding sites capable of binding a first epitope. The third and fourth polypeptide chains of such diabodies associate together to form a VL2/VH2 binding site that is capable of binding to a second epitope, as well as a VL3/VH3 binding site that is capable of binding to 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 Region. Such bispecific diabodies have enhanced potency. **Figures 5A-5D** illustrate 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. However, as provided herein, these domains are preferably selected so as to bind CD137 and a **TA**.

[00308] 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 may be selected such that a bispecific 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 **Figures 5A-5D**). The general structure of the polypeptide chains of representative five-chain Fc Region-containing diabodies of invention is provided in **Table 4**:

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

(– © – denotes a cysteine-containing polypeptide domain that possesses one, two, or more than two cysteine residues. The representation is intended to be illustrative and non-limiting. Cysteine residues may be present in additional or alternative domains, such as within the Heterodimer-Promoting Domain (HPD))

[00309] In a preferred embodiment, **CD137 x TA** Binding Molecules of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-bearing diabodies that are composed of five total polypeptide chains having two epitope-binding sites immunospecific for CD137 (which may be capable of binding to the same epitope of CD137 or to different epitopes of CD137), and two epitope-binding sites immunospecific for a **TA** (which may be capable of binding to the same epitope of a **TA** or to different epitopes of a **TA** or different epitopes of different **TAs**). In another embodiment, the **CD137 x TA** Binding Molecules of the invention are bispecific, tetravalent, Fc-bearing diabodies that comprise three epitope-binding sites immunospecific for CD137 (which may be capable of binding to the same epitope of CD137 or to two or three different epitopes of CD137), and one epitope-binding site specific for a **TA**.

B. Trivalent CD137 x TA Binding Molecules

[00310] In one embodiment, the **CD137 x TA Binding Molecules** of the present invention are trivalent and will comprise a first epitope-binding site (*e.g.*, a VL1 and VH1), a second epitope-binding site (*e.g.*, a VL2 and VH2), and a third epitope-binding site (*e.g.*, a VL3 and VH3), and will thus be able to bind to an epitope of **TA**, an epitope of **CD137**, and a third epitope, which third epitope may be:

- (a) the same or a different epitope of the **TA**;
- (b) the same or a different epitope of **CD137**; or
- (c) an epitope of a different **TA**.

[00311] Preferably, such “**Trivalent CD137 x TA Binding Molecules**” of the present invention will comprise two epitope-binding sites for an epitope of **CD137** (which epitopes may be the same or different), and one epitope-binding site for an epitope of a **TA**.

[00312] In general, such **Trivalent CD137 x TA Binding Molecules** of the present invention are composed of three, four, five or more than five polypeptide chains that, by virtue of one or more disulfide bonds between pairs of such polypeptides, form a covalently bonded molecular complex that comprises a “**Diabody-Type Binding Domain**” and a “**Non-Diabody-Type Binding Domain**.”

[00313] A “**Diabody-Type Binding Domain**” is the Epitope-Binding Domain of a diabody, and especially, a DART® diabody. The terms “**diabody**” and “**DART® diabody**” have been discussed above. A “**Non-Diabody-Type Binding Domain**” is intended to denote a Binding Domain that does not have the structure of a Diabody-Type Binding Domain. Preferably, a Non-Diabody-Type Binding Domain is a **Fab-Type Binding Domain** or an **ScFv-Type Binding Domain**. As used herein, the term “**Fab-Type Binding Domain**” refers to an Epitope-Binding Domain that is 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 Domain in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single Epitope-Binding Domain, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two Epitope-Binding Domains. ScFv-Type Binding Domains differ from Diabody-Type Binding Domain in that VL and VH Domains of the same polypeptide chain interact to form an Epitope-Binding Domain. Thus, as used herein, Fab-Type Binding Domains and ScFv-Type Binding Domains are distinct from Diabody-Type Binding Domain.

[00314] Thus, the **Trivalent CD137 x TA Binding Molecules** of the present invention preferably comprise:

- (I) a “first” Epitope-Binding Domain that is capable of immunospecifically binding to a “first” epitope;
- (II) a “second” Epitope-Binding Domain that is capable of immunospecifically binding to a “second” epitope;
- (III) a “third” Epitope-Binding Domain that is capable of immunospecifically binding to a “third” epitope; and
- (IV) an Fc Domain that is formed by the association of two CH2-CH3 Domains to one another;

wherein:

- (A) the “first” Epitope-Binding Domain and the “second” Epitope-Binding Domain are both “Diabody-Type Binding Domains;
- (B) the “third” Epitope-Binding Domain is a Non-Diabody-Type Binding Domain; and
- (C) one of such “first,” “second,” or “third” Epitope-Binding Domains binds an epitope of **TA**, and another of such “first,” “second,” or “third” Epitope-Binding Domains binds an epitope of CD137;

[00315] The epitope that is bound by the remaining Epitope-Binding Domain may be any desired epitope, preferably an epitope of CD137. Such epitope which may be the same or different from the CD137 epitope that is bound by other Epitope-Binding Domains of the molecule.

[00316] **Figures 6A-6H** provide a diagrammatic representation of the Domains of preferred **Trivalent CD137 x TA Binding Molecules**. **Figures 6A-6D** illustrate schematically the Domains of preferred **Trivalent CD137 x TA Binding Molecules** that are composed from the covalent complexing of four polypeptide chains and possess one Non-Diabody-Type Binding Site (VL3/VH3 and thus being monovalent for such epitope), and two Diabody-Type Binding Sites (VL1/VH1 and VL2/VH2, and thus being monovalent for each of such epitopes). **Figures 6E-6H** illustrate schematically the Domains of preferred **Trivalent CD137 x TA Binding Molecules** that are composed from the covalent complexing of three polypeptide chains and possess one Non-Diabody-Type Binding Site (VL3/VH3 and thus being monovalent for such epitope), and two Diabody-Type Binding Sites (VL1/VH1 and VL2/VH2, and thus being

monovalent for each of such epitopes). The Non-Diabody-Type Binding Site shown in **Figures 6A-6H** is a **Fab-Type Binding Domain** in **Figures 6A-6D** and is an **scFv-Type Binding Domain** in **Figures 6E-6H**. As provided below, VL/VH binding sites formed by the association of the polypeptide chains may be the same or different so as to permit trivalent binding that is monospecific, bispecific, or trispecific.

IV. Exemplary CD137 x TA Binding Molecules

[00317] The invention provides **CD137 x TA Binding Molecule** that are bispecific tetravalent Fc diabodies capable of simultaneously and specifically binding to CD137 and to a **TA**. As indicated above, the **CD137 x TA Binding Molecules** of the present invention may comprise four or five polypeptide chains. The polypeptide chains of seven exemplary **CD137 x TA Binding Molecules** capable of binding to CD137 and to the **TA**, HER2/neu are provided below (designated “**DART-A**,” “**DART-B**,” “**DART-C**,” “**DART-D**,” “**DART-E**,” “**DART-F**,” “**DART-G**,” “**DART-G1**,” “**DART-G2**,” “**DART-G3**,” and “**DART-G4**,” respectively). The invention further provides **CD137 x TA Binding Molecule** that are bispecific trivalent binding molecules capable of simultaneously and specifically binding to CD137 and to a **TA**. As indicated above, the **Trivalent CD137 x TA Binding Molecules** of the present invention may comprise four polypeptide chains. The polypeptide chains of an exemplary **CD137 x TA Trispecific Binding Molecule** capable of binding to CD137 and to the **TA**, HER2/neu, and optimized variants of the same are provided below (designated “**TRIDENT-A**,” “**TRIDENT-A1**,” “**TRIDENT-A2**,” “**TRIDENT-A3**,” “**TRIDENT-A5**,” “**TRIDENT-B**,” “**TRIDENT-B2**,” and “**TRIDENT-B5**” respectively).

A. DART-A

[00318] **DART-A** is composed of four polypeptide chains, in which the first and third polypeptide chains are the same and the second and fourth polypeptide chains are the same (see **Figure 3B**).

[00319] The first and third polypeptide chains of **DART-A** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL2 (SEQ ID NO:68)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding to CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1 (SEQ ID NO:76)**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting

(E-coil) Domain (EVAACKEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:38)), a linker (LEPKSADKTHTCPPCP (SEQ ID NO:30)), the CH2-CH3 Domain of an exemplary human IgG1 that substantially lacks effector function (SEQ ID NO:40), and a C-terminus:

[00320] The amino acid sequence of the first and third polypeptide chains of **DART-A** is (SEQ ID NO:98):

```
DIQMTQSPSS LSASVGDRVT ITCKASQDIN TYLSWFQQKP GKAPKTLIYR
ANRLVEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
LRSEDVAVYY CTRDYGSSYS FDYWGQGTIV TVSSGGCGGG EVAACEKEVA
ALEKEVALE KEVALEKLE PKSADKTHC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP
VLDSGDSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
```

[00321] Thus, the first and third polypeptide chain of **DART-A** is composed of: **SEQ ID NO:68 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:18 — SEQ ID NO:38 — SEQ ID NO:30 — SEQ ID NO:40**.

[00322] The second and fourth polypeptide chain of **DART-A** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD137 (VL_{CD137} (**hCD137 MAB-3 VL1** (SEQ ID NO:87)), an intervening linker peptide (**Linker 1**; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu} (**hHER2 MAB-1 VH1**, SEQ ID NO:64)), an intervening linker peptide (**Linker 2**; GGCGGG (SEQ ID NO:18)), a Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALEK-KVAALEK-KVAALEK (SEQ ID NO:39), and a C-terminus.

[00323] Thus, the second and fourth polypeptide chain of **DART-A** is composed of: **SEQ ID NO:87 — SEQ ID NO:16 — SEQ ID NO:64 — SEQ ID NO:18 — SEQ ID NO:39**.

[00324] The amino acid sequence of the second and fourth polypeptide chain of **DART-A** is (SEQ ID NO:99):

```
DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTNYGMN
WVRQAPGQGL EWMGWINTNI GEPTYTEEFK GRVTMTRDTS ISTAYMELSR
LRSDDTAVYY CARDDGYGNR VSYWGQGTIV TVSSGGCGGG KVAACEKEVA
ALKEKVAALK EKVAALKE
```

B. DART-B

[00325] **DART-B** is composed of four polypeptide chains, in which the first and third polypeptide chains are the same and the second and fourth polypeptide chains are the same (see **Figure 3B**).

[00326] The first and third polypeptide chain of **DART-B** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL3 (SEQ ID NO:69)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding to CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1 (SEQ ID NO:76)**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting (E-coil) Domain (**EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:38)**), a linker (**LEPKSADKTHTCPPCP (SEQ ID NO:30)**), the CH2-CH3 Domain of an exemplary human IgG1 that substantially lacks effector function (**SEQ ID NO:40**), and a C-terminus.

[00327] Thus, the first and third polypeptide chain of **DART-B** is composed of: **SEQ ID NO:69 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:18 — SEQ ID NO:38 — SEQ ID NO:30 — SEQ ID NO:40**.

[00328] The amino acid sequence of the first and third polypeptide chains of **DART-B** is (**SEQ ID NO:100**):

```
DIQMTQSPSS LSASVGDRVT ITCKASQDIS NYLSWFQQKP GKAPKTLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
GTKLEIKGGG SGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
LRSEDVAVYY CTRDYGSSYS FDYWGQGTIV TVSSGGCGGG EVAACEKEVA
ALEKEVAALE KEVAALEKLE PKSADKTHTC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSLT CLVKGFPYPSD IAVEWESNGQ PENNYKTTTP
VLDSGDSFLL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG
```

[00329] The second and fourth polypeptide chain of **DART-B** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3 (SEQ ID NO:89)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1, SEQ ID NO:64**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting

(K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:39), and a C-terminus.

[00330] Thus, the second and fourth polypeptide chain of **DART-B** is composed of: SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:64 — SEQ ID NO:18 — SEQ ID NO:39.

[00331] The amino acid sequence of the second and fourth polypeptide chain of **DART-B** is (SEQ ID NO:101):

```
DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTNYGMN
WVRQAPGQGL EWMGWINTNI GEPTYTEEFK GRVTMTRDTS ISTAYMELSR
LRSDDTAVYY CARDDGYG NR VSYWGQGLV TVSSGGCGGG KVAACKEKVA
ALKEKVAALK EKVAALKE
```

C. DART-C

[00332] **DART-C** is composed of four polypeptide chains, in which the first and third polypeptide chains are the same and the second and fourth polypeptide chains are the same (see **Figure 3B**).

[00333] The first and third polypeptide chain of **DART-C** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD137 (VL_{CD137} (**hCD137 MAB-3 VL3** (SEQ ID NO:89)), an intervening linker peptide (**Linker 1**; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1** (SEQ ID NO:64)), an intervening linker peptide (**Linker 2**; GGCGGG (SEQ ID NO:18)), a Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:38)), a linker (LEPKSADKTHTCPPCP (SEQ ID NO:30)), the CH2-CH3 Domain of an exemplary human IgG1 that substantially lacks effector function (SEQ ID NO:40), and a C-terminus.

[00334] Thus, the first and third polypeptide chain of **DART-C** is composed of: SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:64 — SEQ ID NO:18 — SEQ ID NO:38 — SEQ ID NO:30 — SEQ ID NO:40.

[00335] The amino acid sequence of the first and third polypeptide chains of **DART-C** is (SEQ ID NO:102):

```
DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
```

```

GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTNYGMN
WVRQAPGQGL EWMGWINTNI GEPTYTEEFK GRVTMTRDTS ISTAYMELSR
LRSDDTAVYY CARDDGYGMR VSYWGQGTLLV TVSSGGCGGG EVAACEKEVA
ALEKEVAALKE KEVAALKELE PKSADKTHTC PPCAPEAAG GPSVFLFPPK
PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY
NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRE EMTKNQVSLT CLVKGFPYPSD IAVEWESNGQ PENNYKTTTP
VLDSGDSFFL YSKLTVDKSR WQQGNVFCSS VMHEALHNHY TQKSLSLSPG

```

[00336] The second and fourth polypeptide chain of **DART-C** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to HER2/neu (VL_{HER2/neu} (**hHER2 MAB-1 VL3**, SEQ ID NO:69)), an intervening linker peptide (**Linker 1**; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137} (**hCD137 MAB-3 VH1** (SEQ ID NO:76)), an intervening linker peptide (**Linker 2**; GGCGGG (SEQ ID NO:18)), a Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:39), and a C-terminus.

[00337] Thus, the second and fourth polypeptide chain of **DART-C** is composed of: SEQ ID NO:69 – SEQ ID NO:16 – SEQ ID NO:76 – SEQ ID NO:18 – SEQ ID NO:39.

[00338] The amino acid sequence of the second and fourth polypeptide chain of **DART-C** is (SEQ ID NO:103):

```

DIQMTQSPSS LSASVGDRVT ITCKASQDIS NYLSWFQQKP GKAPKTLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
LRSEDTAVYY CTRDYGSSYS FDYWGQGTTV TVSSGGCGGG KVAACEKVA
ALKEKVAALK EKVAALKE

```

D. DART-D

[00339] **DART-D** is composed of five polypeptide chains, of which the second and fifth are identical (see, **Figure 5A**, wherein VL2/VH2 are the same as VL3/VH3, and **Figure 5B**).

[00340] The first polypeptide chain of **DART-D** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1** (SEQ ID NO:64)), a human IgG1 CH1 Domain (SEQ ID NO:3), a human IgG1 Hinge Region (SEQ ID NO:7), and a “hole-bearing” CH2 and CH3 Domain (SEQ ID NO:47).

[00341] Thus, the first polypeptide chain of **DART-D** is composed of: **SEQ ID NO:64** — **SEQ ID NO:3** — **SEQ ID NO:7** — **SEQ ID NO:47**.

[00342] The amino acid sequence of the first polypeptide chain of **DART-D** is (**SEQ ID NO:104**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
GYGNRVSYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTPPV
DSDGSFFLVS KLTVDKSRWQ QGNVFSCSVM HEALHNRVTQ KSLSLSPGK
```

[00343] The second and fifth polypeptide chains of **DART-D** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL3 (SEQ ID NO:69)**), a human IgG CL Kappa Domain (**SEQ ID NO:1**), and a C-terminus.

[00344] Thus, the second and fifth polypeptide chains of **DART-D** are composed of: **SEQ ID NO:69** — **SEQ ID NO:1**.

[00345] The amino acid sequence of the second and fifth polypeptide chains of **DART-D** is (**SEQ ID NO:105**):

```
DIQMTQSPSS LSASVGDRVT ITCKASQDIS NYLSWFQQKP GKAPKTLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC
```

[00346] The third polypeptide chain of **DART-D** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1, SEQ ID NO:64**), a human IgG1 CH1 Domain (**SEQ ID NO:3**), a human IgG1 Hinge Region (**SEQ ID NO:7**), a “knob-bearing CH2 and CH3 Domain (**SEQ ID NO:44**), an intervening linker peptide (GGGSGGGSGGG (**SEQ ID NO:24**)), a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**CD137 MAB-3 VL (SEQ ID NO:75)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**CD137 MAB-3 VH (SEQ ID NO:74)**), an intervening linker peptide (**Linker 2**; GGCGGG

(SEQ ID NO:18)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:36), and a C-terminus.

[00347] Thus, the third polypeptide chain of **DART-D** is composed of: SEQ ID NO:64 — SEQ ID NO:3 — SEQ ID NO:7 — SEQ ID NO:44— SEQ ID NO:24 — SEQ ID NO:75 — SEQ ID NO:16 — SEQ ID NO:74— SEQ ID NO:18 — SEQ ID NO:36.

[00348] The amino acid sequence of the third polypeptide chains of **DART-D** is (SEQ ID NO:106):

```

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
GYGNRVSYWG QGTLTVVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFP AVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IQMTQTTSSL SASLGDRVTI SCRPSQDISN YLNWYQQKPD
GTVKLLIYYT SRLRSGVPSR FSGSGSGTDY SLTISNLEQE DIATYFCQQG
DTLPYTFGGG TKLEIKGGGS GGGGQVQLQQ PGAELVRPGA SVKL SCKASG
YTFTSYWINW VKQRPQGGL WIGNIYPSDS YTNYNQKFKD KATLTVDKSS
STAYMQLSSP TSEDSAVYYC TRDYGSSYSF DYWGQGTTLT VSSGGCGGGE
VAALEKEVAA LEKEVAALEK EVAALEK

```

[00349] The fourth polypeptide chain of **DART-D** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**CD137 MAB-3 VL** (SEQ ID NO:75)), an intervening linker peptide (**Linker 1**; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**CD137 MAB-3 VH** (SEQ ID NO:74)), an intervening linker peptide (**Linker 2**; GGCGGG (SEQ ID NO:18)), a Heterodimer-Promoting (K-coil) Domain (KVAALEK-KVAALEK-KVAALEK-KVAALEK) (SEQ ID NO:37) and a C-terminus.

[00350] Thus, the fourth polypeptide chain of **DART-D** is composed of: SEQ ID NO:75 — SEQ ID NO:16 — SEQ ID NO:74 — SEQ ID NO:18— SEQ ID NO:37.

[00351] The amino acid sequence of the fourth polypeptide chain of **DART-D** is (SEQ ID NO:107):

```

DIQMTQTTSS LSASLGDRVT ISCRPSQDIS NYLNWYQQKP DGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GDTLPYTFGG
GTKLEIKGGG SGGGGQVQLQ QPGAELVRPG ASVKLSCKAS GYTFTSYWIN
WVKQRPQGGL EWIGNIYPSD SYTNYNQKFK DKATLTVDKS SSTAYMQLSS

```

PTSEDSAVYY CTRDYGSSYS FDYWGQGTTL TVSSGGCGGG KVAALKEKVA
ALKEKVAALK EKVAALKE

E. DART-E

[00352] **DART-E** is composed of five polypeptide chains, of which the second and fifth are identical (see, **Figure 5A**, wherein VL2/VH2 are the same as VL3/VH3, and **Figure 5C**).

[00353] The first polypeptide chain of **DART-E** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to CD137 (VH_{CD137}) (**CD137 MAB-3 VH (SEQ ID NO:74)**), an IgG1 CH1 Domain (**SEQ ID NO:3**), an IgG1 Hinge Domain (**SEQ ID NO:7**), a “hole-bearing CH2 and CH3 Domain (**SEQ ID NO:47**), and a C-terminus.

[00354] Thus, the first polypeptide chain of **DART-E** is composed of: **SEQ ID NO:74** — **SEQ ID NO:3** — **SEQ ID NO:7** — **SEQ ID NO:47**.

[00355] The amino acid sequence of the first polypeptide chain of **DART-E** is (**SEQ ID NO:108**):

QVQLQQPGAE LVRPGASVKL SCKASGYTFT SYWINWVKQR PGQGLEWIGN
IYPSDSYTNV NQKFQDKATL TVDKSSSTAY MQLSSPTSED SAVYYCTRDY
GSSYSFDYWG QGTTTLTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPAYLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVSVSLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLSCA VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLVV KLTVDKSRWQ QGNVFSCSVM HEALHNRYTQ KSLSLSPGK

[00356] The second and fifth polypeptide chains of **DART-E** comprise, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD137 (VL_{CD137u}) (**CD137 MAB-3 VL (SEQ ID NO:75)**), a human IgG CL Kappa Domain (**SEQ ID NO:1**), and a C-terminus.

[00357] Thus, the second and fifth polypeptide chains of **DART-E** are composed of: **SEQ ID NO:75** — **SEQ ID NO:1**.

[00358] The amino acid sequence of the second and fifth polypeptide chains of **DART-E** is (**SEQ ID NO:109**):

DIQMTQTTSS LSASLGDRVT ISCRPSQDIS NYLNWYQQKP DGTVKLLIYY
TSRLRSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GDTLPYTFGG
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV

DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEK

[00359] The third polypeptide chain of **DART-E** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to CD137 (VH_{CD137}) (**CD137 MAB-3 VH, SEQ ID NO:74**), a human IgG1 CH1 Domain (**SEQ ID NO:3**), a human IgG1 Hinge Region (**SEQ ID NO:7**), a “knob-bearing CH2 and CH3 Domain (**SEQ ID NO:44**), an intervening linker peptide (GGGSGGGSGGG (**SEQ ID NO:24**)), a VL domain of a monoclonal antibody capable of binding HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL3 (SEQ ID NO:69)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1 (SEQ ID NO:64)**), an intervening linker peptide (**Linker 2**; GCGGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:36**), and a C-terminus.

[00360] Thus, the third polypeptide chain of **DART-E** is composed of: **SEQ ID NO:74 — SEQ ID NO:3 — SEQ ID NO:7 — SEQ ID NO:44— SEQ ID NO:24 — SEQ ID NO:69 — SEQ ID NO:16 — SEQ ID NO:64— SEQ ID NO:18 — SEQ ID NO:36**.

[00361] The amino acid sequence of the third polypeptide chains of **DART-E** is (**SEQ ID NO:110**):

QVQLQQPGAE LVRPGASVKL SCKASGYTFT SYWINWVKQR PGQGLEWIGN
IYPSDSYTNV NQKFQDKATL TVDKSSSTAY MQLSSPTSED SAVYYCTRDY
GSSYSFDYWG QGTTTLTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IQMTQSPSSL SASVGDRVTI TCKASQDISN YLSWFQKPG
KAPKTLIYRA NRLQSGVPSR FSGSGSGTDF TLTISSLQPE DFATYYCLQH
DEFPWTFGQG TKLEIKGGGS GGGGQVQLVQ SGAEVKKPGA SVKVSCKASG
YTFTNYGMNW VRQAPGGGLE WMGWINTNIG EPTYTEEFKG RVTMTRDTSI
STAYMELSRL RSDDTAVYYC ARDDGYGNRV SYWGQGTLLV VSSGGCGGGE
VAALEKEVAA LEKEVAALEK EVAALEK

[00362] The fourth polypeptide chain of **DART-E** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL3 (SEQ ID NO:69)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody

capable of binding HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1 (SEQ ID NO:64)**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting (K-coil) Domain (**KVAALKE-KVAALKE-KVAALKE-KVAALKE**) (**SEQ ID NO:37**) and a C-terminus.

[00363] Thus, the fourth polypeptide chain of **DART-E** is composed of: **SEQ ID NO:69** — **SEQ ID NO:16** — **SEQ ID NO:64** — **SEQ ID NO:18** — **SEQ ID NO:37**.

[00364] The amino acid sequence of the fourth polypeptide chain of **DART-E** is (**SEQ ID NO:111**):

```
DIQMTQSPSS LSASVGDRVT ITCKASQDIS NYLSWFQQKP GKAPKTLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCLQ HDEFPWTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVCKAS GYTFTNYGMN
WVRQAPGQGL EWMGWINTNI GEPTYTEEFK GRVTMTRDTS ISTAYMELSR
LRSDDTAVYY CARDDGYGNR VSYWGQGLV TVSSGGCGGG KVAALKEKVA
ALKEKVAALK EKVAALKE
```

F. DART-F

[00365] **DART-F** is composed of five polypeptide chains, of which the second and fifth are identical (see, **Figure 5A**, wherein VL2/VH2 are the same as VL3/VH3, and **Figure 5B**).

[00366] The first polypeptide chain of **DART-F** is the same as the first polypeptide chain of **DART-D (SEQ ID NO:104)**.

[00367] The second and fifth polypeptide chains of **DART-F** are the same as the second and fifth polypeptide chains of **DART-D (SEQ ID NO:105)**.

[00368] The third polypeptide chain of **DART-F** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1 (SEQ ID NO:64)**), an IgG1 CH1 Domain (**SEQ ID NO:3**), an IgG1 Hinge Domain (**SEQ ID NO:7**), a “knob-bearing CH2 and CH3 Domain (**SEQ ID NO:44**), an intervening linker peptide (GGGSGGGSGGG (**SEQ ID NO:24**)), a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**CD137 MAB-4 VL (SEQ ID NO:91)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**CD137 MAB-4 VH (SEQ ID NO:90)**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting (E-coil) Domain (**EVAALEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:36)**), and a C-terminus.

[00369] Thus, the third polypeptide chain of **DART-F** is composed of: **SEQ ID NO:64** — **SEQ ID NO:3** — **SEQ ID NO:7** — **SEQ ID NO:44** — **SEQ ID NO:24** — **SEQ ID NO:91** — **SEQ ID NO:16** — **SEQ ID NO:90** — **SEQ ID NO:18** — **SEQ ID NO:36**.

[00370] The amino acid sequence of the third polypeptide chain of **DART-F** is (**SEQ ID NO:112**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSLRSDD TAVYYCARD
GYGNRVSYWG QGTLTVVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHNKPSN TKVDKRVPEK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IQMTQTTSSL SASLGDRVTI SCRASQDISN YLNWYQQKPD
GTVKLLIYYT SRLHSGVPSR FSGSGSGTDY SLTISNLEQE DIATYFCQQG
NTLPYTFGGG TKLEIKGGGS GGGGQVQLQQ PGAELVRPGA SVKLSCASG
YTFTSYWINW VKQRPQGGL E WIGNIYPSDS YTNVDQKFKD KATLTVDKSS
STAYMQLSSP TSEDSAVYYC TKSGEYGKIG YYAMDYWGQ TSVTVSSGGC
GGGEVAALEK EVAALEKEVA ALEKEVAALE K
```

[00371] The fourth polypeptide chain of **DART-F** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**CD137 MAB-4 VL (SEQ ID NO:91)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**CD137 MAB-4 VH (SEQ ID NO:90)**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:18**)), a Heterodimer-Promoting (K-coil) Domain (**KVAALKE-KVAALKE-KVAALKE-KVAALKE**) (**SEQ ID NO:37**) and a C-terminus.

[00372] Thus, the fourth polypeptide chain of **DART-F** is composed of: **SEQ ID NO:91** — **SEQ ID NO:16** — **SEQ ID NO:90** — **SEQ ID NO:18** — **SEQ ID NO:37**.

[00373] The amino acid sequence of the fourth polypeptide chain of **DART-F** is (**SEQ ID NO:113**):

```
DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTISNLEQ EDIATYFCQQ GNTLPYTFGG
GTKLEIKGGG SGGGQVQLQ QPGAELVRPG ASVKLSCKAS GTFTSYWIN
WVKQRPQGGL EWIGNIYPSD SYTNVDQKFK DKATLTVDKS SSTAYMQLSS
PTSEDSAVYY CTKSGEYGI GYYAMDYWGQ GTSVTVSSGG CGGGKVAALK
EKVAALKEKV AALKEKVAAL KE
```

G. DART-G

[00374] **DART-G** is composed of five polypeptide chains, of which the second and fifth are identical (see, **Figure 5A**, wherein VL2/VH2 are the same as VL3/VH3, and **Figure 5B**).

[00375] The first polypeptide chain of **DART-G** is the same as the first polypeptide chain of **DART-D** (SEQ ID NO:104).

[00376] The second and fifth polypeptide chains of **DART-G** are the same as the second and fifth polypeptide chains of **DART-D** (SEQ ID NO:105).

[00377] The third polypeptide chain of **DART-G** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1** (SEQ ID NO:64)), an IgG1 CH1 Domain (SEQ ID NO:3), an IgG1 Hinge Domain (SEQ ID NO:7), a “knob-bearing CH2 and CH3 Domain (SEQ ID NO:44), an intervening linker peptide (GGGSGGGSGGG (SEQ ID NO:24)), a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3** (SEQ ID NO:89)), an intervening linker peptide (**Linker 1**; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1** (SEQ ID NO:76)), an intervening linker peptide (**Linker 2**; GGCGGG (SEQ ID NO:18)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (SEQ ID NO:36), and a C-terminus.

[00378] Thus, the third polypeptide chain of **DART-G** is composed of: SEQ ID NO:64 — SEQ ID NO:3 — SEQ ID NO:7 — SEQ ID NO:44 — SEQ ID NO:24 — SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:18 — SEQ ID NO:36.

[00379] The amino acid sequence of the third polypeptide chain of **DART-G** is (SEQ ID NO:114):

```

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
GYGNRVSYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFPVQLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
DTLMISRTPE VTCVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IQMTQSPSSL SASVGDRTI TCRPSQDISN YLNWYQQKPD
KTVKLLIYYT SRLRSGVPSR FSGSGSGTDF TFTISSLOPE DIATYFCQQG

```

DTLPYTFGQG TKLEIKGGGS GGGGQVQLVQ SGAEVKKPGA SVKVSCKASG
 YTFTSYWINW VKQAPGQGLE WIGNIYPSDS YTNYNQKFKD KATITADKST
 STAYMELSSL RSEDVAVYYC TRDYGSSYSF DYWGQGT TTVT VSSGGCGGGE
 VAALEKEVAA LEKEVAALEK EVAALEK

[00380] Alternative **DART-G** third polypeptide chains may be employed in which the amino acid residues of **SEQ ID NO:76** (the VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137})) are replaced with the amino acid residues of **SEQ ID NO:83** (hCD137 MAB-3 VH1A), **SEQ ID NO:84** (hCD137 MAB-3 VH1B), **SEQ ID NO:85** (hCD137 MAB-3 VH1C), or **SEQ ID NO:86** (hCD137 MAB-3 VH1D). Optimized molecules comprising such polypeptide chains are described below.

The fourth polypeptide chain of **DART-G** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3 (SEQ ID NO:89)**), an intervening linker peptide (**Linker 1; GGGSGGGG (SEQ ID NO:16)**), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1 (SEQ ID NO:76)**), an intervening linker peptide (**Linker 2; GGCGGG (SEQ ID NO:18)**), a Heterodimer-Promoting (K-coil) Domain (**KVAAALKE-KVAAALKE-KVAAALKE-KVAAALKE**) (**SEQ ID NO:37**) and a C-terminus.

[00381] Thus, the fourth polypeptide chain of **DART-G** is composed of: **SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:18 — SEQ ID NO:37**.

[00382] The amino acid sequence of the fourth polypeptide chain of **DART-G** is (**SEQ ID NO:119**):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDVAVYY CTRDYGSSYS FDYWGQGT TTVT VSSGGCGGG KVAALKEKVA
 ALKEKVAALK EKVAALKE

[00383] Alternative **DART-G** fourth polypeptide chains may be employed in which the amino acid residues of **SEQ ID NO:76** (the VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137})) are replaced with the amino acid residues of **SEQ ID NO:83** (hCD137 MAB-3 VH1A), **SEQ ID NO:84** (hCD137 MAB-3 VH1B), **SEQ ID NO:85** (hCD137 MAB-3 VH1C), or **SEQ ID NO:86** (hCD137 MAB-3 VH1D). Optimized molecules comprising such polypeptide chains are described below.

H. Optimized DART-G

[00384] Optimized variants of **DART-G** designated “**DART-G1**,” “**DART-G2**,” “**DART-G3**,” and “**DART-G4**” are composed of five polypeptide chains, and contain the HER2/neu binding domains of antibody **hHER2 MAB-1 (1.3)** and the CD137 binding domains of any of antibody **hCD137 MAB-3 (1A.3)-(1D.3)**.

[00385] The first polypeptide chain of such optimized variants of **DART-G** have the same amino acid sequence as the first polypeptide chain of **DART-G (SEQ ID NO:104)**.

[00386] The second and fifth polypeptide chains of such optimized variants of **DART-G** have the same amino acid sequence as the second and fifth polypeptide chains of **DART-G (SEQ ID NO:105)**.

[00387] The third and fourth polypeptide chains of such optimized variants of **DART-G** have the amino acid sequences of **SEQ ID NO:115** and **SEQ ID NO:120 (DART-G1)**; of **SEQ ID NO:116** and **SEQ ID NO:121 (DART-G2)**; of **SEQ ID NO:117** and **SEQ ID NO:122 (DART-G3)**; or of **SEQ ID NO:118** and **SEQ ID NO:123 (DART-G4)**, as provided below.

[00388] The amino acid sequence of the third polypeptide chain of optimized **DART-G1**, comprising **SEQ ID NO:83 (hCD137 MAB-3 VH1A)**, is **SEQ ID NO:115**:

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
GYGNRVSYWG QGTLTVVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
YFPEPVTVSW NSGALTSGVH TFP AVLQSSG LYSLSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKRVEPK SCDKTHTCP CPAP EAAGGP SVFLFPPKPK
DTLMISRTP E VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL
DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
GSGGGSGGGD IQMTQSPSSL SASVGDRVTI TCRPSQDISN YLNWYQQKPD
KTVKLLIYYT SRLRSGVPSR FSGSGSGTDF TFTISSLQPE DIATYFCQQG
DTLPYTFGQG TKLEIKGGGS GGGGQVQVQL VQSGAEVKKP GASVKVSCA
SGYTFTSYWI NWVKQAPGQG LEWIGNIYPS DSYTNYNQKF KDKATITADK
STSTAYMELS SLRSED TAVY YCTRDYGS AY SFHPWGQGT VTVSSGGCGG
GEVAALEKEV AALEKEVAAL EKEVAALEK
```

[00389] The amino acid sequence of the third polypeptide chain of optimized **DART-G2**, comprising **SEQ ID NO:84 (hCD137 MAB-3 VH1B)**, is **SEQ ID NO:116**:

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
```

GYGNRVSYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
 YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVSVTV PSSSLGTQTY
 ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
 DTLNISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
 GSGGGSGGGD IQMTQSPSSL SASVGDRVTI TCRPSQDISN YLNWYQQKPD
 KTVKLLIYYT SRLRSGVPSR FSGSGSGTDF TFTISSLOPE DIATYFCQQG
 DTLPYTFGQG TKLEIKGGGS GGGGQVQVQL VQSGAEVKKP GASVKVSCA
 SGYTFTSYWI NWVKQAPGQG LEWIGNIYPS DSYTNYNQKF KDKATITADK
 STSTAYMELS SLRSEDVAVY YCTRDYGSAY SMSTWGQGT VTVSSGGCGG
 GEVAALEKEV AALEKEVAAL EKEVAALEK

[00390] The amino acid sequence of the third polypeptide chain of optimized **DART-G3**, comprising **SEQ ID NO:85** (hCD137 MAB-3 VH1C), is **SEQ ID NO:117**:

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
 INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
 GYGNRVSYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
 YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVSVTV PSSSLGTQTY
 ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
 DTLNISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
 GSGGGSGGGD IQMTQSPSSL SASVGDRVTI TCRPSQDISN YLNWYQQKPD
 KTVKLLIYYT SRLRSGVPSR FSGSGSGTDF TFTISSLOPE DIATYFCQQG
 DTLPYTFGQG TKLEIKGGGS GGGGQVQVQL VQSGAEVKKP GASVKVSCA
 SGYTFTSYWI NWVKQAPGQG LEWIGNIYPS DSYTNYNQKF KDKATITADK
 STSTAYMELS SLRSEDVAVY YCTRDYGSAY SYSLWGQGT VTVSSGGCGG
 GEVAALEKEV AALEKEVAAL EKEVAALEK

[00391] The amino acid sequence of the third polypeptide chain of optimized **DART-G4**, comprising **SEQ ID NO:86** (hCD137 MAB-3 VH1D), is **SEQ ID NO:118**:

QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGMNWVRQA PGQGLEWMGW
 INTNIGEPTY TEEFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARD
 GYGNRVSYWG QGTLVTVSSA STKGPSVFPL APSSKSTSGG TAALGCLVKD
 YFPEPVTVSW NSGALTSGVH TFPVAVLQSSG LYSLSVSVTV PSSSLGTQTY
 ICNVNHNKPSN TKVDKRVEPK SCDKTHTCPP CPAPEAAGGP SVFLFPPKPK
 DTLNISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV
 YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGGG
 GSGGGSGGGD IQMTQSPSSL SASVGDRVTI TCRPSQDISN YLNWYQQKPD
 KTVKLLIYYT SRLRSGVPSR FSGSGSGTDF TFTISSLOPE DIATYFCQQG
 DTLPYTFGQG TKLEIKGGGS GGGGQVQVQL VQSGAEVKKP GASVKVSCA
 SGYTFTSYWI NWVKQAPGQG LEWIGNIYPS DSYTNYNQKF KDKATITADK
 STSTAYMELS SLRSEDVAVY YCTRDYGSSY SYNVWGQGT VTVSSGGCGG
 GEVAALEKEV AALEKEVAAL EKEVAALEK

[00392] The amino acid sequence of the fourth polypeptide chain of optimized **DART-G1**, comprising **SEQ ID NO:83** (hCD137 MAB-3 VH1A), is **SEQ ID NO:120**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS FHPWGQTTV TVSSGGCGGG KVAALKEKVA
 ALKEKVAALK EKVAALKE

[00393] The amino acid sequence of the fourth polypeptide chain of optimized **DART-G2**, comprising **SEQ ID NO:84** (hCD137 MAB-3 VH1B), is **SEQ ID NO:121**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS MSTWGQTTV TVSSGGCGGG KVAALKEKVA
 ALKEKVAALK EKVAALKE

[00394] The amino acid sequence of the fourth polypeptide chain of optimized **DART-G3**, comprising **SEQ ID NO:85** (hCD137 MAB-3 VH1C), **SEQ ID NO:122**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS YSLWGQTTV TVSSGGCGGG KVAALKEKVA
 ALKEKVAALK EKVAALKE

[00395] The amino acid sequence of the fourth polypeptide chain of optimized **DART-G4**, comprising **SEQ ID NO:86** (hCD137 MAB-3 VH1D), is **SEQ ID NO:123**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSSYS YNVWGQTTV TVSSGGCGGG KVAALKEKVA
 ALKEKVAALK EKVAALKE

I. TRIDENT-A

[00396] **TRIDENT-A** is a Trivalent **CD137 x CD137 x TA** Binding Molecule having two CD137 binding sites and one binding site for the exemplary **TA**, HER2/neu. **TRIDENT-A** is composed of four polypeptide chains (see, **Figure 6A**, wherein VL1/VH1 (Site A) are the same as VL2/VH2 (Site B) and bind CD137, and VL3/VH3 (Site C) bind HER2/neu).

[00397] The first polypeptide chain of **TRIDENT-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding

CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3 (SEQ ID NO:89)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1 (SEQ ID NO:76)**), an intervening linker peptide (**Linker 2**; ASTKG (**SEQ ID NO:19**)), a cysteine containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:38**)), an intervening linker peptide (GGGDKTHTCPPCP (**SEQ ID NO:21**)), a “knob-bearing CH2 and CH3 Domain (**SEQ ID NO:44**), and a C-terminus.

[00398] Thus, the first polypeptide chain of **TRIDENT-A** is composed of: **SEQ ID NO:89** — **SEQ ID NO:16** — **SEQ ID NO:76** — **SEQ ID NO:19** — **SEQ ID NO:38** — **SEQ ID NO:21** — **SEQ ID NO:44**.

[00399] The amino acid sequence of the first polypeptide chain of **TRIDENT-A** is (**SEQ ID NO:192**):

```

DIQMTQSPSS LSASVGDRVIT ITCRPSQDIS NYLNWYQQKP DKTIVKLLIYY
TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
LRSEDYAVYY CTRDYGSSYS FDYWGQGTTV TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKGGG DKTHTCPPCP APEAAGGPSV FLFPPKPKDT
LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTIVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDL
DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

```

[00400] The second polypeptide chain of **TRIDENT-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3 (SEQ ID NO:89)**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1 (SEQ ID NO:76)**), an intervening linker peptide (**Linker 2**; ASTKG (**SEQ ID NO:19**)), a cysteine containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:39**)), and a C-terminus.

[00401] Thus, the second polypeptide chain of **TRIDENT-A** is composed of: **SEQ ID NO:89** — **SEQ ID NO:16** — **SEQ ID NO:76** — **SEQ ID NO:19** — **SEQ ID NO:39**.

[00402] The amino acid sequence of the second polypeptide chain of **TRIDENT-A** is (**SEQ ID NO:197**):

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFGSGSGSTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSS**SY** **FDY**WGQGTTV TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

[00403] Alternative **TRIDENT-A** first and second polypeptide chains may be employed in which the amino acid residues of **SEQ ID NO:76** (the VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137})) are replaced with the amino acid residues of **SEQ ID NO:83** (hCD137 MAB-3 VH1A), **SEQ ID NO:84** (hCD137 MAB-3 VH1B), **SEQ ID NO:85** (hCD137 MAB-3 VH1C), **SEQ ID NO:86** (hCD137 MAB-3 VH1D), **SEQ ID NO:208** (hCD137 MAB-3 VH1E), **SEQ ID NO:209** (hCD137 MAB-3 VH1F), or **SEQ ID NO:210** (hCD137 MAB-3 VH1G) and/or the amino acid residues of **SEQ ID NO:89** (the VL domain of a monoclonal antibody capable of binding CD137 (VH_{CD137})) are replaced with the amino acid residues of **SEQ ID NO:211** (hCD137 MAB-3 VL4), **SEQ ID NO:212** (hCD137 MAB-3 VL5), **SEQ ID NO:213** (hCD137 MAB-3 VL6), **SEQ ID NO:214** (hCD137 MAB-3 VL7), **SEQ ID NO:215** (hCD137 MAB-3 VL8), **SEQ ID NO:216** (hCD137 MAB-3 VL9), **SEQ ID NO:217** (hCD137 MAB-3 VL10), **SEQ ID NO:218** (hCD137 MAB-3 VL11), **SEQ ID NO:219** (hCD137 MAB-3 VL12), **SEQ ID NO:220** (hCD137 MAB-3 VL13), **SEQ ID NO:221** (hCD137 MAB-3 VL14), or **SEQ ID NO:222** (hCD137 MAB-3 VL15). Optimized/deimmunized molecules comprising many of such polypeptide chains are described below.

[00404] The third polypeptide chain of **TRIDENT-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to HER2/neu (VH_{HER2/neu}) (**hHER2 MAB-1 VH1 (SEQ ID NO:64)**), a human IgG1 CH1 Domain (**SEQ ID NO:3**), a human IgG1 Hinge Region (**SEQ ID NO:7**), and a “hole-bearing” CH2 and CH3 Domain (**SEQ ID NO:47**). Thus, the third polypeptide chain of **TRIDENT-A** is composed of: **SEQ ID NO:64 — SEQ ID NO:3 — SEQ ID NO:7 — SEQ ID NO:47**, and has the same amino acid sequence as the first polypeptide chain of **DART-D (SEQ ID NO:104)** provided above.

[00405] The fourth polypeptide chain of **TRIDENT-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding

to HER2/neu (VL_{HER2/neu}) (**hHER2 MAB-1 VL3 (SEQ ID NO:69)**), a human IgG CL Kappa Domain (**SEQ ID NO:1**), and a C-terminus. Thus, the fourth polypeptide chain of **TRIDENT-A** is composed of: **SEQ ID NO:69 — SEQ ID NO:1**, and has the same amino acid sequence as the second and fifth polypeptide chains of **DART-D (SEQ ID NO: 105)** provided above.

1. Optimized Variants of TRIDENT-A

[00406] Optimized variants of **TRIDENT-A** designated “**TRIDENT-A1**,” “**TRIDENT-A2**,” “**TRIDENT-A3**,” and “**TRIDENT-A4**” are composed of four polypeptide chains, and contain the HER2/neu binding domains of antibody **hHER2 MAB-1 (1.3)** and the CD137 binding domains of any of antibody **hCD137 MAB-3 (1A.3)-(1D.3)**.

[00407] The third polypeptide chain of such optimized **TRIDENT-A** have the same amino acid sequence as the third polypeptide chain of **TRIDENT-A**, which as noted above is the same as the first polypeptide chain of **DART-D (SEQ ID NO:104)**.

[00408] The fourth polypeptide chain of such optimized **TRIDENT-A** have the same amino acid sequence as the fourth polypeptide chains of **TRIDENT-A**, which as noted above is the same as the second and fifth polypeptide chains of **DART-D (SEQ ID NO:105)**.

[00409] The first and second polypeptide chains of such optimized variants of **TRIDENT-A** have the amino acid sequence of **SEQ ID NO:193** and **SEQ ID NO:198 (TRIDENT-A1)**; of **SEQ ID NO:194** and **SEQ ID NO:199 (TRIDENT-A2)**; of **SEQ ID NO:195** and **SEQ ID NO:200 (TRIDENT-A3)**; or of **SEQ ID NO:196** and **SEQ ID NO:201 (TRIDENT-A4)**, as provided below.

[00410] The amino acid sequence of the first polypeptide chain of **TRIDENT-A1**, comprising **SEQ ID NO:83 (hCD137 MAB-3 VH1A)**, is **SEQ ID NO:193**:

DIQMTQSPSS	LSASVGDRVT	ITCRPSQDIS	NYLNWYQQKP	DKTVKLLIYY
TSRLRSGVPS	RFSGSGSGTD	FTFTISSLQP	EDIATYFCQQ	GDTPPYTFGQ
GTKLEIKGGG	SGGGGQVQLV	QSGAEVKKPG	ASVKVSCAS	GYTFTSYWIN
WVKQAPGQGL	EWIGNIYPSD	SYTNYNQKFK	DKATITADKS	TSTAYMELSS
LRSED ~~TAVYY~~	CTRDYGS ~~AYS~~	~~FHP~~ WGQTTV	TVSSASTKGE	~~VAACEKEVAA~~
~~LEKEVAALEK~~	~~EVAALEKGGG~~	DKTHTCPPCP	APEAAGGPSV	FLFPPKPKDT
LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY
RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT
LPPSREEMTK	NQVSLWCLVK	GFYP ~~SDIAVE~~	WESNGQPENN	YKTTTPVLDS
DGSFFLYSKL	TVDKSRWQQG	NVFSCSV ~~MHE~~	ALHNHYTQKS	LSLSPGK

[00411] The amino acid sequence of the first polypeptide chain of **TRIDENT-A2**, comprising **SEQ ID NO:84** (hCD137 MAB-3 VH1B), is **SEQ ID NO:194**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSED~~TAVYY~~ CTRDYGS**AYS** **MST**WGQGT TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKGGG DKHTCPPCP APEAAGGPSV FLFPPKPKDT
 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
 LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

[00412] The amino acid sequence of the first polypeptide chain of **TRIDENT-A3**, comprising **SEQ ID NO:85** (hCD137 MAB-3 VH1C), is **SEQ ID NO:195**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSED~~TAVYY~~ CTRDYGS**AYS** **YSL**WGQGT TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKGGG DKHTCPPCP APEAAGGPSV FLFPPKPKDT
 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
 LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

[00413] The amino acid sequence of the first polypeptide chain of **TRIDENT-A4**, comprising **SEQ ID NO:86** (hCD137 MAB-3 VH1D), is **SEQ ID NO:196**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSED~~TAVYY~~ CTRDYGS**SYS** **YNV**WGQGT TVSSASTKGE VAACEKEVAA
LEKEVAALEK EVAALEKGGG DKHTCPPCP APEAAGGPSV FLFPPKPKDT
 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
 LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

[00414] The amino acid sequence of the second polypeptide chain of **TRIDENT-A1**, comprising **SEQ ID NO:83** (hCD137 MAB-3 VH1A), is **SEQ ID NO:198**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSED~~TAVYY~~ CTRDYGS**AYS** **FHP**WGQGT TVSSASTKGE VAACKEKVAA
LKEKVAALKE KVAALKE

[00415] The amino acid sequence of the second polypeptide chain of **TRIDENT-A2**, comprising **SEQ ID NO:84** (hCD137 MAB-3 VH1B), is **SEQ ID NO:199**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS MSTWGQGT TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

[00416] The amino acid sequence of the second polypeptide chain of **TRIDENT-A3**, comprising **SEQ ID NO:85** (hCD137 MAB-3 VH1C), is **SEQ ID NO:200**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS YSLWGQGT TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

[00417] The amino acid sequence of the second polypeptide chain of **TRIDENT-A4**, comprising **SEQ ID NO:86** (hCD137 MAB-3 VH1D), is **SEQ ID NO:201**:

DIQMTQSPSS LSASVGDRVT ITCRPSQDIS NYLNWYQQKP DKTVKLLIYY
 TSRLRSGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
 WVKQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSSYS YNVWGQGT TVSSASTKGK VAACKEKVAA
LKEKVAALKE KVAALKE

2. Deimmunized Variants of TRIDENT-A

[00418] Deimmunized variants of **TRIDENT-A** are composed of four polypeptide chains, and contain the HER2/neu binding domains of antibody **hHER2 MAB-1 (1.3)** and the CD137 VH Domain of any of **hCD137 MAB-3 VH1E-VH1G** and the CD137 VL Domain of any of **hCD137 MAB-3 VL4-VL15**.

[00419] An exemplary deimmunized variant of **TRIDENT-A** designated “**TRIDENT-A5**,” is composed of four polypeptide chains, and contains the HER2/neu binding domains of antibody **hHER2 MAB-1 (1.3)** and the CD137 binding domains of any of antibody **hCD137 MAB-3 (1E.15)**.

[00420] The amino acid sequence of the first polypeptide chain of **TRIDENT-A5** (comprising **hCD137 MAB-3 VH1E** and **hCD137 MAB-3 VL15**), is (**SEQ ID NO:229**):

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTVKLLIYY
TGRASGVPS RFSGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ

GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
 WVRQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS MSTWGQGT TVSSASTKGE VAACEKEVAA
LEKEVAALKE EVAALEKGGG DKTHTCPPCP APEAAGGPSV FLFPPKPKDT
 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 RVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
 LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS
 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK

[00421] The amino acid sequence of the second polypeptide chain of **TRIDENT-A5**, (comprising **hCD137 MAB-3 VH1E** and **hCD137 MAB-3 VL15**), is **SEQ ID NO:230**:

DIQMTQSPSS LSASVGDRVT ITCQASQDIS NYLNWYQQKP DKTVKLLIYY
 TGRARASGVPS RFGSGSGTD FTFTISSLQP EDIATYFCQQ GDTLPYTFGQ
 GTKLEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCAS GYTFTSYWIN
 WVRQAPGQGL EWIGNIYPSD SYTNYNQKFK DKATITADKS TSTAYMELSS
 LRSEDTAVYY CTRDYGSAYS MSTWGQGT TVSSASTKGE VAACEKEVAA
LKEKVAALKE KVAALKE

[00422] The third polypeptide chain of such deimmunized **TRIDENT-A5** have the same amino acid sequence as the third polypeptide chain of **TRIDENT-A**, which as noted above is the same as the first polypeptide chain of **DART-D** (**SEQ ID NO:104**).

[00423] The fourth polypeptide chain of such deimmunized **TRIDENT-A5** have the same amino acid sequence as the fourth polypeptide chains of **TRIDENT-A**, which as noted above is the same as the second and fifth polypeptide chains of **DART-D** (**SEQ ID NO:105**).

J. TRIDENT-B

[00424] **TRIDENT-B** is a Trivalent **CD137 x CD137 x TA** Binding Molecule having two CD137 binding sites and one binding site for the exemplary **TA**, 5T4. **TRIDENT-B** is composed of four polypeptide chains (see, **Figure 6A**, wherein VL1/VH1 (Site A) are the same as VL2/VH2 (Site B) and bind CD137, and VL3/VH3 (Site C) bind 5T4).

[00425] The first polypeptide chain of **TRIDENT-B** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (**hCD137 MAB-3 VL3** (**SEQ ID NO:89**)), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:16**)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (**hCD137 MAB-3 VH1** (**SEQ ID NO:76**)), an intervening linker peptide (**Linker 2**; ASTKG (**SEQ ID NO:19**)), a cysteine containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:38**)), an intervening linker peptide (GGGDKTHTCPPCP (**SEQ ID NO:21**)), a “knob-bearing CH2 and

CH3 Domain (SEQ ID NO:44), and a C-terminus. Thus, the first polypeptide chain of TRIDENT-B is composed of: SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:19 — SEQ ID NO:38 — SEQ ID NO:21 — SEQ ID NO:44, and has the same amino acid sequence as the first polypeptide chain of TRIDENT-A (SEQ ID NO: 192) provided above

[00426] The second polypeptide chain of TRIDENT-B comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137}) (hCD137 MAB-3 VL3 (SEQ ID NO:89)), an intervening linker peptide (Linker 1; GGGSGGGG (SEQ ID NO:16)), a VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137}) (hCD137 MAB-3 VH1 (SEQ ID NO:76)), an intervening linker peptide (Linker 2; ASTKG (SEQ ID NO:19)), a cysteine containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (SEQ ID NO:39)), and a C-terminus. Thus, the second polypeptide chain of TRIDENT-B is composed of: SEQ ID NO:89 — SEQ ID NO:16 — SEQ ID NO:76 — SEQ ID NO:19 — SEQ ID NO:39, and has the same amino acid sequence as the second polypeptide chain of TRIDENT-A (SEQ ID NO: 197) provided above.

[00427] Alternative TRIDENT-B first and second polypeptide chains may be employed in which the amino acid residues of SEQ ID NO:76 (the VH domain of a monoclonal antibody capable of binding CD137 (VH_{CD137})) are replaced with the amino acid residues of SEQ ID NO:83 (hCD137 MAB-3 VH1A), SEQ ID NO:84 (hCD137 MAB-3 VH1B), SEQ ID NO:85 (hCD137 MAB-3 VH1C), SEQ ID NO:86 (hCD137 MAB-3 VH1D), SEQ ID NO:208 (hCD137 MAB-3 VH1E), SEQ ID NO:209 (hCD137 MAB-3 VH1F), or SEQ ID NO:210 (hCD137 MAB-3 VH1G) and/or the amino acid residues of SEQ ID NO:89 (the VL domain of a monoclonal antibody capable of binding CD137 (VL_{CD137})) are replaced with the amino acid residues of SEQ ID NO:211 (hCD137 MAB-3 VL4), SEQ ID NO:212 (hCD137 MAB-3 VL5), SEQ ID NO:213 (hCD137 MAB-3 VL6), SEQ ID NO:214 (hCD137 MAB-3 VL7), SEQ ID NO:215 (hCD137 MAB-3 VL8), SEQ ID NO:216 (hCD137 MAB-3 VL9), SEQ ID NO:217 (hCD137 MAB-3 VL10), SEQ ID NO:218 (hCD137 MAB-3 VL11), SEQ ID NO:219 (hCD137 MAB-3 VL12), SEQ ID NO:220 (hCD137 MAB-3 VL13), SEQ ID NO:221 (hCD137 MAB-3 VL14), or SEQ ID NO:222 (hCD137 MAB-3 VL15). Optimized/deimmunized molecules comprising many of such polypeptide chains are described

herein and have the same amino acid sequences as the first and second polypeptide chains of optimized/deimmunized **TRIDENT-A** molecules (*e.g.*, **TRIDENT-A1 – TRIDENT-A5**)

[00428] The third polypeptide chain of **TRIDENT-B** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VH domain of a monoclonal antibody capable of binding to 5T4 (VH_{5T4}) (**h5T4 MAB-1 VH1 (SEQ ID NO:134)**), a human IgG1 CH1 Domain (**SEQ ID NO:3**), a human IgG1 Hinge Region (**SEQ ID NO:7**), and a “hole-bearing” CH2 and CH3 Domain (**SEQ ID NO:47**). Thus, the first polypeptide chain of **TRIDENT-B** is composed of: **SEQ ID NO:134 – SEQ ID NO:3 – SEQ ID NO:7 – SEQ ID NO:47**.

[00429] The amino acid sequence of the third polypeptide chain of **TRIDENT-B** is (**SEQ ID NO:231**):

```
QVQLVQSGAE VKKPGASVKV SCKASGYTFT SFWMHWVRQA PGQGLEWMGR
IDPNRGGTEY NEKAISRVTM TADKSTSTAY MELSSLRSED TAVYYCAGGN
PYYPMQDYWGQ GTTVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
CNVNHKPSNT KVDKRVEPKS CDKTHTCPPC PAPEAAGGPS VFLFPPKPKD
TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
TLPPSREEMT KNQVSLSCAV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD
SDGSFFLVSK LTVDKSRWQQ GNVFSCSVMH EALHNRYTQK SLSLSPGK
```

[00430] The fourth polypeptide chain of **TRIDENT-B** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to 5T4 (VL_{5T4}) (**h5T4 MAB-1 VL1 (SEQ ID NO:135)**), a human IgG CL Kappa Domain (**SEQ ID NO:1**), and a C-terminus. Thus, the fourth polypeptide chain of **TRIDENT-B** is composed of: **SEQ ID NO:135 – SEQ ID NO:1**.

[00431] The amino acid sequence of the fourth polypeptide chains of **TRIDENT-B** is (**SEQ ID NO:232**):

```
DIQMTQSPSS LSASVGDRVT ITCRASQGIS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFSGSGSGTD FTLTISSLQP EDVATYYCLQ YDDFPWTFGQ
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTGSFN RGEC
```

1. Optimized and Deimmunized Variants **TRIDENT-B**

[00432] Optimized and deimmunized variants of **TRIDENT-B** are composed of four polypeptide chains, and contain the 5T4 binding domains of antibody **h5T4 MAB-1** and the

CD137 VH Domain of any of **hCD137 MAB-3 VH1A-VH1G** and the CD137 VL Domain of any of **hCD137 MAB-3 VL3-VL15**.

[00433] Exemplary optimized and deimmunized variants of **TRIDENT-B** designated “**TRIDENT-B2**,” and “**TRIDENT-B5**” are composed of four polypeptide chains, and contain the 5T4 binding domains of antibody **h5T4 MAB-1** and the CD137 binding domains of antibody **hCD137 MAB-3 (1B.3)**, and **hCD137 MAB-3 (1E.15)**, respectively.

[00434] The first polypeptide chain of **TRIDENT-B2** has the same amino acid sequence as the first polypeptide chain of **TRIDENT-A2 (SEQ ID NO:194)**, as provided above.

[00435] The second polypeptide chain of **TRIDENT-B2** has the same amino acid sequence as the second polypeptide of **TRIDENT-A2 (SEQ ID NO:199)**, as provided above.

[00436] The first polypeptide chain of **TRIDENT-B5** has the same amino acid sequence as the first polypeptide chain of **TRIDENT-A5 (SEQ ID NO:229)**, as provided above.

[00437] The second polypeptide chain of **TRIDENT-B5** has the same amino acid sequence as the second polypeptide of **TRIDENT-A5 (SEQ ID NO:230)**, as provided above.

[00438] The third polypeptide chains of both exemplary optimized and deimmunized variants of **TRIDENT-B** have the same amino acid sequence as the third polypeptide chain of **TRIDENT-B (SEQ ID NO:231)**.

[00439] The fourth polypeptide chain of both exemplary optimized and deimmunized variants of **TRIDENT-B** have the same amino acid sequence as the fourth polypeptide chains of **TRIDENT-B (SEQ ID NO:232)**.

K. Alternative CD137 x TA Binding Molecules

[00440] As will be recognized in view of the instant disclosure, additional **CD137 x TA** binding molecules having the general structure of any of the above exemplary molecules and comprising a binding site for an alternative Tumor Antigen and/or having an optimized/deimmunized CD137 binding site may be constructed by employing the VL and VH domains of alternative Tumor Antigen antibodies in lieu of the VL and VH domains of the anti-HER2/neu or anti-5T4 antibodies, and/or the VL and VH domains of any of the optimized/deimmunized CD137 antibodies disclosed herein. Similarly, as provided herein,

alternative **CD137 x TA** binding molecules may likewise be constructed incorporating alternative linkers and/or heterodimer promoting domains.

V. Control Molecules

[00441] In order to more meaningfully demonstrate the properties of the **CD137 x TA** Binding Molecules of the present invention, control antibodies, whose VL and VH domains may be used to produce control Fc-bearing diabodies and other control binding molecules, are presented below.

[00442] The anti-fluorescein antibody 4-4-20 (Gruber, M. *et al.* (1994) “*Efficient Tumor Cell Lysis Mediated By A Bispecific Single Chain Antibody Expressed In Escherichia coli*,” J. Immunol. 152(11):5368-5374; Bedzyk, W.D. *et al.* (1989) “*Comparison Of Variable Region Primary Structures Within An Anti-Fluorescein Idiotypic Family*,” J. Biol. Chem. 264(3): 1565-1569) is a suitable control antibody, diabodies. The amino acid sequences of the variable light and variable heavy Domains of anti-fluorescein antibody 4-4-20 are as follows:

[00443] The amino acid sequence of the VH Domain of anti-fluorescein antibody 4-4-20 (SEQ ID NO:124) is shown below (CDR_H residues are shown underlined):

EVKLD~~ET~~GGG LVQPGRPMKL SCVASGFTFS DYWMNWVRQS PEKGLEWVAQ
IRNKPYNYET YYSDSVKGRF TISRDDSKSS VYLQMN~~N~~LRV EDMGIYYCTG
SYYGMDYWGQ GTSVTVSS

[00444] The amino acid sequence of the VL Domain of anti-fluorescein antibody 4-4-20 (SEQ ID NO:125) is shown below (CDR_L residues are shown underlined):

DVMTQT~~P~~FSS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK
 VLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP
WTFGGGTKLE IK

[00445] Palivizumab (see, *e.g.*, Protein Data Bank (PDB) ID No. 2HWZ) is a humanized monoclonal antibody (IgG) directed against an epitope in the A antigenic site of the F protein of RSV, and is a suitable control antibody, whose VL and VH domains may be used to produce control diabodies and other control binding molecules. Alternative anti-RSV glycoprotein F antibodies include motavizumab (see, *e.g.*, PDB ID No. 3IXT) and a variant of palivizumab engineered to remove a cysteine residues from CDR 1 of the light chain. The variant of palivizumab was used for generation of the control molecules below

[00446] The amino acid sequence of the VH Domain of palivizumab and the variant (SEQ ID NO:126) is shown below (CDR_H residues are shown underlined):

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMSVGWIR QPPGKALEWL
 ADIWDDDKD YNPSLKSRILT ISKDTSKNQV VLKVTNMDPA DTATYYCARS
MITNWFFDVW GAGTTVTVSS

[00447] The amino acid sequence of the VL Domain of the variant of palivizumab (SEQ ID NO:127) is shown below (CDRL residues are shown underlined):

DIQMTQSPST LSASVGDRVT ITCRASQSVG YMHWYQQKPG KAPKLLIYDT
SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG
 TKLEIK

VI. Summary of CD137 x TA Binding and Control Molecules

[00448] Table 5 summarizes the domain attributes of DART-A – DART-G, TRIDENT-A-A5:

Table 5					
Name (No. of Chains)	Parental mAbs	Fc Domain	Chain No.	SEQ ID NOs.	Other Components
DART-A (4 Chains)	hHER2 MAB-1 (1.2) hCD137 MAB-3 (1.1)	IgG1 (AA)	1	98	E/K Coils
			2	99	
			3	98	
			4	99	
DART-B (4 Chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1.3)	IgG1 (AA)	1	100	E/K Coils
			2	101	
			3	100	
			4	101	
DART-C (4 Chains)	hCD137 MAB-3 (1.3) hHER2 MAB-1 (1.3)	IgG1 (AA)	1	102	E/K Coils
			2	103	
			3	102	
			4	103	
DART-D (5 Chains)	hHER2 MAB-1 (1.3) CD137 MAB-3	IgG1 (AA) (knob/hole)	1	104	CL/CH1 and E/K Coils
			2	105	
			3	106	
			4	107	
			5	105	
DART-E (5 Chains)	CD137 MAB-3 hHER2 MAB-1 (1.3)	IgG1 (AA) (knob/hole)	1	108	CL/CH1 and E/K Coils
			2	109	
			3	110	
			4	111	
			5	109	
DART-F (5 Chains)	hHER2 MAB-1 (1.3) CD137 MAB-4	IgG1 (AA) (knob/hole)	1	104	CL/CH1 and E/K Coils
			2	105	
			3	112	
			4	113	
			5	105	
DART-G (5 Chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1.3)	IgG1 (AA) (knob/hole)	1	104	CL/CH1 and E/K Coils
			2	105	
			3	114	
			4	119	
			5	105	

Table 5					
Name (No. of Chains)	Parental mAbs	Fc Domain	Chain No.	SEQ ID NOs.	Other Components
Optimized DART-G1- G4 (5 Chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1A.3)-(1D.3)	IgG1 (AA) (knob/hole)	1	104	CL/CH1 and E/K Coils
			2	105	
			3/4	115/120	
			3/4	116/121	
			3/4	117/122	
			3/4	118/123	
			5	105	
TRIDENT-A (4 Chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1.3)	IgG1 (AA) (knob/hole)	1	192	CL/CH1 and E/K Coils
			2	197	
			3	104	
			4	105	
Optimized TRIDENT- A1-A4 (4 Chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1A.3)-(1D.3)	IgG1 (AA) (knob/hole)	1/2	193/198	CL/CH1 and E/K Coils
			1/2	194/199	
			1/2	195/200	
			1/2	196/201	
			3	104	
Deimmunized TRIDENT A5 (4 chains)	hHER2 MAB-1 (1.3) hCD137 MAB-3 (1E.15)	IgG1 (AA) (knob/hole)	1	229	CL/CH1 and E/K Coils
			2	230	
			3	104	
			4	105	
TRIDENT-B	h5T4 MAB-1 hCD137 MAB-3 (1.3)	IgG1 (AA) (knob/hole)	1	192	CL/CH1 and E/K Coils
			2	197	
			3	231	
			4	232	
Optimized TRIDENT- B2	h5T4 MAB-1 hCD137 MAB-3 (1B.3)	IgG1 (AA) (knob/hole)	1	194	CL/CH1 and E/K Coils
			2	199	
			3	231	
			4	232	
Deimmunized TRIDENT- B5	h5T4 MAB-1 hCD137 MAB-3 (1E.15)	IgG1 (AA) (knob/hole)	1	229	CL/CH1 and E/K Coils
			2	230	
			3	231	
			4	232	

[00449] Table 6 shows the attributes of additional DART and TRIDENT molecules that were prepared:

Table 6			
Name (No. of Chains)	Parental mAbs	Fc Domain	Other Components
DART-1 (4 Chains)	hHER2 MAB-1 (1.2) CD137 MAB-1	IgG1 (AA)	Same as DART-A except comprising VH/VL of CD137 MAB-1 in place of the VH/VL of hCD137 MAB-3(1.1)
DART-2 (4 Chains)	hHER2 MAB-1 (1.2) CD137 MAB-2	IgG1 (AA)	Same as DART-A except comprising VH/VL of CD137 MAB-2 in place of the VH/VL of hCD137 MAB-3(1.1)

Table 6			
Name (No. of Chains)	Parental mAbs	Fc Domain	Other Components
DART-3 (4 Chains)	hHER2 MAB-1 (1.2) variant palivizumab	IgG1 (AA)	Same as DART-A except comprising VH/VL of variant palivizumab in place of the VH/VL of hCD137 MAB-3(1.1)
DART-4 (5 Chains)	hHER2 MAB-1 (1.3) CD137 MAB-1	IgG1 (AA) (knob/hole)	Same as DART-D except comprising VH/VL of CD137 MAB-1 in place of the VH/VL of CD137 MAB-3
DART-5 (5 Chains)	hHER2 MAB-1 (1.3) CD137 MAB-2	IgG1 (AA) (knob/hole)	Same as DART-D except comprising VH/VL of CD137 MAB-2 in place of the VH/VL of CD137 MAB-3
DART-6 (5 Chains)	variant palivizumab CD137 MAB-3	IgG1 (AA) (knob/hole)	Same as DART-D except comprising VH/VL of variant palivizumab in place of the VH/VL of hHER2 MAB-1 (1.3)
DART-7 (4 Chains)	Humanized EphA2 MAB-3 CD137 MAB-1	IgG1 (AA)	Same as DART-1 except comprising VH/VL of a humanized variant of EphA2 MAB-3 in place of hHER2 MAB-1 (1.2)
DART-8 (4 Chains)	Humanized EphA2 MAB-3 variant palivizumab	IgG1 (AA)	Same as DART-3 except comprising VH/VL of a humanized variant of EphA2 MAB-3 in place of hHER2 MAB-1 (1.2)
TRIDENT-1 (4 Chains)	variant palivizumab CD137 MAB-3 (1B.3)	IgG1 (AA) (knob/hole)	Same as TRIDENT A2 except comprising VH/VL of variant palivizumab in place of the VH/VL of hHER2 MAB-1 (1.3)
TRIDENT-2 (4 Chains)	hHER2 MAB-1 (1.3) 4-4-20 variant palivizumab	IgG1 (AA) (knob/hole)	Same as TRIDENT-A except the CD137 MAB-3 (1.3) VH and VL Domains are replaced with the VH/VL of variant palivizumab, and the VH/VL of 4-4-20
TRIDENT-3 (4 Chains)	5T4 MAB-1 4-4-20 Palivizumab	IgG1 (AA) (knob/hole)	Same as TRIDENT-2 except the hHER2 MAB-1 (1.3) VH and VL Domains are replaced with the VH/VL of 5T4 MAB-1
TRIDENT-4 (4 Chains)	Palivizumab CD137 MAB-3 (1E.15)	IgG1 (AA) (knob/hole)	Same as TRIDENT A5 except comprising VH/VL of variant palivizumab in place of the VH/VL of hHER2 MAB-1 (1.3)

VII. Pharmaceutical Compositions

[00450] 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) which can be used in the preparation of unit dosage forms. Such compositions comprise a **CD137 x TA** Binding Molecule of the present invention, 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 **CD137 x TA** bispecific Fc-bearing diabody of the invention and a pharmaceutically acceptable carrier.

[00451] The invention also encompasses pharmaceutical compositions comprising a **CD137 x TA Binding Molecule** of the invention and one or more additional molecules that are effective in stimulating an immune response (*e.g.*, an immune checkpoint inhibitor) and/or in combination with one or more additional molecules that specifically bind a tumor antigen (*e.g.*, a tumor-specific monoclonal antibody or diabody) that is specific for at least one particular tumor antigen (**TA**, as described above), and a pharmaceutically acceptable carrier.

[00452] 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. Such pharmaceutical carriers can be sterile liquids. Aqueous carriers, such as saline solutions, aqueous dextrose and glycerol solutions are preferred when the pharmaceutical composition is administered intravenously.

[00453] 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.

[00454] The invention also provides a pharmaceutical pack or kit comprising one or more containers containing a **CD137 x TA Binding Molecule** of the present invention alone or with other agents, preferably with a 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.

[00455] A kit can comprise a **CD137 x TA** Binding Molecule of the 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; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more tumor antigens (TAs). In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

VIII. Methods of Administration

[00456] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with cancer or other disease, or disorder by administering to a subject an effective amount of a molecule of the invention, or a pharmaceutical composition comprising a molecule 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.

[00457] Various delivery systems are known and can be used to administer the molecules and compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) "Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System," J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00458] Methods of administering a molecule 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 **CD137 x TA** Binding Molecules of the 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.

[00459] The invention also provides that the **CD137 x TA** Binding Molecules of the invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, the **CD137 x TA** Binding Molecules of the invention 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 **CD137 x TA** Binding Molecules of the invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

[00460] The lyophilized **CD137 x TA** Binding Molecules of the present invention should be stored at between 2 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, **CD137 x TA** Binding Molecules of the invention are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, the liquid form of the **CD137 x TA** Binding Molecules of the invention are supplied in a hermetically sealed container.

[00461] The amount of the composition of the invention which 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.

[00462] As used herein, an “**effective amount**” of a pharmaceutical composition, in one embodiment, 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 disease (*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.

[00463] Such 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 reduce the proliferation of (or the effect of) viral presence and to reduce and/or delay the development of the disease (*e.g.*, cancer) 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.

[00464] For the **CD137 x TA 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. The dosage administered is typically from about 0.01 µg/kg to about 150 mg/kg, or more, of the subject’s body weight.

[00465] The dosage and frequency of administration of the **CD137 x TA Binding Molecules** of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the **CD137 x TA Binding Molecules** by modifications such as, for example, lipidation.

[00466] The dosage of the **CD137 x TA Binding Molecules** of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the **CD137 x TA Binding Molecules** of the invention are used in combination with other therapeutic compositions such that the dosage administered to a patient is lower than when said molecules are used as a single agent therapy.

[00467] Treatment of a subject with a therapeutically or prophylactically effective amount of a **CD137 x TA Binding Molecules** of the invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody one time per week, one time bi-weekly (*i.e.*, once every other week), or one time every three weeks, for between about 1 to 52 weeks. The pharmaceutical compositions of the invention can be administered once a day, twice a day, or three times a day. Alternatively, the pharmaceutical compositions can be administered 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. 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.

IX. Uses of the Compositions of the Invention

[00468] The **CD137 x TA** Binding Molecules of the present invention have the ability to bind T cells (APCs) (for example, by binding to CD137 expressed on the surfaces of such T cells) and the ability to bind **TA**-expressing tumor cells (for example, by binding to a **TA** expressed on the surfaces of such tumor cells). Thus, the **CD137 x TA** Binding Molecules of the present invention have the ability to co-localize T cells to **TA**-expressing tumor cells, and thus may be used to treat any disease or condition associated with or characterized by the expression of a **TA**. Thus, without limitation, pharmaceutical compositions comprising such molecules may be employed in the diagnosis or treatment of cancers that express a **TA**, including such cancers characterized by the presence of a cancer cell, including but not limited to a cancer cell, of an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, 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, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, or a uterine cancer.

[00469] In particular, **CD137 x TA** Binding Molecules of the present invention are useful for the treatment of squamous cell cancers of the head and neck (SCCHN), bladder cancers, breast cancers, colorectal cancers, gastric cancers, glioblastomas, kidney cancers, lung cancers including non-small cell lung cancers (NSCLC), melanomas, ovarian cancers, pancreatic cancers, pharyngeal cancers, prostate cancers, renal cell carcinomas, and small round blue cell tumors of childhood including neuroblastomas and rhabdomyosarcomas, each of which highly express TAs.

[00470] The **CD137 x TA** Binding Molecules of the present invention may additionally be used in the manufacture of medicaments for the treatment of the above-described conditions.

[00471] As demonstrated herein, the **CD137 x TA** Binding Molecules of the present invention enhance the activity of tumor targeting agents. According, the **CD137 x TA** Binding Molecules of the present invention may additionally be used in combination with other a tumor targeting agents, including but not limited to an antibody, an antigen binding fragment of an antibody (*e.g.*, an scFv, a Fab, a F(ab)₂, *etc.*), a TandAb, *etc.*), a multispecific binding molecule (*e.g.*, a diabody, a bispecific antibody, a trivalent binding molecule, *etc.*), capable of binding a desired **TA**. It is specifically contemplated that the tumor targeting agent may bind the same or a different **TA** as the **CD137 x TA** Binding Molecule used in such combinations. In particular embodiments, the tumor targeting agent is a multispecific molecule that binds to a **TA** and to an epitope expressed on T-cells including, for example, **CD3**, and/or **CD8**. Exemplary tumor targeting agents include, but are not limited to, molecules that bind to a **TA** and **CD3** ("**TA x CD3**"). Exemplary **TA x CD3** binding molecules (*e.g.*, bispecific antibodies, DART® molecules, BiTe® molecules, TandAbs, *etc.*), and methods for making the same, which may be used in such combinations are well known in the art. (see for *e.g.*, WO2013026835, WO2013158856, WO2014047231; WO2014110601; WO2014131711; WO 2015/026894; WO2015026892; WO 2015/184203; WO 2016/036937; WO2016182751; WO2017091656; WO2017/142928; WO2017118675).

[00472] As provided herein, the use of **CD137 x TA** Binding Molecules of the present invention in combination with a tumor targeting agent (*e.g.*, a **TA x CD3** binding molecule) can lead to up-regulation of the inhibitory immune modulator Programmed Death-1 ("**PD-1**," also known as "CD279"). PD-1 mediates its inhibition of the immune system by binding **PD-L1** and **PD-L2** (also known as B7-H1 and B7-DC) (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). However, as demonstrated herein, the further addition of an agent that inhibits the inhibitory activity of PD-1 (“**PD-1/PD-L1 Checkpoint Inhibitor**”) down regulates the expression of PD-1 and further enhances the activity of the **CD137 x TA** and tumor targeting agents such as **TA x CD3** Binding Molecules. The invention particularly encompasses PD-1/PD-L1 Checkpoint Inhibitors comprising an epitope-binding site of an antibody that binds PD-1.

[00473] Accordingly, the **CD137 x TA** Binding Molecules of the present invention may additionally be used in combination with other tumor targeting agents, in further combination with a PD-1/PD-L1 checkpoint inhibitor. **PD-1/PD-L1 Checkpoint Inhibitors** include, but not limited to, an antibody, an antigen binding fragment of an antibody (*e.g.*, an scFv, a Fab, a F(ab)₂, *etc.*), a TandAb, *etc.*), a multispecific binding molecule (*e.g.*, a diabody, a bispecific antibody, a trivalent binding molecule, *etc.*), capable of binding to PD-1 and/or PD-L1. Exemplary **PD-1/PD-L1 Checkpoint Inhibitors** and methods for making the same, which may be used in such combinations are well known in the art. (see for *e.g.*, US Patent Nos. 9,617,338; 9,273,135; 9,062,112; 8,981,063; 8,779,108; 8,609,089; 8,552,154; 8,460,927; 8,008,449; US Patent Publication Nos: 2015/0197571; 2016/0075782; 2016/0159905; 2016/0319019; 2017/0044259; PCT Patent Publication Nos. WO 2004/056875; WO 2006/121168; WO 2008/156712; WO 2012/135408; WO 2012/145549; WO 2013/014668; WO 2014/055897; WO 2013/079174; WO 2014/179664; WO 2014/194302; WO 2015/109124; WO 2015/112800; WO 2015/112805; WO 2016/000619; WO 2016/007235; WO 2016/061142; WO 2016/111645; WO 2016/210262; WO 2016/014688; WO 2016/077397; WO 2017/019846; WO 2017/079112; WO 2017/087547; and WO 2017/106656).

[00474] Where such combinations are employed, it is specifically contemplated that, one or more of the molecules may be administered to a subject “concurrently” (*e.g.*, a **CD137 x TA** Binding Molecule may be administered at the same time as a **TA x CD3** binding molecule and/or a **PD-1/PD-L1 Checkpoint Inhibitor** is administered) and/or that one or more of the molecules may be administered “sequentially” (*e.g.*, a **CD137 x TA** Binding Molecule is administered and, at a later time, a **TA x CD3** binding molecule and/or a **PD-1/PD-L1 Checkpoint Inhibitor** is administered, or vice versa).

X. Particular Embodiments of the Invention

[00475] Having now generally described the invention, the same will be more readily understood through reference to the following numbered Embodiments (“E”), which are provided by way of illustration and are not intended to be limiting of the present invention unless specified:

E1. A **CD137 x TA** Binding Molecule, wherein such Binding Molecule is capable of specific binding to an epitope of CD137 and an epitope of a tumor antigen (**TA**), and wherein such **CD137 x TA** Binding Molecule comprises a first Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a first Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; and wherein,

- (A) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL15 (SEQ ID NO:222)**; and
 - (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (B) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL14 (SEQ ID NO:221)**; and
 - (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (C) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL11 (SEQ ID NO:218)**; and
 - (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (D) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL10 (SEQ ID NO:217)**; and
 - (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (E) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL6 (SEQ ID NO:213)**; and

- (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (F)
 - (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL4 (SEQ ID NO:211)**; and
 - (2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (G)
 - (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH (SEQ ID NO:74)**;
- (H)
 - (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-4 VL (SEQ ID NO:91)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-4 VH (SEQ ID NO:90)**;
- (I)
 - (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-5 VL (SEQ ID NO:97)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-5 VH (SEQ ID NO:96)**;
- (J)
 - (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1A (SEQ ID NO:83)**;
- (K)
 - (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;

- (L) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1C (SEQ ID NO:85)**;
 - or
 - (M) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1D (SEQ ID NO:86)**.
- E2.** The **CD137 x TA** Binding Molecule of **E1**, wherein the first Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 (SEQ ID NO:77)**; or
 - (B) **hCD137 MAB-4 (SEQ ID NO:92)**.
- E3.** The **CD137 x TA** Binding Molecule of **E1** or **E2**, wherein the first Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 (SEQ ID NO:82)**; or
 - (B) **hCD137 MAB-4 (SEQ ID NO:93)**.
- E4.** The **CD137 x TA** Binding Molecule of any one of **E1-E3**, wherein the first Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 VH1E (SEQ ID NO:208)**;
 - (B) **hCD137 MAB-3 VH1B (SEQ ID NO:84)**;
 - (C) **hCD137 MAB-3 VH1A (SEQ ID NO:83)**;
 - (D) **hCD137 MAB-3 VH1 (SEQ ID NO:76)**;
 - (E) **hCD137 MAB-3 VH1C (SEQ ID NO:85)**;
 - (F) **hCD137 MAB-3 VH1D (SEQ ID NO:86)**;
 - (G) **hCD137 MAB-3 VH1F (SEQ ID NO:209)**;
 - (H) **hCD137 MAB-3 VH1G (SEQ ID NO:210)**; or
 - (I) **hCD137 MAB-4 VH1 (SEQ ID NO:92)**.
- E5.** The **CD137 x TA** Binding Molecule of any one of **E1-E4**, wherein the first Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 VL15 (SEQ ID NO:222)**;
 - (B) **hCD137 MAB-3 VL14 (SEQ ID NO:221)**;

- (C) hCD137 MAB-3 VL1 (SEQ ID NO:87);
- (D) hCD137 MAB-3 VL2 (SEQ ID NO:88);
- (E) hCD137 MAB-3 VL3 (SEQ ID NO:89);
- (F) hCD137 MAB-3 VL4 (SEQ ID NO:211);
- (G) hCD137 MAB-3 VL5 (SEQ ID NO:212);
- (H) hCD137 MAB-3 VL6 (SEQ ID NO:213);
- (I) hCD137 MAB-3 VL7 (SEQ ID NO:214);
- (J) hCD137 MAB-3 VL8 (SEQ ID NO:215);
- (K) hCD137 MAB-3 VL9 (SEQ ID NO:216);
- (L) hCD137 MAB-3 VL10 (SEQ ID NO:217);
- (M) hCD137 MAB-3 VL11 (SEQ ID NO:218);
- (N) hCD137 MAB-3 VL12 (SEQ ID NO:219);
- (O) hCD137 MAB-3 VL13 (SEQ ID NO:220);
- (P) hCD137 MAB-4 VL1 (SEQ ID NO:94); or
- (Q) hCD137 MAB-4 VL2 (SEQ ID NO:95).

- E6.** The **CD137 x TA** Binding Molecule of any one of **E1-E5**, wherein the tumor antigen (**TA**) is selected from the group of tumor antigens consisting of: 19.9; oncofetal protein 5T4; antigen 4.2; A33; AFP; ALCAM; BAGE; beta-catenin; CA125; Carboxypeptidase M; B1; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD30; CD33; CD36; CD46; CD52; CD79a/CD79b; CD123; CD317; CEA; CEACAM5; CEACAM6; CO-43; CO-514; CTLA-1; CTLA-4; Cytokeratin 8; E1 series; EGF-R; an Ephrin receptor; Erb; F3; FC10.2; a GAGE GD2; GD3; GD49; GM2; GM3; GICA 19-9; gp37; gp75; gp100; HER-2/neu; human B-lymphoma antigen-CD20; human milk fat globule antigen; human papillomavirus-E6/human papillomavirus-E7; HMW-MAA; I antigen; ITGB6; IL13R α 2; JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; KS 1/4; KSA; L6; L20; LEA; LUCA-2; M1:22:25:8; M18; M39; a MAGE; MART; Myl; MUC-1; MUM-1; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; PSA; PSMA; PEMA; PIPA; prostatic acid phosphatase; R24; ROR1; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; TAG-72; TL5; TNF- α receptor; TNF- β receptor; TNF- γ receptor; TRA-1-85; Transferrin Receptor; TSTA; and VEGF-R.
- E7.** The **CD137 x TA** Binding Molecule of any one of **E1-E5**, wherein the tumor antigen (**TA**) is selected from the tumor antigens of **Table 1**.

- E8.** The **CD137 x TA** Binding Molecule of any one of **E1-E7**, wherein the tumor antigen (**TA**) is: HER2/neu, EphA2 or 5T4.
- E9.** The **CD137 x TA** Binding Molecule of **E8**, wherein the tumor antigen (**TA**) is HER2/neu and wherein the **CD137 x TA** Binding Molecule comprises a second Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a first Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; and wherein:
- (A) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **HER2 MAB-1 VL (SEQ ID NO:63)**; and
 - (B) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **HER2 MAB-1 VH (SEQ ID NO:62)**.
- E10.** The **CD137 x TA** Binding Molecule of **E9**, wherein:
- (A)
 - (1) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
 - (2) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL2 (SEQ ID NO:68)**;
 - or
 - (3) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL3 (SEQ ID NO:69)**;
 - and
 - (B)
 - (1) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
 - (2) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH2 (SEQ ID NO:65)**;
 - or
 - (3) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.
- E11.** The **CD137 x TA** Binding Molecule of **E10**, wherein the second Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
 - (B) **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
 - (C) **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

- E12.** The **CD137 x TA** Binding Molecule of **E10** or **E11**, wherein the second Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
 - (B) **hHER2 MAB-1 VL2 (SEQ ID NO:68)**; or
 - (C) **hHER2 MAB-1 VL3 (SEQ ID NO:69)**.
- E13.** The **CD137 x TA** Binding Molecule of **E8**, wherein the tumor antigen (**TA**) is **5T4** and wherein the **CD137 x TA** Binding Molecule comprises a second Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a first Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; and wherein:
- (I) (A) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **5T4 MAB-1 VL (SEQ ID NO:135)**; and
 - (B) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **5T4 MAB-1 VH (SEQ ID NO:134)**; or
 - (II) (A) the second Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **5T4 MAB-2 VL (SEQ ID NO:137)**; and
 - (B) the second Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **5T4 MAB-2 VH (SEQ ID NO:136)**.
- E14.** The **CD137 x TA** Binding Molecule of **E13**, wherein the second Heavy Chain Variable Domain comprises the amino acid sequence of: **MAB-1 VH1 (SEQ ID NO:135)**.
- E15.** The **CD137 x TA** Binding Molecule of **E13** or **E14**, wherein the second Light Chain Variable Domain comprises the amino acid sequence of: **MAB-1 VH1 (SEQ ID NO:136)**.
- E16.** The **CD137 x TA** Binding Molecule of any one of **E1-E15**, wherein the molecule is a bispecific tetravalent Fc-bearing diabody comprising a first, a second, a third, and a fourth polypeptide chain, wherein the polypeptide chains form a covalently bonded complex.
- E17.** The **CD137 x TA** Binding Molecule of **E16**, wherein the tumor antigen (**TA**) is **HER2/neu** and wherein:
- (I) (A) the first and the third polypeptide chain have the amino acid sequence of **SEQ ID NO:100**; and
 - (B) the second and the fourth polypeptide chain have the amino acid sequence of **SEQ ID NO:101**;

or

- (II) (A) the first and the third polypeptide chain have the amino acid sequence of **SEQ ID NO:102**; and
- (B) the second and the fourth polypeptide chain have the amino acid sequence of **SEQ ID NO:103**.

E18. The **CD137 x TA** Binding Molecule of any one of **E1-E15**, wherein the molecule is bispecific and tetravalent, and comprises a first, a second, a third, a fourth, and a fifth polypeptide chain, wherein the polypeptide chains form a covalently bonded complex.

E19. The **CD137 x TA** Binding Molecule of **E15**, wherein the tumor antigen (**TA**) is HER2/neu and wherein:

- (I) (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:104**;
- (B) the second and the fifth polypeptide chain have the amino acid sequence of **SEQ ID NO:105**;
- (C) the third polypeptide chain has the amino acid sequence of **SEQ ID NO:106**; and
- (D) the fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:107**;

or

- (II) (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:104**;
- (B) the second and the fifth polypeptide chain have the amino acid sequence of **SEQ ID NO:105**;
- (C) the third polypeptide chain has the amino acid sequence of **SEQ ID NO:114**, **SEQ ID NO:115**, **SEQ ID NO:116**, **SEQ ID NO:117**, or **SEQ ID NO:118**; and
- (D) the fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:119**, **SEQ ID NO:120**, **SEQ ID NO:121**, **SEQ ID NO:122**, or **SEQ ID NO:123**.

E20. The **CD137 x TA** Binding Molecule of any one of **E1-15**, wherein said molecule is bispecific and trivalent, and comprises a first, a second, a third, and a fourth, polypeptide chain, wherein said polypeptide chains form a covalently bonded complex.

- E21.** The **CD137 x TA** Binding Molecule of **E20**, wherein said tumor antigen (**TA**) is HER2/neu and wherein:
- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, or SEQ ID NO:229**;
 - (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, or SEQ ID NO:230**;
 - (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:104**; and
 - (D) said fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:105**.
- E23.** The **CD137 x TA** Binding Molecule of **E20**, wherein said tumor antigen (**TA**) is 5T4 and wherein:
- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, or SEQ ID NO:229**;
 - (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:197, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:200, SEQ ID NO:201, or SEQ ID NO:230**;
 - (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:231**; and
 - (D) said fourth polypeptide chain has the amino acid sequence of **SEQ ID NO:232**.
- E24.** A pharmaceutical composition comprising the **CD137 x TA** Binding Molecule of and one **E1-E23**, and a physiologically acceptable carrier.
- E25.** Use of the **CD137 x TA** Binding Molecule of any one of **E1-E23**, or the pharmaceutical composition of **E24**, in the treatment of a disease or condition associated with or characterized by the expression of the tumor antigen (**TA**).
- E26.** The use of **E25**, wherein the disease or condition associated with or characterized by the expression of the tumor antigen (**TA**) is cancer.

E27. A CD137 binding molecule that comprises a Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; wherein:

- (A) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL15 (SEQ ID NO:222)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (B) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL14 (SEQ ID NO:221)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (C) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL11 (SEQ ID NO:218)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (D) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL10 (SEQ ID NO:217)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (E) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL6 (SEQ ID NO:213)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (F) (1) said first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL4 (SEQ ID NO:211)**; and
(2) said first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
- (G) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
(2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH (SEQ ID NO:74)**;

- (H) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-4 VL (SEQ ID NO:91)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-4 VH (SEQ ID NO:90)**;
 - (I) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-5 VL (SEQ ID NO:97)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-5 VH (SEQ ID NO:96)**;
 - (J) (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1A (SEQ ID NO:83)**;
 - (K) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1B (SEQ ID NO:84)**;
 - (L) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1C (SEQ ID NO:85)**;
 or
 - (M) (1) the first Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **CD137 MAB-3 VL (SEQ ID NO:75)**; and
 - (2) the first Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **CD137 MAB-3 VH1D (SEQ ID NO:86)**.
- E28.** The CD137 Binding Molecule of **E27**, wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 (SEQ ID NO:77)**; or
 - (B) **hCD137 MAB-4 (SEQ ID NO:92)**.
- E29.** The CD137 Binding Molecule of **E27** or **E28**, wherein the Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 (SEQ ID NO:82)**; or
 - (B) **hCD137 MAB-4 (SEQ ID NO:93)**.

- E30.** The CD137 Binding Molecule of any one of **E27-E29**, wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 VH1 (SEQ ID NO:76);**
 - (B) **hCD137 MAB-3 VH1A (SEQ ID NO:83);**
 - (C) **hCD137 MAB-3 VH1B (SEQ ID NO:84);**
 - (D) **hCD137 MAB-3 VH1C (SEQ ID NO:85);**
 - (E) **hCD137 MAB-3 VH1D (SEQ ID NO:86);**
 - (F) **hCD137 MAB-3 VH1E (SEQ ID NO:208);**
 - (G) **hCD137 MAB-3 VH1F (SEQ ID NO:209);**
 - (H) **hCD137 MAB-3 VH1G (SEQ ID NO:210);** or
 - (I) **hCD137 MAB-4 VH1 (SEQ ID NO:92).**
- E31.** The CD137 Binding Molecule of any one of **E27-E30**, wherein the Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hCD137 MAB-3 VL15 (SEQ ID NO:222);**
 - (B) **hCD137 MAB-3 VL14 (SEQ ID NO:221);**
 - (C) **hCD137 MAB-3 VL1 (SEQ ID NO:87);**
 - (D) **hCD137 MAB-3 VL2 (SEQ ID NO:88);**
 - (E) **hCD137 MAB-3 VL3 (SEQ ID NO:89);**
 - (F) **hCD137 MAB-3 VL4 (SEQ ID NO:211);**
 - (G) **hCD137 MAB-3 VL5 (SEQ ID NO:212);**
 - (H) **hCD137 MAB-3 VL6 (SEQ ID NO:213);**
 - (I) **hCD137 MAB-3 VL7 (SEQ ID NO:214);**
 - (J) **hCD137 MAB-3 VL8 (SEQ ID NO:215);**
 - (K) **hCD137 MAB-3 VL9 (SEQ ID NO:216);**
 - (L) **hCD137 MAB-3 VL10 (SEQ ID NO:217);**
 - (M) **hCD137 MAB-3 VL11 (SEQ ID NO:218);**
 - (N) **hCD137 MAB-3 VL12 (SEQ ID NO:219);**
 - (O) **hCD137 MAB-3 VL13 (SEQ ID NO:220);**
 - (P) **hCD137 MAB-4 VL1 (SEQ ID NO:94);** or
 - (Q) **hCD137 MAB-4 VL2 (SEQ ID NO:95).**
- E32.** The CD137 Binding Molecule of and one **E27-31**, wherein the molecule is an antibody or an antigen binding fragment thereof.
- E33.** A pharmaceutical composition comprising the CD137 Binding Molecule of any one **E27-E32**, and a physiologically acceptable carrier.

- E34.** Use of the CD137 Binding Molecule of any one of **E27-E31**, or the pharmaceutical composition of **E33**, in the treatment of a disease or condition associated with a suppressed immune system or characterized by the expression of a tumor antigen (**TA**).
- E35.** The use of **E34**, wherein the condition associated with a suppressed immune system or characterized by the expression of the tumor antigen (**TA**) is cancer.
- E36.** An anti-HER2/neu Binding Molecule that comprises a Light Chain Variable Domain that comprises a CDR_{L1}, CDR_{L2} and CDR_{L3}, and a Heavy Chain Variable Domain that comprises a CDR_{H1}, CDR_{H2} and CDR_{H3}; wherein:
- (A) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **HER2 MAB-1 VL (SEQ ID NO:63)**; and
 - (B) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **HER2 MAB-1 VH (SEQ ID NO:62)**.
- E37.** The anti-HER2/neu Binding Molecule of **E36**, wherein:
- (A)
 - (1) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL1 (SEQ ID NO:67)**;
 - (2) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL2 (SEQ ID NO:68)**; or
 - (3) the Light Chain Variable Domain CDR_{L1}, CDR_{L2}, and CDR_{L3} are the Light Chain CDRs of **hHER2 MAB-1 VL3 (SEQ ID NO:69)**;
- and
- (B)
 - (1) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
 - (2) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
 - (3) the Heavy Chain Variable Domain CDR_{H1}, CDR_{H2}, and CDR_{H3} are the Heavy Chain CDRs of **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.
- E38.** The anti-HER2/neu Binding Molecule of **E337**, wherein the Heavy Chain Variable Domain comprises the amino acid sequence of:
- (A) **hHER2 MAB-1 VH1 (SEQ ID NO:64)**;
 - (B) **hHER2 MAB-1 VH2 (SEQ ID NO:65)**; or
 - (C) **hHER2 MAB-1 VH3 (SEQ ID NO:66)**.

- E39.** The anti-HER2/neu Binding Molecule of **E37-E38**, wherein the Light Chain Variable Domain comprises the amino acid sequence of:
- (A) **hHER2 MAB-1 VL1 (SEQ ID NO:67);**
 - (B) **hHER2 MAB-1 VL2 (SEQ ID NO:68);** or
 - (C) **hHER2 MAB-1 VL3 (SEQ ID NO:69).**
- E40.** The anti-HER2/neu Binding Molecule of and one **E36-E39**, wherein the molecule is an antibody or an antigen binding fragment thereof.
- E41.** A pharmaceutical composition comprising the anti-HER2/neu Binding Molecule of any one **E36-E40** and a physiologically acceptable carrier.
- E42.** Use of the anti-HER2/neu Binding Molecule of any one of **E33-E37**, or the pharmaceutical composition of **E41**, in the treatment of a disease or condition associated with or characterized by the expression of HER2/neu.
- E43.** The use of **E42**, wherein the condition associated with or characterized by the expression of HER2/neu is cancer.
- E44.** A method of enhancing the activity of a tumor targeting agent comprising administering said tumor targeting agent in combination with the **CD137 x TA** Binding Molecule of any one of **E1-E23**, or the pharmaceutical composition of embodiment **E24**.
- E45.** A method of treating a disease or condition associated with a suppressed immune system or characterized by the expression of a tumor antigen (**TA**) comprising administering to a subject in need thereof the **CD137 x TA** Binding Molecule of any one of **E1-E23**, or the pharmaceutical composition of **E24**.
- E46.** The method of **E45**, wherein the condition associated with a suppressed immune system or characterized by the expression of the tumor antigen (**TA**) is cancer.
- E47.** The method of **E45** or **E46**, further comprising administering a tumor targeting agent.
- E48.** The method of any one of **E44-E47**, further comprising administering a PD-1/PD-L1 checkpoint inhibitor.
- E49.** The method of **E44** or **E47**, wherein said tumor targeting agent is an antibody, an epitope binding fragment of an antibody, or an agent that mediates T-cell redirected killing of a target cell.
- E50.** The method of **E48** or **E49**, wherein said PD-1/PD-L1 checkpoint inhibitor is an anti-PD-1 antibody or an anti-PD-L1 antibody.

E51. The method of any one of **E48-E50**, wherein said PD-1/PD-L1 checkpoint inhibitor comprises the VH and VL Domains of an antibody selected from those listed in **Table 7**:

Table 7	
PD-1 and PD-L1 Antibodies	Reference / Source
nivolumab	CAS Reg. No.:946414-94-4; WHO Drug Information, 2013, Recommended INN: List 69, 27(1):68-69
pembrolizumab	CAS Reg. No.:1374853-91-4; WHO Drug Information, 2014, Recommended INN: List 75, 28(3):407
PD1-17; PD1-28; PD1-33; PD1-35; and PD1-F2	US Patent Nos. 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 Patent Nos. 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 Patent Nos. 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
mAb1; mAb2; mAb3; mAb4; mAb7; mAb8; mAb9; mAb10; mAb11; mAb12; mAb13; mAb14; mAb15; and mAb16	US Patent Publication 2016/0159905

Table 7	
PD-1 and PD-L1 Antibodies	Reference / Source
246A10; 244C8; 413D2; 393C5; 388D4; 413E1; 244C8-1; 244C8-2; 244C8-3; 388D4-1; 388D4-2; and 388D4-3	US Patent Publication 2016/0319019
22A5; 6E1; IOD1, 4C1; 7D3; 13F1; 14A6; 15H5; 5A8; 7A4; and humanized versions of the same	PCT Publication No. WO 2016/014688
1E9; h1E9-1; h1E9-2; h1E9-4; h1E9-5; 4B10; h4B10-1; h4B10-2; h4B10-3; 1B10; 10B4; A09; C07; F09; G08; G10; H08; and H09	PCT Publication No. WO 2016/077397
M136-M13-MHC723; m136-M14-MHC724; m136-M19-MHC725; m245-M3-MHC728; m245-M5-MHC729; A1.0; A1.6; Ba2; Bb2/C1.1; and D4	US Patent Publication 2017/0044259
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; hPD-1 mAb 15; and humanized versions of the same; particularly anti-PD-1 mAb (hPD-1 mAb 7(1.2) IgG4 (P))	PCT Publication No. WO 2017/019846 (particularly SEQ ID NOs: 264 and 266)
atezolizumab	CAS Reg No. 1380723-44-3; (WHO Drug Information, 2015, Recommended INN: List 74, 29(3):387
durvalumab	CAS Reg No. 1428935-60-7; WHO Drug Information, 2015, Recommended INN: List 74, 29(3):393-394
avelumab, MDX1105	CAS Reg No. 1537032-82-8; WHO Drug Information, 2016, Recommended INN: List 74, 30(1):100-101
A09-188-1, and affinity matured and optimized variants: A09-204-1, A09-211-1, A09-212-1, A09-213-1, A09-214-1, A09-215-1, A09-216-1 A09-219-1, A09-220-1, A09-221-1, A09-222-1 A09-223-1, A09-202-1, A09-248-2, A09-239-2, A09-240-2, A09-241-2, A09-242-2, A09-243-2, A09-244-2, A09-245-2, A09-246-2, and A09-247-2	PCT Patent Publication WO 2013/079174
1B9.2E11.2, 4H1.G10.15, 1A8, 1E4, 8G2, 1D11, 3A2, 3B11, 3F4, 3H6, 4C1, 4E1, 5A6, 9C12, 1B4, 1B11, 1F6, 1H8, 1H12, 2D5, 2H11, 3D12, 4C8, 4C9, 5E10, 5H4, 5H5, 8A1, 9G9, 10A7, and 10H6	US Patent Publication US2015/0197571
1D05, 84G09, 411B08, 411C04, 411D07, 386H03, 386A03, 385F01, 413D08, 413G05, 413F09, 414B06	US Patent 9,617,338
3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4	US Publication 2016/0075782

Table 7	
PD-1 and PD-L1 Antibodies	Reference / Source
A1, C2, C4, H12, and H12-GL	US Publication 2017/0319690; PCT Patent Publication WO 2016/111645
Ab- 14, Ab-16, Ab-22, Ab-30, Ab-31, Ab-32, Ab-38, Ab-42, Ab-46, Ab-50, Ab-52, Ab-55, Ab-56, and Ab- 65.	PCT Patent Publication WO 2014/055897
R2κA3, R2κA4, R2κA6, R2κF4, R2κH5, R2κH6, R2κH3, sR3κA8, sR3κA9, sR3κB2, sR3κB5, tccR3KA8, tccR3KA11, tccR3KB7, tccR3KD9, tccKF10, tctR3KA4, tctR3KF8, R2λA7, R2λB12, R2λ12, sR3λD7, sR3λE1, tccAF8, tccAD7, tctR3λH4, and others	PCT Patent Publication WO 2015/109124
H2M8306N, H2M8307N, H2M8309N, H2M8310N, H2M8312N, H2M8314N, H2M8316N, H2M8317N, H2M8321N, H2M8323N, H2M8718N, H2M8718N2, and H2M8719N, H1H9323P, H1 H9327P, H1 H9329P, H1H9336P, H1H9344P2, 1H9345P2, H1H9351P2, H1H9354P2, H1 H9364P2, H1H9373P2, H1H9382P2, H1H9387P2, and H1H9396P2	PCT Patent Publication WO 2015/112805
Mu333, Mu277, and humanized variants thereof including: hu333-2B, hu333-3A2, hu333-3C2 and hu333-3H2	PCT Patent Publication WO 2016/000619
BAP058 and humanized variants thereof including: BAP058-hum01, BAP058-hum02, BAP058-hum03, BAP058-hum04, BAP058-hum05, BAP058-hum06, BAP058-hum07, BAP058-hum08, BAP058-hum09, BAP058-hum10, BAP058-hum11, BAP058-hum12, BAP058-hum13, BAP058-hum14, BAP058-hum15, BAP058-hum16, and BAP058-hum17	PCT Patent Publication WO 2016/061142
332M1 and humanized variants there of including: 332M7, 332M72, and 332M8	PCT Patent Publication WO 2017/087547

E52. The use of **E26**, **E35**, **E42** or the method of any one of **E46-E51**, wherein the cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, 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, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile

duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

- E53.** The use of **E26**, **E35**, **E42** or the method of any one of **E46-E51**, wherein the cancer is selected from the group consisting: bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, lung cancer, melanoma, neuroblastoma, ovarian cancer, pancreatic cancer, pharyngeal cancer, prostate cancer, renal cell carcinoma, rhabdomyosarcoma, and squamous cell cancer of the head and neck (SCCHN).

EXAMPLES

[00476] The following examples illustrate various methods for compositions in the diagnostic or treatment methods of the invention. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Example 1 The Humanization of HER2 MAB-1

[00477] PCT Publication WO 2001/036005 discloses an anti-HER2/neu antibody, designated therein as "8H11," however, the sequences of the VL and VH Domains provided therein are inaccurate. The 8H11 antibody binds a HER2/neu epitope that is distinct from the epitope bound by trastuzumab, margetuximab, and pertuzumab.

[00478] The correct sequences for the VH and VL Domains of anti-HER2/neu antibody 8H11 were deduced (to thereby provide **HER2-MAB-1 VH (SEQ ID NO:60)** and **HER2-**

MAB-1 VL (SEQ ID NO:61)), and multiple rounds of humanization were then undertaken in order to obtain the above-described humanized VH Domain “**hHER2 MAB-1 VH1**” (SEQ ID NO:64) and humanized VL Domain “**hHER2 MAB-1 VL1**” (SEQ ID NO:67). In the course of such efforts, several potential sequence liabilities were identified in the **hHER2 MAB-1 VH1** and **hHER2 MAB-1 VL1** Domains, and substitutions were made that removed these liabilities while maintaining binding affinity. Specifically:

- (1) an aspartic acid isomerization site was identified at Kabat position 96 of the **hHER2 MAB-1 VH1** Domain, and was corrected either by the substitution D96E or by the substitution G97I, as in **hHER2 MAB-1 VH2 (SEQ ID NO:65)** and **hHER2 MAB-1 VH3 (SEQ ID NO:66)**, respectively;
- (2) a potential asparagine deamidation site was identified at Kabat position 30 of the **hHER2 MAB-1 VL1** Domain and was corrected either by the substitution N30S or by the substitution S31T, as in **hHER2 MAB-1 VL3 (SEQ ID NO:69)** and **hHER2 MAB-1 VL2 (SEQ ID NO:68)**, respectively;
- (3) potential aspartic acid isomerization site was identified at Kabat position D56 of the **hHER2 MAB-1 VL1** Domain and was corrected by a substitution: V55Q, D56E, or D56S, as in **hHER2 MAB-1 VL3 (SEQ ID NO:69)**, and **hHER2 MAB-1 VL2 (SEQ ID NO:68)**.

[00479] The humanized heavy and light chain variable domains of **hHER2 MAB-1** may be used in any combination and particular combinations of humanized chains are referred to by reference to the specific VH/VL Domains. For example, a humanized antibody comprising **hHER2 MAB-1 VH1** and **hHER2 MAB-1 VL3** is referred to herein as “**hHER2 MAB-1 (1.3)**.”

[00480] The humanized variants were selected to have improved binding affinity as compared to the parental murine antibody and as described may be further engineered to eliminate sequence liabilities (e.g., isomerization, deamidation sites) as described above. The binding affinity of several combinations of humanized VH and VL domains was examined by BIACORE®. Thus, an extracellular portion of human HER2 fused to a histidine-containing peptide (“**huHER2**”) was passed over a surface coated with immobilized antibody. Briefly, each test molecule was captured onto an F(ab)₂ goat-anti-human Fc-coated surface and then incubated in the presence of different concentrations (6.25 nM - 50 nM) of huHER2. The kinetics of binding were determined via BIACORE® analysis binding (normalized 1:1

Langmuir binding model). The calculated k_a , k_d and K_D from these studies are presented in **Table 8**.

Table 8					
Antibody	VH	VL	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
HER2 MAB-1	murine	murine	5.1×10^5	6.54×10^{-4}	1.27
hHER2 MAB-1 (1.2)	1	2	6.2×10^5	2.80×10^{-4}	0.45
hHER2 MAB-1 (1.3)	1	3	6.18×10^5	2.23×10^{-4}	0.36

[00481] In a separate study, the binding kinetics of the **CD137 x TA** bispecific diabody, **DART-B** (described above), were determined for binding to human HER2 and cynomolgus monkey HER2, essentially as described above. The calculated k_a , k_d and K_D from these studies are presented in **Table 9**.

Table 9			
HER2/neu	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
Human	4.8×10^5	1.82×10^{-4}	0.38
Cynomolgus Monkey	4.44×10^5	2.94×10^{-4}	0.66

Example 2

The Isolation and Characterization of Murine Anti-CD137 mAbs

[00482] A panel of murine monoclonal antibodies specific for human CD137 were screened for antibodies exhibiting high affinity binding. Three antibodies were selected for further study. For such evaluations, purified pan T-cells (see above) were stimulated with anti-CD3/CD28 beads (cells : beads=1:1) for 72 hours in the presence of IL-2 (100 U/mL). 100 μ L of activated T cells (1.0×10^6 cells/mL) and 100 μ L of serially diluted (5- or 10-fold dilutions) test article (antibody or bispecific diabody) was added to each well of microtiter assay plate(s), mixed and incubated at RT for 45 min. The cells were washed with FACS Buffer and secondary antibody (anti-human-Fc region-APC) was then added to each well; mixed and incubated at RT for 30 min. Cells were then washed FACS Buffer and T cell surface marker antibodies (anti-CD4 labeled with V510, and anti-CD8 labeled with FITC) were then added to each well; mixed and incubated at RT for 30 min. Cells were washed and resuspended in 100 μ L FACS Buffer and analyzed by flow cytometry (BD LSR Fortessa or FACSCanto II) for cell event collection. Data analysis were performed via FloJo v10.

[00483] Two of such selected antibodies, **CD137 MAB-3** and **CD137 MAB-4**, were found to exhibit strong binding to human CD137 and were able to block binding between CD137 and its natural ligands. The third of such selected antibodies, **CD137 MAB-5**, exhibited lower

binding to human CD137 and was not capable of blocking binding between CD137 and its natural ligands.

[00484] Chimeric antibodies having human IgG1 Fc regions and murine VH/VL domains were generated (designated: **chCD137 MAB-1**, **chCD137 MAB-2**, **chCD137 MAB-3**, **chCD137 MAB-4** and **chCD137 MAB-5**, respectively), and were tested for their ability to bind to activated CD8⁺ T cells. As shown in **Figure 7**, the selected antibodies exhibited a range of binding affinities.

[00485] Epitope binning was performed by cross-competition studies. The results of these and ligand binding competition studies indicate that:

- (1) **CD137 MAB-2**, **CD137 MAB-3** and **CD137 MAB-4** compete with CD137L for binding to CD137, but that **CD137 MAB-2** recognizes an epitope of CD137 that is distinct from the epitope recognized by **CD137 MAB-1**, **CD137 MAB-3**, **CD137 MAB-4** and **CD137 MAB-5**;
- (2) **CD137 MAB-1** and **CD137 MAB-5** do not compete with CD137L for binding to CD137;
- (3) **CD137 MAB-3** and **CD137 MAB-4** exhibit competition with **CD137 MAB-1**, but bind a distinct epitope as demonstrated by their ability to compete for ligand binding;
- (4) **CD137 MAB-5** recognizes an epitope that is distinct from the epitopes recognized by **CD137 MAB-1**, **CD137 MAB-2**, **CD137 MAB-3** or **CD137 MAB-4**.

[00486] The chimeric antibodies were also tested for their ability to induce cytokine (*e.g.*, IFN- γ , TNF- α) release from T cells (*i.e.*, their agonist activity) in the absence of ligand. For such evaluations, purified pan T cells (see above) were resuspended in assay media and placed in a tissue culture incubator overnight. Tumor target cells (including JIMT-1, N87) were obtained from culture. After washing, the cell pellets were resuspended in culture media at cell density of 1.0×10^5 cells/mL. 10^4 cells were then added to each well of white flat-bottom assay plate placed in a tissue culture incubator overnight. The next day, rested human pan T cells were measured for density and viability by trypan blue exclusion using a Beckman Coulter Vi-Cell counter and adjusted to a density of 2×10^6 cells/mL. Tumor target cells pre-seeded on plate were washed once with assay media.

[00487] For the cytokine release assays: 50 μ L of serially diluted test article (antibodies (+/- cross-linking with anti-human Fc (Fab) $'_2$), diabodies, trivalent molecules, *etc.*), 50 μ L of

prewashed Dynabead α CD3 (REF 11151D; Invitrogen by Thermo Fisher Scientific) at 2×10^6 beads/mL, 50 μ L/well of human pan T cells at 2×10^6 cells/mL, and 50 μ L of assay media alone or containing 1.6 μ g/mL α CD28 (Cat: 555725; BD Pharmingen) were added to each well of the assay plate. The final volume of each well on the plate was 200 μ L. For those control wells that did not contain test article or α CD3 beads, assay media was added to bring up the total volume to 200 μ L and the plates were incubated for 72 hours in a tissue culture incubator. The supernatants were then collected from each well and the released cytokines of IFN- γ , TNF- α and IL-2R were measured using a Cytokine ELISA Kit from R&D System (Human IL-2 DuoSet ELISA (Cat: DY202), Human IFN-gamma DuoSet ELISA (Cat: DY285) and Human TNF-alpha DuoSet ELISA (Cat: DY210) or similar commercial reagents. The ELISA methods were provided with the kits. Microsoft Excel and SoftMax Pro were used for data analysis to extrapolate cytokine levels, which were plotted with Prism.

[00488] As shown in **Figure 8**, **CD137 MAB-1**, **chCD137 MAB-3** and **chCD137 MAB-4** each exhibited strong agonist activity, **CD137 MAB-2** exhibited less agonist activity and **CD137 MAB-5** exhibited no agonist activity in the absence of ligand.

[00489] Chimeric antibodies **chCD137 MAB-3** and **chCD137 MAB-4** were also tested for their ability to induce cytokine (*e.g.*, IFN- γ , TNF- α) release from pan T cells in the absence and presence of ligand and/or target cells. Human pan T cells were purified from donor PBMC using Dynabeads Untouched Human T Cells Kit (Invitrogen Cat# 11344D) per manufacture's protocol. The results for IFN- γ release are on shown in **Figure 9**. **Figure 9** shows the induction of IFN- γ by pan T cells 72 hours after stimulation with CD3 coated beads in the presence or absence of 1 μ g/mL chimeric anti-CD137 antibodies (**chCD137 MAB-3** or **chCD137 MAB-5**), and in the presence or absence of 1 μ g/mL CD-137L-His and 4 μ g/mL hFc F(ab)'₂. The figure shows that IFN- γ was induced in the presence or absence of CD137-His, and that greater induction was observed with JIMT-1/pan T cells relative to that observed with pan T cells alone. Similar results were seen for TNF- α .

[00490] In the presence of both T-cells and Target cells, or T-cells alone, **chCD137 MAB-3** exhibited strong agonist activity which was similar in the presence or absence of ligand. In contrast, **chCD137 MAB-5** exhibited minimal agonist activity in the absence of ligand and a greatly enhanced agonist activity in the presence of ligand.

Example 3 The Humanization of Murine Anti-CD137 mAbs

[00491] Antibodies **CD137 MAB-3** and **CD137 MAB-4** were selected for humanization. Four rounds of humanization were undertaken for **CD137 MAB-3**, which yielded one humanized VH domain, designated as “**hCD137 MAB-3 VH1**” (SEQ ID NO:76), and three humanized VL domains, designated as “**hCD137 MAB-3 VL1**” (SEQ ID NO:87), “**hCD137 MAB-3 VL2**” (SEQ ID NO:88) and “**hCD137 MAB-3 VL3**” (SEQ ID NO:89).

[00492] Three rounds of humanization were undertaken for **CD137 MAB-4**, which yielded one humanized VH domain, designated as “**hCD137 MAB-4 VH1**” (SEQ ID NO:92), and two humanized VL domains, designated as “**hCD137 MAB-4 VL1**” (SEQ ID NO:94) and “**hCD137 MAB-4 VL2**” (SEQ ID NO:95).

[00493] The humanized heavy and light chain variable domains of a particular anti-CD137 antibody (*e.g.*, **CD137 MAB-3**) may be used in any combination and particular combinations of humanized chains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising **hCD137 MAB-3 VH1** and **hCD137 MAB-3 VL3** is referred to herein as “**hCD137 MAB-3 (1.3)**.”

[00494] The binding affinities of the chimeric antibodies having the murine variable domains and fully humanized antibodies were examined by Biacore.

[00495] Thus, an extracellular portion of human CD137 fused to a histidine-containing peptide (“**huCD137**”) was passed over a surface coated with immobilized antibody. Briefly, each test molecule was captured onto an F(ab)₂ goat-anti-human Fc-coated surface and then incubated in the presence of different concentrations (25 nM and 100 nM) of huCD137. The kinetics of binding were determined via BIACORE® analysis binding (normalized 1:1 Langmuir binding model). The calculated k_a , k_d and K_D from these studies are presented in **Table 10** and show that the humanized mAbs exhibited about a 2-fold decrease in binding affinity relative to the murine antibodies.

Table 10					
Antibody	VH	VL	k_a (M⁻¹s⁻¹)	k_d (s⁻¹)	K_D (nM)
chCD137 MAB-3	murine	murine	8.9×10^5	0.020	23
chCD137 MAB-3 (1.1)	1	1	6.7×10^5	0.031	46
chCD137 MAB-3 (1.2)	1	2	6.1×10^5	0.030	49
chCD137 MAB-3 (1.3)	1	3	6.6×10^5	0.033	50

Table 10					
Antibody	VH	VL	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
chCD137 MAB-4	murine	murine	1.5×10^5	9.2×10^{-4}	6.1
chCD137 MAB-4 (1.1)	1	1	1.3×10^5	2.1×10^{-3}	16.2
chCD137 MAB-4 (1.2)	1	2	1.4×10^5	1.9×10^{-3}	13.6

Example 4
Characterization of CD137 x TA Binding Molecules

[00496] As described above a number of exemplary **CD137 x TA** Binding Molecules capable of binding to CD137 and to the exemplary **TA**, HER2/neu, were generated. In particular, six bispecific diabodies (designated “**DART-A**,” “**DART-B**,” and “**DART-C**,” “**DART-D**,” “**DART-E**,” and “**DART-G**”) comprising **CD137 MAB-3** or **hCD137 MAB-3** domains, one bispecific diabody (designated “**DART-F**”) comprising **CD137 MAB-4** domains, two bispecific diabodies (designated “**DART-1**,” and “**DART-4**”) comprising **CD137 MAB-1** domains, and two bispecific diabodies (designated “**DART-2**,” and “**DART-5**”) comprising **CD137 MAB-2** domains, were generated, each comprising **hHER2 MAB-1** domains. **DART-A**, **DART-B**, **DART-C**, **DART-1**, and **DART-2** have the general structure shown in **Figure 3B** (**DART-A**, **DART-B**, **DART-1** and **DART-2** have the structure shown in **Figure 3E**; **DART-C** has the structure shown in **Figure 3D**). **DART-D**, **DART-E**, **DART-F**, **DART-G**, **DART-4**, and **DART-5** have the general structure shown in **Figure 5A** (**DART-D**, **DART-F**, **DART-G**, **DART-4**, and **DART-5** have the structure shown in **Figure 5B**; **DART-E** has the structure shown in **Figure 5C**).

[00497] Another exemplary **CD137 x TA** Binding Molecule capable of binding to CD137 and to the exemplary **TA**, EphA2 (designated “**DART-7**”) was generated. **DART-7** is a bispecific diabody having the structure presented in **Figure 3B**, comprising **CD137 MAB-1** humanized **EphA2 MAB-3** domains.

[00498] In addition, three control molecules, “**DART-3**,” comprising **hHER2 MAB-1** and variant palivizumab domains (**HER2 x RSV**), “**DART-6**,” comprising variant palivizumab and **CD137 MAB-3** domains (**RSV x CD137**), and “**DART-8**,” comprising humanized **EphA2 MAB-3** and variant palivizumab domains (**EphA2 x RSV**), were generated.

[00499] **CD137 x TA** Binding Molecules having two binding sites for CD137 and 2 binding sites for HER2/neu, and the **CD137 x RSV** control molecule (**DART-6**) bound to CD137 present on the surface of activated T cells. In contrast, the **HER2/neu x RSV** control molecule

(**DART-3**) did not exhibit any binding. **DART-1** exhibited the highest binding and **DART-5** exhibited the lowest binding in this assay, with the remaining molecules tested (**DART-B**, **DART-C**, **DART-D**, **DART-E**, **DART-F**, **DART-2**, **DART-4** and **DART-6**) exhibiting comparable binding (**Figure 10**). The relative positions of the HER2/neu and CD137 binding domains had minimal to no impact on CD137 binding in this assay for molecules having the structure presented in **Figure 3B** (compare **DART-B/DART-C**) or the structure presented in **Figures 5B-5C** (compare **DART-D/DART-E**).

[00500] Similarly, the **CD137 x TA Binding Molecules** were evaluated for their ability to bind to HER2/neu present on the surface of target N87 gastric cancer cells (**Figures 11A-11B**). The **RSV x CD137** control molecule (**DART-6**) did not exhibit any binding. The relative positions of the HER2/neu and CD137 binding domains had minimal to no impact on HER2/neu binding in this assay for molecules having the structure presented in **Figure 3B** (compare **DART-B/DART-C**). In contrast, **DART-E** exhibited reduced HER2/neu binding as compared to **DART-D**, suggesting that there may be a positional effect on HER2/neu binding for molecules having the structure presented in **Figures 5B-5C**, or that the binding mediated by the HER2/neu binding domains of **hHER2 MAB-1 (1.3)** in particular, exhibits a positional effect.

[00501] **CD137 x TA Binding Molecules**, **DART-B**, **DART-D**, **DART-G** and the control molecules **DART-3** and **DART-6** were additionally evaluated for their ability to bind to CD137 expressed by CHO cells (**Figure 12A**) and activated T cells (**Figure 12B**).

[00502] **CD137 x TA Binding Molecules**, **DART-B**, **DART-D**, **DART-G** and the control molecules **DART-3** and **DART-6** were additionally evaluated for their ability to bind to HER2/neu expressed on N87 cells (**Figure 13A**) and JIMT-1 cells (**Figure 13B**). For such evaluation, 100 μ L of HER2/neu expressing target cells (1.0×10^6 cells/mL) and 100 μ L of serially diluted (5- or 10-fold dilutions) test article (bispecific diabody) were added to each well of microtiter assay plate(s), mixed and incubated at RT for 45 min. The cells were washed with FACS Buffer and secondary antibody (anti-human-Fc region-APC) was then added to each well; mixed and incubated at RT for 30 min. Cells were washed and resuspended in 100 μ L FACS Buffer and analyzed by flow cytometry (BD LSR Fortessa or FACSCanto II) for cell event collection. Data analysis were performed via FloJo v10. The **HER2 x CD137 Binding Molecules** having the structure presented in **Figure 3B** exhibited slightly better binding to HER2/neu than those having the structure presented in **Figures 5A-5C** (compare **DART-**

B/DART-D). The **RSV x CD137** control molecule (**DART-6**) did not exhibit any binding. **CD137 x TA** bispecific diabodies having a murine or humanized CD137 binding site exhibited similar binding to HER2/neu (compare **DART-D/DART-G**).

[00503] **Figures 14A-14B** provide the results of a first representative assay of the ability of **CD137 x TA** Binding Molecules to mediate a co-stimulatory activity in a T cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN- γ) in the presence or absence of target cells expressing the exemplary TA, HER2/neu. The Figures show results for **DART-1, DART-2, DART-3, DART-4, DART-5, DART-6, DART-A, DART-D, DART-E** and **DART-F**, tested (using the above-described protocols) in the presence of HER2/neu-expressing N87 (3⁺) (**Figure 14A**) or JIMT-1 (2⁺) target cells (**Figure 14B**), HER2/neu-negative Hs700T target cells, or no target cells. The results show that the control molecules, **DART-3** and **DART-6** exhibited no co-stimulatory activity. The **CD137 x TA** Binding Molecules did not exhibit any observable co-stimulatory activity in the absence of target cells or with HER2/neu-negative target cells. **DART-D** and **DART-F**, comprising novel anti-CD137 antibodies (CD137 MAB-3 and MAB-4, respectively) exhibited the highest co-stimulatory activity in the presence of either target cell line. **DART-E** (comprising swapped HER2/neu and CD137 domains) exhibited lower co-stimulatory activity, particularly in presence of N87 target cells. Similar patterns were seen with IL-2 and TNF- α .

[00504] **Figures 15A-15B** provide the results of a second representative assay of the ability of **CD137 x TA** Binding Molecules to mediate a co-stimulatory activity in a T cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN- γ). The Figures show results for **DART-1, DART-2, DART-3, DART-4, DART-5, DART-6, DART-B, DART-D** and **DART-G**, tested (using the above-described protocols) in the presence of HER2/neu-expressing N87 (**Figure 15A**) or JIMT-1 target cells (**Figure 15B**), HER2/neu-negative Hs700T target cells, or no target cells.

[00505] The results show that the control molecules, **DART-3** and **DART-6** exhibited no co-stimulatory activity. The **CD137 x TA** Binding Molecules did not exhibit any observable co-stimulatory activity in the absence of target cells or with HER2/neu-negative target cells. **DART-D** and **DART-G**, comprising the novel anti-CD137 antibody, **CD137 MAB-3** (murine and humanized, respectively) exhibited the highest co-stimulatory activity in the presence of either target cell line. **DART-G** exhibited the highest co-stimulatory activity despite exhibiting

slightly lower binding to cell surface expressed CD137. As detailed above, **DART-B** and **DART-G** comprise the same HER2 and CD137 binding sites but have the structures shown in **Figure 3B** and **Figure 5B**, respectively. **DART-G** exhibited higher co-stimulatory activity than **DART-B** in the presence of either target cell line.

[00506] The **CD137 x TA Binding Molecule, DART-G** and the control molecules, **DART-3** and **DART-6**, were additionally evaluated for their ability to mediate dose dependent T-cell signal transduction of the NF/ κ B pathway in a CD137 expressing reporter cell line (Jurkat-NF- κ B-Luc) in the presence of target cells positive or negative for the exemplary TA, HER2/neu. Briefly, Jurkat-NF- κ B-Luc reporter cells overexpressing CD137 were co-cultured with HER2/neu expressing JIMT-1 cells (**Figure 16A**) or HER2/neu-negative KG-1 cells (**Figure 16B**) in the presence of increasing concentrations of **DART-G**, **DART-3**, or **DART-6**. After incubation, signal transduction was measured by luminescence with luminescence relative light unit (RLU) as the read-out. The results show that **CD137 x TA Binding Molecules** such as **DART-G**, mediate dose dependent T-cell signal transduction that is dependent on the presence of TA-expressing target cells.

[00507] The **CD137 x TA Binding Molecule, DART-A** and the control molecules, **DART-3** and **DART-6**, were additionally evaluated for their ability to mediate enhanced T-cell proliferation in the presence of cells expressing different levels of the exemplary TA, HER2/neu. Briefly, CFSE-labeled human T cells $\pm$ sub-optimal α CD3/ α CD28 stimulation were co-cultured with HER2/neu-high N87 target cells (**Figure 17, Panels A-C and J-K**), HER2/neu-low MCF-7 target cells (**Figure 17, Panels D-F and L-M**), or were cultured alone (**Figure 17, Panels G-I and N-O**) in the presence of **DART-A** (**Figure 17, Panels A, D and G**), **DART-3** (**Figure 17, B, E and H**), **DART-6** (**Figure 17, Panels C, F and I**), or no molecule (**Figure 17, Panels J, L and N**) and monitored for T cell proliferation. The results show that **CD137 x TA Binding Molecules**, such as **DART-A**, enhance T-cell signal proliferation. Such enhanced T cell proliferations is TA-expression dependent and correlates with TA expression levels.

[00508] Margetuximab is an Fc-optimized anti-HER2/neu monoclonal antibody that can induce enhanced antibody dependent cell-mediated cytotoxicity after binding target tumor cell. As noted above, **HER2 MAB-1** binds to a HER2/neu epitope that is distinct from that bound by margetuximab. The target cell killing activity of the anti-TA antibody margetuximab was examined in combination with the **CD137 x TA Binding Molecule DART-1** or the control

molecule **DART-3 (HER2 x RSV)**. Briefly, target N87 cells that had been engineered to express the luciferase (luc) reporter gene (N87/GFP/Luc cells) were incubated with purified NK effector cells (at an effector:target (E:T) ratio of 2:1, increasing concentrations of margituximab in combination with a fixed concentration (0.1 µg/mL) of **DART-1** or **DART-3** for 72 hours, and cytotoxicity was determined by luminescence (LUM) assay measuring cellular luciferase activity of the target cells with luminescence relative light unit (RLU) as the read-out (**Figure 18A**). In addition, NK cells (CD3⁺/CD56⁺) from this assay were evaluated for expression of the activation marker, CD69 (**Figure 18B**). The results of this study demonstrate that the **CD137 x TA Binding Molecule DART-1** enhanced both margituximab-mediated ADCC activity against N87/Luc target cells, and margituximab-mediated CD69 upregulation on NK cells as compared to the control molecule having just HER2/neu binding.

[00509] The ability of a **CD137 x TA Binding Molecule** designated “**DART-7**,” which binds the illustrative **TA** EphA2, to mediate a co-stimulatory activity in a T cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN-γ) was examined. The **CD137 x TA Binding Molecule DART-7** and the control molecule **DART-8**, were tested (using the above-described protocols) in the presence of EphA2-expressing Hs700T target cells (**Figure 19A**) or EphA2-negative Hs700T (EphA2.KO) target cells (**Figure 19B**), or no target cells. The results show that the **CD137 x TA Binding Molecule DART-7** exhibited co-stimulatory activity in the presence of EphA2-expressing cells but did not exhibit any observable co-stimulatory activity in the absence of target cells or with EphA2-negative target cells. The control molecule, **DART-8** exhibited no co-stimulatory activity.

[00510] The co-stimulatory activity of **CD137 x TA Binding Molecules** on T cell populations was examined by evaluating the the fraction of Central Memory T cells (Tcm) and Effector Memory T Cells (Tem). Briefly, human T cells were co-cultured with EphA2 positive colon adenocarcinoma Colo205 target cells for 5 days under sub-optimal stimulation conditions in the presense of the **CD137 x TA Binding Molecule DART-7** or the control molecules **DART-8** or **DART-6**. Following co-culture, the percentage of Tcm (CCR7⁺CD45RA⁻) and Tem (CCR7⁻CD45RA⁺) cells was determined in gated CD4⁺ and gated CD8⁺ subsets. The results, summarized in **Table 11**, show that there is a substantial increase in the fraction of CD8⁺ Tcm and Tem cell types and that this increase requires the binding of both CD137 and the **TA** (e.g., EphA2). These results indicate that **CD137 x TA Binding**

Molecules induce a substantial increase in the fraction of CD8⁺ Tcm and Tem cells in the presence of the proper tumor antigen expressing cells. The targeted T-cell agonism exhibited by **CD137 x TA** Binding Molecules may offer an opportunity to induce tumor-cell anchored CD137 activation, limiting systemic immune-cell activation and related side effects.

Table 11				
Treatment	Gated CD4⁺		Gated CD8⁺	
	Tcm (%) (CCR7 ⁺ CD45RA ⁻)	Tem (%) (CCR7 ⁺ CD45RA ⁻)	Tcm (%) (CCR7 ⁺ CD45RA ⁻)	Tem (%) (CCR7 ⁺ CD45RA ⁻)
DART-7	50.1	17.7	20.5	38.6
DART-8	44.8	10.4	6.10	23.5
DART-6	44.4	9.95	6.67	25.2

[00511] In addition, **CD137 x TA** Binding Molecules having one binding site for CD137 and one or more binding sites for HER2/neu (see, *e.g.*, **Figures 4A, 5A, and 6A**) were constructed and tested. Such molecules are monovalent for CD137, and exhibited reduced binding to activated T cells as expected in view of their reduced avidity. Such molecules exhibited a range of binding to target N87 cells. However, none of the molecules monovalent for CD137 exhibited any co-stimulatory activity in T cell cytokine release assays. This observation suggests that at least two CD137 binding sites may be required for co-stimulatory activity in these assays.

Example 5 **Optimization of Humanized CD137 MAB-3**

[00512] As noted above humanization of **CD137 MAB-3** resulted in approximately a 2-fold loss in binding affinity (see, *e.g.*, **Table 9** above). Optimization was used to identify humanized **CD137 MAB-3** clones having similar or better binding affinity than the parental murine antibody. Briefly, random mutagenesis was used to introduce substitutions within the Heavy Chain CDR_{H3} (Kabat positions 99-102) domains of **hCD137 MAB-3 (1.3)**. Three rounds of selection screening using increasing stringency were used to identify clones having enhanced binding to CD137. 48 clones were selected from rounds 2 and 3, and were evaluated as diabodies (having the structure shown in **Figure 5B**) for affinity. **Table 12** provides an alignment of the amino acid sequence of CDR_{H3} Kabat residues 99-102 from four clones selected for enhanced binding to hCD137.

Table 12			
Clone ID	Clone SEQ ID NO.	CDR _{H3} (Kabat 99-102)	CDR _{H3} SEQ ID NO.
hCD137 MAB-3 VH1	76	SYSFDY	160
hCD137 MAB-3 VH1A	83	AYSFHP	78
hCD137 MAB-3 VH1B	84	AYSMST	79
hCD137 MAB-3 VH1C	85	AYSYSL	80
hCD137 MAB-3 VH1D	86	SYSYNV	81

[00513] The amino acid sequences of these clones were incorporated into the 5 chain diabody DART-G to yield optimized **DART-G** molecules (designated as **DART-G1**, **DART-G2**, **DART-G3**, and **DART-G4**). The binding affinity of **DART-G2**, **DART-G3**, and **DART-G4** were evaluated via BIAcore (Table 11). For such evaluation, His-tagged soluble human CD137 (huCD137) or cynomolgus monkey CD137 (cynoCD137) (containing an extracellular portion of human or cynomolgus monkey CD137 fused to a histidine-containing peptide) was passed over a surface coated with immobilized antibody. Briefly, each test molecule was captured onto an F(ab)₂ goat-anti-human Fc-coated surface and then incubated in the presence of different concentrations (6.25-100 nM) of huCD137 or cynoCD137. The kinetics of binding were determined via BIAcore® analysis binding (normalized 1:1 Langmuir binding model). The calculated k_a , k_d and K_D from these studies are presented in Table 13.

Table 13							
Molecule	hCD137 MAB-3 VH Domain	huCD137			cynoCD137		
		k_a ($\times 10^5$) ($M^{-1} s^{-1}$)	k_d ($\times 10^{-3}$) (s^{-1})	K_D (nM)	k_a ($\times 10^5$) ($M^{-1} s^{-1}$)	k_d ($\times 10^{-4}$) (s^{-1})	K_D (nM)
DART-D	murine	4.7	25	53	4.6	31	67
DART-G	VH1	3.7	42	114	3.9	45	115
DART-G2	VH1B	3.5	21	60	3.9	25	64
DART-G3	VH1C	2.2	14	64	2.3	18	78
DART-G4	VH1D	3.1	8.7	28	3.5	14	40

[00514] The diabodies were evaluated for their ability to bind CD137 in the same manner as described above. The results of this study are shown in **Figures 20A-20B**. As noted above **DART-G**, having hCD137 MAB-3 (1.3) binding sites for CD137 exhibited slightly lower binding as compared to constructs having the same structure but comprising the CD137 binding sites of murine CD137 MAB-3 (**DART-6**). **HER2 x CD137** diabodies comprising the optimized hCD137 MAB-3 VH domains exhibited higher binding, the relative binding activity of the molecules to human CD137 was: **DART-G4** > **DART-G3** > **DART-G2** $\approx$ **DART-6** > **DART-G**. **DART-G** and the optimized variants all exhibited nearly identical binding to **HER2** on the surface of multiple target cell types including: N87, JIMT-1 and MDA-MB231 cells.

[00515] **Figures 21A-21C** show the results of a representative assay of the ability of **DART-3**, **DART-6**, **DART-B**, **DART-D**, **DART-G**, **DART-G2**, **DART-G3** and **DART-G4** to mediate co-stimulatory activity in a T cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by the release of IFN- γ). Cytokine release was measured as described above in the presence of HER2/neu-expressing N87 target cells (**Figure 21A**), JIMT-1 target cells (**Figure 21B**), or MDA-231 (**Figure 21C**) target cells, or with no target cells present.

[00516] The results show that the control diabodies, **DART-3** and **DART-6** exhibited no co-stimulatory activity. The **CD137 x TA** Binding Molecules did not exhibit any observable co-stimulatory activity in the absence of target cells or with HER2/neu-negative target cells. **DART-G**, comprising the novel anti-CD137 humanized antibody, **hCD137 MAB-3**, exhibited the highest co-stimulatory activity in the presence of either target cell line. TNF- α and IL-2 show similar release patterns.

[00517] The co-stimulatory activity of the optimized variants **DART-G2**, **DART-G3** and **DART-G4** was found to be inversely related to the increased binding to cell surface expressed CD137, indicating that higher binding affinity does not appear to directly correlate with co-stimulatory activity in this assay. However, as shown below, the optimized variants perform comparably in other functional assays. These attributes make the affinity optimized variants particularly useful for detection of CD137 expression and for diagnostic assays that measure the expression of CD137 in addition to their use as co-stimulatory agents.

[00518] The ability of the **CD137 x TA** Binding Molecules to mediate redirected cell killing of tumor target cells was examined. Briefly, N87/GFP/Luc target cells (engineered to express the luciferase (luc) reporter gene) were incubated with pan T cells under sub-optimal stimulation conditions for 72 hours in the absence or presence of **DART-B**, **DART-G**, **DART-G1**, **DART-G2**, **DART-G3**, **DART-G4** or the control molecules **DART-3** or **DART-6** (each at 0.001, 0.01, 0.1, and 1 μ g/mL), essentially as described above for the co-stimulation assays. At the end of the incubation cytotoxicity was determined by luminescence (LUM) assay measuring cellular luciferase activity of the target cells with luminescence relative light unit (RLU) as the read-out (**Figure 22**). Each of the **CD137 x TA** Binding Molecules examined exhibited some redirected cell killing activity with **DART-G** exhibiting the highest activity and **DART-B**, exhibiting the lowest activity. In contrast, the control molecules exhibited no

detectable redirected cell killing activity. The results of this study demonstrate that the **CD137 x TA Binding Molecules** are able to mediate redirected cell killing of tumor cells.

Example 6

Characterization of Trivalent CD137 x TA Binding Molecules

[00519] As noted above, **CD137 x TA Binding Molecules** having one binding site for CD137 and one or more binding sites for HER2/neu did not exhibit any co-stimulatory activity in T cell cytokine release assays. This observation suggests that at least two CD137 binding sites may be required for co-stimulatory activity. To address this question, a set of exemplary Trivalent **CD137 x TA Binding Molecules** capable of binding to CD137 and to the exemplary **TA**, HER2/neu, were generated. These molecules included four bispecific trivalent binding molecules “**TRIDENT-A**,” “**TRIDENT-A2**,” “**TRIDENT-A3**,” and “**TRIDENT-A4**,” respectively, each of which comprises two **hCD137 MAB-3** domains and one **hHER2 MAB-1** domain (**CD137 x CD137 x HER2**). The structure and sequences of these exemplary **CD137 x TA Binding Molecules** are provided in detail above. In addition, two control molecules, “**TRIDENT-1**,” each comprising one variant palivizumab domain and two optimized **hCD137 MAB-3** domains (**CD137 x CD137 x RSV**), and “**TRIDENT-2**” comprising one **hHER2 MAB-1** domain, one **4-4-20** domain, and one variant palivizumab domain (**RSV x FITC x HER2**) were generated. The trivalent molecules have the general structure shown in **Figure 6A**, and were generated and characterized as detailed below for their ability to bind to HER2/neu present on the surface of target N87(3⁺), JIMT-1(2⁺) and MCF-7(1⁺) by FACS analysis essentially as described above. **CD137 x TA Binding Molecules** having two binding sites for CD137 and two binding sites for HER2/neu (**DART-G**, **DART-G2**, **DART-G3**, and **DART-G4**), were also evaluated

[00520] As shown in **Figures 23A-23C**, the molecules that comprised one or two HER2/neu binding domains bound to all three target cell types. It will be noted that the binding curves of molecules having two HER2/neu binding sites (**DART-G**, **DART-G2**, **DART-G3**, and **DART-G4**) reach saturation sooner particularly on cells having high levels of HER2/neu on their cell surface. This indicates that some molecules are exhibiting bivalent binding (*i.e.*, binding two HER2/neu molecules on the surface). Bivalent bonding is more likely in the presence of high concentrations of target ligand.

[00521] The **CD137 x TA Binding Molecules** having two binding sites for CD137 and one binding site for HER2/neu (**TRIDENT-A**, **TRIDENT-A2**, **TRIDENT-A3** and **TRIDENT-**

A4), the **CD137 x CD137 x RSV** and **TA x FITC x RSV** control molecules (**TRIDENT-1** and **TRIDENT-2**), and the **CD137 x TA** Binding Molecules having two binding sites for CD137 and 2 binding sites for HER2/neu (**DART-G**, **DART-G2**, **DART-G3**, and **DART-G4**), were also evaluated by FACS analysis essentially as described in Example 2 above for their ability to bind CD137 present on the surface of activated CD4⁺ (**Figure 24A**) and CD8⁺ (**Figure 24B**) T cells. The molecules that comprised two CD137 binding domains exhibited strong binding to activated T-cells. Improved binding was observed for the exemplary **HER2 x CD137** diabodies comprising the optimized **hCD137 MAB-3 VH** domains described above. The **TA x FITC x RSV** control molecule (**TRIDENT-2**) did not exhibit any binding to activated T-cells, as expected.

[00522] In another study, the **CD137 x TA** Binding Molecules having two binding sites for CD137 and one binding site for HER2/neu (**TRIDENT-A**, **TRIDENT-A2**, **TRIDENT-A3** and **TRIDENT-A4**), a **CD137 x TA** Binding Molecule having two binding sites for CD137 and two binding sites for HER2/neu (**DART-G4**) and the **TA x FITC x RSV** control molecule (**TRIDENT-2**), were evaluated for their ability to mediate cell-cell conjugation between CD137 expressing cells and HER2/neu expressing target cells. For such evaluation, 1.5×10^4 CD137-expressing CHO cells labeled with PKH26 were co-incubated with 1.5×10^4 HER2/neu-expressing target cells (N87(HER2 expression level: 3⁺), JIMT-1 (HER2 expression level: 2⁺), or MCF-7(HER2 expression level: 1⁺)), labeled with CFSE in a 1:1 ratio in the presence of serially diluted (10-fold dilutions) test article for 30 at room temperature. The samples were then analyzed by flow cytometry to determine the percentage of conjugated cells (CFSE+/PKH26+) as a readout for cell-cell conjugation. As shown in **Figures 25A-25C**, the molecules that comprised at least one HER2/neu binding domain and two CD137 binding domains were able to mediate cell-cell conjugation while the control molecule was inactive. For all active molecules, cell-cell conjugation activity correlated with the level of HER2/neu expression.

[00523] In another study, the **CD137 x TA** Binding Molecules having two binding sites for CD137 and one binding site for HER2/neu (**TRIDENT-A**, **TRIDENT-A2**, **TRIDENT-A3** and **TRIDENT-A4**), the **CD137 x RSV** and **TA x FITC x RSV** control molecules (**TRIDENT-1** and **TRIDENT-2**), and the **CD137 x TA** Binding Molecules having two binding sites for CD137 and two binding sites for HER2/neu (**DART-G**, **DART-G2**, **DART-G3**, and **DART-G4**), were evaluated for their ability to mediate a co-stimulatory activity in a

T cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN- γ) in the presence or absence of target cells expressing the exemplary TA, HER2/neu. Cytokine release was measured as described above in the presence of HER2/neu-expressing N87 target cells (**Figure 26A**), JIMT-1 target cells (**Figure 26B**), or with no target cells present (**Figure 26C**).

[00524] The results show that the exemplary CD137 x TA Binding Molecules exhibited co-stimulatory activity in the presence of either target cell line, with activity correlated with the level of HER2/neu expression. **TRIDENT-A, TRIDENT-A2, TRIDENT-A3 and TRIDENT-A4** (each having two binding sites for CD137 and one binding site for HER2/neu) exhibited higher co-stimulatory activity on JIMT-1 (HER2⁺⁺) cells than **DART-G, DART-G2, DART-G3 or DART-G4** (having two binding sites for CD137 and 2 binding sites for HER2/neu). The CD137 x TA Binding Molecules did not exhibit any observable co-stimulatory activity in the absence of target cells or with HER2/neu-negative target cells. the control molecules, **TRIDENT-1 and TRIDENT-2**, exhibited no co-stimulatory activity.

Example 7 CD137 x TA Binding Molecules Enhance T-cell Activation

[00525] As described above, the **CD137 x TA** Binding Molecules of the present invention can enhance T-cell expansion and activation (see, *e.g.*, **Figures 17A-17O**) and tumor targeting agent-mediated NK effector function (ADCC) (see, *e.g.*, enhancement of Margetuximab-mediated ADCC, **Figures 18A-18B**). **TA x CD3** bispecific molecules (*e.g.*, DART diabodies, BiTE® molecules, *etc.*) which bind to a **TA** and to CD3 are known to mediate T-cell redirected killing of target cells expressing the **TA**, and stimulate T-cells expansion and activation (see, *e.g.*, PCT Publication Nos. WO 2017/030926; WO 2016/048938; and WO 2015/026892). The ability of the **CD137 x TA** Binding Molecules of the invention to enhance T-cell activation and T-cell redirected killing mediated by such tumor targeting agents was assessed in several studies using exemplary **TA x CD3** bispecific molecules.

[00526] In one such study, the ability of the exemplary **CD137 x TA** Binding Molecule **TRIDENT-A4** to enhance T-cell redirected cell killing mediated by a **TA x CD3** diabody that binds to the illustrative **TA** gpA33 was assessed using a luciferase based cytotoxic T lymphocyte (CTL) assay. Briefly, pan T cells were incubated with Colo205 cells engineered to express luciferase (luc) reporter gene (Colo205/luc) target cells at an effector: target (E:T)

ratio of 3:1 in the presence of **TRIDENT-A4** or the control **TRIDENT-1** in combination with a **TA x CD3** diabody or a control **Irrel x CD3** bispecific diabody (comprising an irrelevant binding domain (4-4-20 to fluorescein) in place of gpA33). At the end of the incubation, cytotoxicity was determined by luminescence (LUM) assay measuring cellular luciferase activity of the target cells with luminescence relative light unit (RLU) as the read-out (**Figure 27**). The results of this study demonstrate that the **CD137 x TA** Binding Molecules of the invention are able to enhance T-cell redirected killing mediated by **TA x CD3** bispecific molecules.

[00527] In another study, the ability of the exemplary **CD137 x TA** Binding Molecule **TRIDENT-A2** to enhance T-cell expansion and anti-tumor activity in combination *in vivo* was assessed in an SK-OV3 ovarian carcinoma model (expressing the **TAs** HER2/neu and 5T4). In this study, the activity of **TRIDENT-A2** was examined alone or in combination with a **TA x CD3** diabody that binds to the illustrative **TA** 5T4 (**5T4 x CD3**), and optionally in further combination with an **anti-PD-1 mAb** (hPD-1 mAb 7(1.2) IgG4 (P), see, *e.g.*, **SEQ ID NOs:264** and **266** of PCT Publication WO 2017/019846).

[00528] Briefly, freshly isolated PBMCs were injected retro-orbitally into NSG.MHCI^{-/-} mice (female mice 7 per group for tumor monitoring; 3 per group for T-cell and tumor profiling) on study day 0. SK-OV3 cells (5 x 10⁶) were mixed 1:1 with Matrigel and injected subcutaneously on study day 0. On study day 7, mice were treated with the anti-CD4 antibody OKT4 (initial dose 10 mg/kg subcutaneously, 5 mg/kg twice a week IP thereafter) to eliminate CD4⁺ T-cells. Starting on day 22, mice were treated by intravenous (IV) injection with: vehicle control; **TRIDENT-A2** (2.5 mg/kg, once per week); **TA x CD3** diabody (0.01 mg/kg, twice per week); combination of **TRIDENT-A2** (2.5 mg/kg, once per week) and **TA x CD3** diabody (0.01 mg/kg, twice per week); anti-PD-1 mAb (5 mg/kg, once per week); or the combination of **TRIDENT-A2** (2.5 mg/kg; once per week), **TA x CD3** diabody (0.01 mg/kg, twice per week), and **anti-PD-1 mAb** (5 mg/kg, once per week).

[00529] T-cell markers were evaluated by FACS analysis 7 days post administration of the test articles and tumor growth was monitored over the course of the study. Expression of the T-cell markers CD4, CD8, CD69, and PD-1, present in 1 mg of tumor sampled at day 7 post-post treatment are plotted in **Figure 28A**, **Figure 28B**, **Figure 28C**, and **Figure 28D**, respectively. These data show that the **CD137 x TA** Binding Molecule **TRIDENT-A2** enhanced T-cell activation and expansion (as exemplified by increased expression of CD4,

CD8 and CD69) mediated by the **TA x CD3** diabody. Expression of the immune checkpoint molecule PD-1 was upregulated upon treatment with **TA x CD3** diabody alone. Treatment with the combination of the **CD137 x TA** Binding Molecule **TRIDENT-A2** and the **TA x CD3** diabody further enhanced the expression of the checkpoint molecule PD-1 observed upon treatment with **TA x CD3** diabody alone and suggested that the activity of the combination could be enhanced by the addition of a checkpoint inhibitor. Indeed, addition of the anti-PD-1 mAb, an exemplary **PD-1/PD-L1 check point inhibitor**, to the **TRIDENT-A2/TA x CD3** diabody combination inhibited the upregulation of PD-1 expression and further enhanced T-cell activation and proliferation.

[00530] Tumor growth over the course of the study is plotted in **Figure 29**, which shows that the **CD137 x TA** Binding Molecule **TRIDENT-A2** enhanced the anti-tumor activity of the **TA x CD3** diabody. In addition, the addition of the PD-1/PD-L1 checkpoint inhibitor, anti-PD-1 mAb, to the combination further enhanced the anti-tumor activity of the combination. The results of this study demonstrate that the **CD137 x TA** Binding Molecules of the invention, particularly those having two binding sites for CD137 and one binding site for a TA, enhance T-cell activation, proliferation and target cell killing in combination with a tumor targeting agent. Thus, **CD137 x TA** Binding Molecules of the invention may advantageously be using in combination with tumor targeting agents, particularly those that mediate T cell activation, NK cell activation, and/or stimulate CD137 expression in such cells. Furthermore, these studies demonstrate that the addition of a PD-1/PD-L1 checkpoint inhibitor, such as an anti-PD-1 or anti-PD-L1 antibody, can further enhance T-cell activation, proliferation and target cell killing.

Example 8 **Stability Studies**

[00531] The melting temperature (T_m) and other stability parameters of the exemplary Trivalent **CD137 x TA** Binding Molecules **TRIDENT-A** (comprising **hCD137 MAB-3 (1.3)**), and the optimized variants **TRIDENT-A1** (comprising **hCD137 MAB-3 (1A.3)**), **TRIDENT-A2** (comprising **hCD137 MAB-3 (1B.3)**), **TRIDENT-A3** (comprising **hCD137 MAB-3 (1C.3)**), and **TRIDENT-A4** (comprising **hCD137 MAB-3 (1D.3)**) were evaluated. The T_m of purified material (>95% percent as determined by High Pressure Size Exclusion Chromatography (HP-SEC)) was evaluated by differential scanning calorimetry (DSC). Briefly, purity levels were measured HPLC-SEC using an Agilent 1260 Infinity II HPLC

connected to a Waters Acquity UPLC BEH Column (200Å 1.7µm 4.6 x 150mm). The running buffer was 20 mM sodium phosphate, 0.5 M NaCl, 0.02% sodium azide, pH 7.2. The flow rate was 0.4 ml/minute for 5 minutes. The % purity is determined from the % area of the absorbance tracing at 280nm of the monomer peak. Thermal stability measurements were performed on a MicroCal VP-Capillary DSC (Malvern Instruments, Inc.) between 15-95 °C and ramped at 1 °C /minute.

[00532] To evaluate other stability parameters, purified **CD137 x TA** Binding Molecules (1 mg/mL in PBS pH7.2 unless noted) were stressed and revaluated by HP-SEC and the values compared to those obtained from the unstressed molecule. The following stress conditions were utilized: three rounds of freeze (1hr at -80°C) followed by thaw; after 2 and 7 days of storage at 25°C or 40°C; after 0, 2, 7 days of storage at 25°C in 20 mM Sodium Acetate pH 5.5; at 0, 2, 7 days of storage at 25°C in 10 mM Histidine HCL pH 6.5. The results of these studies are summarized in **Table 14**.

Table 14						
Assay	Stress Type	Optimal Value	TRIDENT-			
			A	A2	A3	A4
HP-SEC pure	none (4°C storage)	> 95% †	99.4	96.6	97.1	99.6
DSC	none	Tm Onset > 50°C	62.19	54.72	48.57	48.35
	none	Tm1 > 60°C	68.19	61.75	54.72	56.61
Stability Assessed	Freeze Thaw 3x	no change	no change	no change	no change	no change
	25°C	no change	no change	no change	no change	no change
	40°C	no change	<i>sensitive</i>	no change	<i>sensitive</i>	<i>very sensitive</i>
	pH5.5 Acetate	no change	<i>sensitive</i>	no change	no change	<i>sensitive</i>
	pH6.5 Histidine	no change	no change	no change	no change	no change
† indicates the percentage purity of the sample as measured by HP-SEC <i>sensitive</i> : < 95% of desired product remains as measured by HP-SEC <i>very sensitive</i> : < 90% desired product remains as measured by HP-SEC						

[00533] The Tm onset and Tm of the CD137 binding regions (Tm1) are in the optimal range for **TRIDENT-A** and **TRIDENT-A2**. However, the Tm onset and Tm1 are lower for both **TRIDENT-A3** and **TRIDENT-A4**. Only **TRIDENT-A2** was stable at 40°C (Day 2 and Day

7) in PBS, the remaining molecules were sensitive to this storage condition. Both **TRIDENT-A** and **TRIDENT-A2** were stable in Acetate pH 5.5, while **TRIDENT-A3** and **TRIDENT-A4** were both sensitive to Acetate pH 5.5 incubation. All the molecules tested were stable in Histidine at pH 6.5. Based on the results from these studies ranking for stability in the tested buffers is **TRIDENT-A2 > TRIDENT-A > TRIDENT-A3 > TRIDENT-A4**.

Example 9 **Co-Stimulatory Activity With Cynomolgus T Cells**

[00534] As noted above, molecules comprising the binding domains of **CD137 MAB-3** (or humanized, optimized variants thereof) bind both human and cynomolgus monkey CD137. Representative **CD137 x TA** Binding Molecules **TRIDENT-A2** and **TRIDENT-B2** having two binding sites for CD137 and one binding site for the for HER2/neu or 5T4 were evaluated for their ability to mediate a co-stimulatory activity in a cynomolgus T cell cytokine release assay. The structure and sequences of these exemplary **CD137 x TA** Binding Molecules are provided in detail above. The control molecules, **TRIDENT-1**, and **TRIDENT-2** were also included. The cytokine release assay was performed essentially as described in Example 2 above, and exemplified by release of IFN- γ) in the presence or absence of target cells except cynomolgus monkey pan T cells were utilized. For these studies JIMT-1 target cells expressing both HER2/neu and 5T4 were utilized.

[00535] The results are plotted in **Figure 30**, and show that the control molecules, **TRIDENT-1** and **TRIDENT-2** exhibited no co-stimulatory activity. While the **CD137 x TA** Binding Molecules **TRIDENT-A2** and **TRIDENT-B2** (comprising the humanized/optimized anti-CD137 antibody **hCD137 MAB-3 (1B.3)**) both exhibited a dose dependent co-stimulatory activity.

Example 10 **CD137 Deimmunization**

[00536] *In silico* analysis of **hCD137 MAB-3 (1B.3)** identified two potential MHC class II binding peptides (T-cell epitopes) in the VH Domain and three potential MHC class II binding peptides in the VL Domain. A panel of amino acid substitutions to **hCD137 MAB-3 (1B.3)** were examined to identify substitutions (which may be single amino acid substitutions or sets of substitutions) to eliminate/reduce the potential immunogenicity of the identified T-cell epitopes. Specifically, amino acid substitutions were introduced at up to two positions (Kabat

residues 38 and 48) for deimmunizing **hCD137 MAB-3 VH1B** and up to six positions (Kabat residues 24, 25, 44, 48, 52, and 54) for deimmunizing **hCD137 MAB-3 VL3**. The substituted amino acid residues in the VH and VL Domains are boxed and the Kabat numbering is indicated by arrows in **Figures 31A** and **31B**, respectively. IgG1 antibodies (having 234A/235A Fc Domain (**SEQ ID NO:40**)) comprising the VH and/or VL variants of **hCD137 MAB-3 (1B.3)** having various substitutions at these positions, separately and/or in combination, were generated and characterized for their ability to bind CD137 by Attana Cell A200 QCM. Briefly, **hCD137 MAB-3 (1B.3)** and the deimmunized variants were independently captured on an Attana sensor chip coated with rabbit anti-human IgG Fc polyclonal antibody and soluble human CD137-His tagged fusion protein was passed over the chip at 11.1, 33.3, 100, and 300 mM. The sensograms of the deimmunized variants were compared to that of **hCD137 MAB-3 (1B.3)** and scored. The antibodies were also characterized for their thermal stability by DSC, performed essentially as described above in Example 8. The results for a number of such variants are summarized in **Table 15**, the amino acid sequences of these variants are provided above.

Table 15				
hCD137 MAB-3 Ref.	VH1B Substitutions[^]	VL3 Substitutions[^]	CD137 binding*	DSC[‡]
(1B.3)	none	none	+++	64.7
(1E.3)	K38R (VH1E)	none	+++	65
(1F.3)	I48A (VH1F)	none	++	55.0
(1B.4)	none	R24Q/P25A (VL4)	+++	65.5
(1B.5)	none	V44A (VL5)	+++	62.6
(1B.6)	none	L54A (VL6)	+++	64.7
(1G.7)	K38R/I48A (VH1G)	R24Q/P25A/V44A (VL7)	+	45.2
(1G.8)	K38R/I48A (VH1G)	R24Q/P25A/V44A/L54A (VL8)	+	n.d.
(1E.4)	K38R (VH1E)	R24Q/P25A (VL4)	+++	64.5
(1G.4)	K38R/I48A (VH1G)	R24Q/P25A (VL4)	+++	55.1
(1B.10)	none	S52T (VL10)	+++	65.2
(1B.11)	none	S52G (VL11)	+++	64.4
(1B.11)	none	I48A/S52T (VL12)	+++	n.d.
(1B.13)	none	I48A/S52G (VL13)	++-	n.d.
(1E.9)	K38R (VH1E)	R24Q/P25A/L54A (VL9)	+++	64
(1E.14)	K38R (VH1E)	R24Q/P25A/S52T/L54A (VL14)	+++	64
(1E.15)	K38R (VH1E)	R24Q/P25A/S52G/L54A (VL15)	+++	62.9

Table 15				
hCD137 MAB-3 Ref.	VH1B Substitutions[^]	VL3 Substitutions[^]	CD137 binding*	DSC[‡]
(1G.14)	K38R/I48A (VH1G)	R24Q/P25A/S52T/L54A (VL14)	++	51.9
(1G.15)	K38R/I48A (VH1G)	R24Q/P25A/S52G/L54A (VL15)	+	50
[^] substitution(s) relative to VH1B or VL3 are listed, the designation of the resulting variable region comprising the indicated substitutions is provided in parenthesis [*] relative binding as compared to hCD137 MAB-3 (1B.3) (set to +++) determined by comparison of binding sensograms [‡] Tm onset “none” indicates no mutations are present n.d. not determined				

[00537] A large number of deimmunized variants were identified that retain both CD137 binding activity and thermal stability. The majority of deimmunized variants comprising the VH Domain I48A substitution exhibited some reduction in both CD137 binding and in the onset of Tm.

[00538] Deimmunized **hCD137 MAB-3 (1E.15)** and **hCD137 MAB-3 (1G.15)**, and the parental humanized **hCD137 MAB-3 (1B.3)** were tested for their ability to bind to activated CD4⁺ and CD8⁺ T cells essentially as described above. As shown in **Figures 32A-32B** the binding curve of the deimmunized antibody **hCD137 MAB-3 (1E.15)** was unchanged, while **hCD137 MAB-3 (1G.15)** exhibited slightly reduced binding to both CD4⁺ and CD8⁺ T cells.

[00539] The *in vitro* agonist activity **hCD137 MAB-3 (1E.15)** and **hCD137 MAB-3 (1G.15)** was evaluated in a T-cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN- γ) in the absence of or with cross-linking. None of the **hCD137 MAB-3** based antibodies exhibited activity was observed in the absence of cross-linking (**Figure 33A**), with crossing linking **hCD137 MAB-3 (1E.15)** exhibited similar agonist activity to **hCD137 MAB-3 (1B.3)**, while **hCD137 MAB-3 (1G.15)** exhibited a slight reduction in agonist activity likely due to the reduced binding for CD137 (**Figure 33B**). As reported above **CD137 MAB-1**, exhibited agonist activity in the presence and absence of cross-linking.

Example 11

Trivalent CD137 x TA Binding Molecules Having Deimmunized CD137 Binding Domains

[00540] As described above the VH and VL Domains of **hCD137 MAB-3** were humanized, optimized and deimmunized. Exemplary bispecific tetravalent and/or trivalent **CD137 x TA Binding Molecules** are generated by incorporating such VH and VL Domains. One such

bispecific trivalent binding molecule “**TRIDENT-B5**” comprising the CD137 binding domains of **hCD137 MAB-3 (1E.15)** was generated and evaluated in a number of assays. The structure and sequence of this exemplary **CD137 x TA** Binding Molecule is provided in detail above. **TRIDENT-B2** and one or more the control molecules: **TRIDENT-1**, **TRIDENT-2**, **TRIDENT-3**, **TRIDENT-4** were included in these studies.

[00541] **TRIDENT-B2**, **TRIDENT-B5**, and the control molecules **TRIDENT-1**, **TRIDENT-3**, and **TRIDENT-4** were evaluated for their ability to bind to activated CD8⁺ T cells, and/or 5T4 present on the surface of target cells (high expressing JIMT-1 breast carcinoma cells and low expressing SKOV-3 ovarian carcinoma cells) essentially as described above. As shown in **Figure 34A**, all the molecules comprising two CD137 binding domains (**TRIDENT-B2**, **TRIDENT-B5**, and the control molecules **TRIDENT-1** and **TRIDENT-4**) exhibited similar binding to activated CD8⁺ T cells, while the control molecule lacking any CD137 binding domains (**TRIDENT-3**) did not bind. Similarly, all the molecules having a 5T4 binding domain (**TRIDENT-B2**, **TRIDENT-B5**, and the control **TRIDENT-3**) exhibited similar strong binding to target cells expressing high levels of 5T4 (JIMT-1, **Figure 34B**), and weaker binding to target cells expressing low levels of 5T4 (SKOV-3, **Figure 34C**), while the control molecules lacking a 5T4 binding domain (**TRIDENT-1** and **TRIDENT-4**) did not bind to either 5T4 expressing target cell.

[00542] The *in vitro* agonist activity of **TRIDENT-B2**, **TRIDENT-B5**, and the control molecules **TRIDENT-1**, **TRIDENT-3**, and **TRIDENT-4** were evaluated in a T-cell cytokine release assay (performed essentially as described in Example 2 above, and exemplified by release of IFN- γ) in the presence or absence of target cells expressing different levels of the exemplary TA, 5T4 (JIMT-1 (5T4^{hi}) cells, or SKOV-3 (5T4^{lo}) cells).

[00543] As shown in **Figures 35A-35C**, **TRIDENT-1**, **TRIDENT-4** (lacking a 5T4 binding domain) exhibited no co-stimulatory activity. **TRIDENT-3** (lacking CD137 binding domains) was also found to lack any co-stimulatory activity. **TRIDENT-B2**, and **TRIDENT-B5** exhibited similar a dose dependent co-stimulatory activity in the presence of high 5T4-expressing JIMT-1 target cells (**Figure 35A**) but little or no co-stimulatory activity in the presence of low 5T4-expressing SKOV-3 target cells (**Figure 35B**), or no target cells (**Figure 35C**).

[00544] The results of these studies indicate that **CD137 x TA** Binding Molecules comprising the deimmunized VH and VL Domains of **hCD137 MAB-3 (1E.15)** exhibit comparable binding and co-stimulatory activity profiles as **CD137 x TA** Binding Molecules comprising the optimized VH and VL Domains of **hCD137 MAB-3 (1B.3)**.

[00545] 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.