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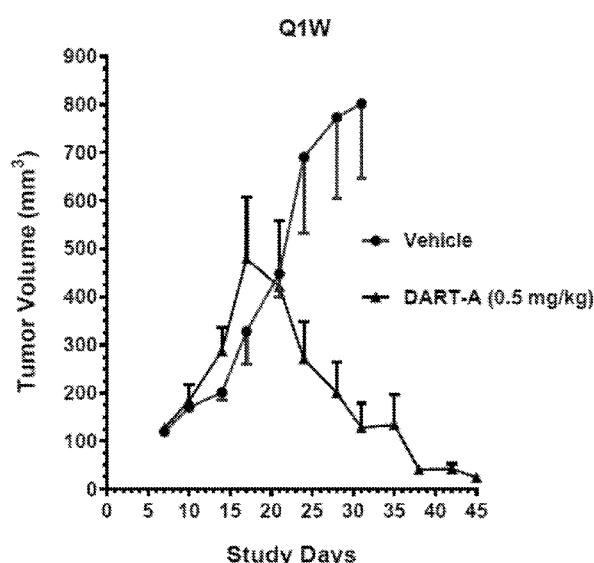
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62/280,318      19 January 2016 (19.01.2016)      US(71) Applicant: **MACROGENICS, INC.** [US/US]; 9704 Medical Center Drive, Rockville, MD 20850 (US).(72) Inventors: **JOHNSON, Leslie, S.**; 14411 Poplar Hill Road, Darnestown, MD 20874 (US). **MOORE, Paul, A.**; 10 Turley Court, North Potomac, MD 20878 (US). **BONVINI, Ezio**; 11136 Powder Horn Drive, Potomac, MD 20854 (US). **HUANG, Ling**; 8210 Moorland Lane, Bethesda, MD 20817 (US). **SHAH, Kalpana**; 13013 Ethel Rose Way, Boyds, MD 20841 (US). **ALDERSON, Ralph**;

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*[Continued on next page]*

(54) Title: BISPECIFIC MONOVALENT DIABODIES THAT ARE CAPABLE OF BINDING B7-H3 AND CD3, AND USES THEREOF



(57) Abstract: The present invention is directed to B7-H3 x CD3 bispecific monovalent diabodies, and particularly, to B7-H3 x CD3 bispecific monovalent Fc diabodies, that are capable of simultaneous binding to B7-H3 and CD3. The invention is also directed to pharmaceutical compositions that contain such bispecific monovalent Fc diabodies. The invention is additionally directed to methods for the use of such diabodies in the treatment of cancer and other diseases and conditions.

**Figure 16A**



SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, —

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**Title Of The Invention:****Bispecific Monovalent Diabodies That Are Capable of Binding B7-H3 and CD3, and Uses Thereof****Cross-Reference to Related Applications**

**[0001]** This application claims priority to U.S. Patent Applications Serial No. 62/206,051 (filed on August 17, 2015; pending) and 62/280,318 (filed on January 19, 2016), each of which applications is herein incorporated by reference in its entirety.

**Reference To Sequence Listing**

**[0002]** This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in computer-readable media (file name: 1301-0123PCT\_ST25.txt, created on August 10, 2016, and having a size of 70,481 bytes), which file is herein incorporated by reference in its entirety.

**Field Of The Invention**

**[0003]** The present invention is directed to bispecific monovalent diabodies that possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 (*i.e.*, a “B7-H3 x CD3 bispecific monovalent diabody”). Most preferably, such B7-H3 x CD3 bispecific monovalent diabodies are composed of three polypeptide chains and possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 and additionally comprise an immunoglobulin Fc Domain (*i.e.*, a “B7-H3 x CD3 bispecific monovalent Fc diabody”). The bispecific monovalent Fc diabodies of the present invention are capable of simultaneous binding to B7-H3 and CD3. The invention is directed to pharmaceutical compositions that contain such bispecific monovalent Fc diabodies. The invention is additionally directed to methods for the use of such diabodies in the treatment of cancer and other diseases and conditions.

**Background Of The Invention****I. The B7 Superfamily and B7-H3**

**[0004]** The growth and metastasis of tumors depends to a large extent on their capacity to evade host immune surveillance and overcome host defenses. Most tumors express antigens that can be recognized to a variable extent by the host immune system, but in many cases, an

inadequate immune response is elicited because of the ineffective activation of effector T cells (Khawli, L.A. *et al.* (2008) “*Cytokine, Chemokine, and Co-Stimulatory Fusion Proteins for the Immunotherapy of Solid Tumors,*” *Exper. Pharmacol.* 181:291-328).

**[0005]** B7-H3 is a member of the B7 superfamily of immunoglobulin molecules. Members of the B7 superfamily possess an immunoglobulin-V-like domain and an immunoglobulin-C-like domain (*e.g.*, IgV and IgC, respectively) (Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily,*” *Nature Rev. Immunol.* 2:116-126). The IgV and IgC domains of B7-superfamily members are each encoded by single exons, with additional exons encoding leader sequences, transmembrane and cytoplasmic domains. The cytoplasmic domains are short, ranging in length from 19 to 62 amino-acid residues and can be encoded by multiple exons (Collins, M. *et al.* (2005) “*The B7 Family Of Immune-Regulatory Ligands,*” *Genome Biol.* 6:223.1-223.7). Members of the B7 superfamily are predicted to form back-to-back, non-covalent homodimers at the cell surface, and such dimers have been found with respect to B7-1 (CD80) and B7-2 (CD86). B7-1 (CD80) and B7-2 (CD86) exhibit have dual specificity for the stimulatory CD28 receptor and the inhibitory CTLA-4 (CD152) receptor (Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily,*” *Nature Rev. Immunol.* 2:116-126).

**[0006]** B7-H3 (CD276) is unique in that the major human form contains two extracellular tandem IgV-IgC domains (*i.e.*, IgV–IgC–IgV–IgC) (Collins, M. *et al.* (2005) “*The B7 Family Of Immune-Regulatory Ligands,*” *Genome Biol.* 6:223.1-223.7). Although initially thought to comprise only 2 Ig domains (IgV–IgC) (Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- $\gamma$  Production,*” *Nature Immunol.* 2:269–274; Sun, M. *et al.* (2002) “*Characterization of Mouse and Human B7-H3 Genes,*” *J. Immunol.* 168:6294-6297) the four immunoglobulin extracellular domain variant (“4Ig-B7-H3”) has been found to be the more common human form of the protein (Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily,*” *Nature Rev. Immunol.* 2:116-126). However, the natural murine form is 2Ig, and it and the human 4Ig form exhibit similar function (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3,*” *Proc. Natl. Acad. Sci. (U.S.A.)* 105(30):10277-10278). The 4Ig-B7-H3 molecule inhibits the natural killer cell-mediated lysis of cancer cells (Castriconi, R. *et al.* (2004) “*Identification Of 4Ig-B7-H3 As A Neuroblastoma-Associated Molecule That Exerts A Protective Role From An NK Cell-Mediated Lysis,*” *Proc. Natl. Acad. Sci. (U.S.A.)* 101(34):12640-12645). The 2Ig form of human B7-H3 has been found to promote T cell activation and IFN- $\gamma$  production by binding to a putative receptor on activated T cells

(Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- $\gamma$  Production,*” *Nature Immunol.* 2:269–274; Xu, H. *et al.* (2009) “*MicroRNA miR-29 Modulates Expression of Immunoinhibitory Molecule B7-H3: Potential Implications for Immune Based Therapy of Human Solid Tumors,*” *Cancer Res.* 69(15):5275-6281). B7-H4 and B7-H3 are both potent inhibitors of immune function when expressed on tumor cells (Flies, D.B. *et al.* (2007) “*The New B7s: Playing a Pivotal Role in Tumor Immunity,*” *J. Immunother.* 30(3):251-260).

**[0007]** The mode of action of B7-H3 is complex, as the protein mediates both T cell co-stimulation and co-inhibition (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3,*” *Proc. Natl. Acad. Sci. (U.S.A.)* 105(30):10277-10278; Martin-Orozco, N. *et al.* (2007) “*Inhibitory Costimulation And Anti-Tumor Immunity,*” *Semin. Cancer Biol.* 17(4):288-298; Subudhi, S.K. *et al.* (2005) “*The Balance Of Immune Responses: Costimulation Verse Coinhibition,*” *J. Mol. Med.* 83:193-202). B7-H3 binds to TREM-like transcript 2 (TLT-2) and co-stimulates T cell activation, but also binds to as yet unidentified receptor(s) to mediate co-inhibition of T cells. In addition, B7-H3, through interactions with unknown receptor(s) is an inhibitor for natural killer cells and osteoblastic cells (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3,*” *Proc. Natl. Acad. Sci. (U.S.A.)* 105(30):10277-10278). The inhibition may operate through interactions with members of the major signaling pathways through which T cell receptors (TCR) regulate gene transcription (e.g., NFTA, NF- $\kappa$ B, or AP-1 factors).

**[0008]** B7-H3 co-stimulates CD4+ and CD8+ T cell proliferation. B7-H3 also stimulates IFN- $\gamma$  production and CD8+ lytic activity (Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- $\gamma$  Production,*” *Nature Immunol.* 2:269–274; Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily,*” *Nature Rev. Immunol.* 2:116-126). However, the protein also possibly acts through NFAT (Nuclear Factor For Activated T Cells), NF- $\kappa$ B (Nuclear Factor Kappa B), and AP-1 (Activator Protein-1) factors to inhibit T cell activation (Yi, K.H. *et al.* (2009) “*Fine Tuning The Immune Response Through B7-H3 And B7-H4,*” *Immunol. Rev.* 229:145-151). B7-H3 is also believed to inhibit Th1, Th2, or Th17 *in vivo* (Prasad, D.V. *et al.* (2004) “*Murine B7-H3 Is A Negative Regulator Of T Cells,*” *J. Immunol.* 173:2500-2506; Fukushima, A. *et al.* (2007) “*B7-H3 Regulates The Development Of Experimental Allergic Conjunctivitis In Mice,*” *Immunol. Lett.* 113:52-57; Yi, K.H. *et al.* (2009) “*Fine Tuning The Immune Response Through B7-H3 And B7-H4,*” *Immunol. Rev.*

229:145-151). Several independent studies have shown that human malignant tumor cells 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).

**[0009]** Molecules that block the ability of a B7 molecule to bind to a T cell receptor (*e.g.*, CD28) inhibit the immune system and have been proposed as treatments for autoimmune disease (Linsley, P.S. *et al.* (2009) “*The Clinical Utility Of Inhibiting CD28-Mediated Co-Stimulation*,” Immunol. Rev. 229:307-321). Neuroblastoma cells expressing 4Ig-B7-H3 treated with anti-4Ig-B7-H3 antibodies were more susceptible to NK cells. However, it is unclear whether this activity can be attributed to only antibodies against the 4Ig-B7-H3 form because all reported antibodies raised against the 4Ig-B7-H3 also bound the two Ig-like form of B7-H3 (Steinberger, P. *et al.* (2004) “*Molecular Characterization of Human 4Ig-B7-H3, a Member of the B7 Family with Four Ig-Like Domains*,” J. Immunol. 172(4): 2352-2359 and Castriconi *et al.* (2004) “*Identification Of 4Ig-B7-H3 As A Neuroblastoma-Associated Molecule That Exerts A Protective Role From An NK Cell-Mediated Lysis*,” Proc. Natl. Acad. Sci. (U.S.A.) 101(34):12640-12645).

**[0010]** B7-H3 is not expressed on resting B or T cells, monocytes, or dendritic cells, but it is induced on dendritic cells by IFN- $\gamma$  and on monocytes by GM-CSF (Sharpe, A.H. *et al.* (2002) “*The B7-CD28 Superfamily*,” Nature Rev. Immunol. 2:116-126). The receptor(s) that bind B7-H3 have not been fully characterized. Early work suggested one such receptor would need to be rapidly and transiently up-regulated on T cells after activation (Loke, P. *et al.* (2004) “*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T Cells*,” Arthritis Res. Ther. 6:208-214). Recently, the TREM-like transcript 2 (TLT-2, or TREML2) receptor (King, R.G. *et al.* (2006) “*Trem-Like Transcript 2 Is Expressed On Cells Of The Myeloid/Gramuloid And B Lymphoid Lineage And Is Up-Regulated In Response To Inflammation*,” J. Immunol. 176:6012-6021; Klesney-Tait, J. *et al.* (2006) “*The TREM Receptor Family And Signal Integration*,” Nat. Immunol. 7:1266-1273; Yi, K.H. *et al.* (2009) “*Fine Tuning The Immune Response Through B7-H3 And B7-H4*,” Immunol. Rev. 229:145-151), which is expressed on myeloid cells has been shown to be capable of binding B7-H3, and

of thereby co-stimulating the activation of CD8+ T cells in particular (Zang, X. *et al.* (2003) “*B7x: A Widely Expressed B7 Family Member That Inhibits T Cell Activation,*” Proc. Natl. Acad. Sci. (U.S.A.) 100:10388–10392; Hashiguchi, M. *et al.* (2008) “*Triggering Receptor Expressed On Myeloid Cell-Like Transcript 2 (TLT-2) Is A Counter-Receptor For B7-H3 And Enhances T Cell Responses,*” Proc. Natl. Acad. Sci. (U.S.A.) 105(30):10495-10500; Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3,*” Proc. Natl. Acad. Sci. (U.S.A.) 105(30):10277-10278).

**[0011]** In addition to its expression on neuroblastoma cells, human B7-H3 is also known to be expressed on a variety of other cancer cells (e.g., gastric, ovarian and non-small cell lung cancers). B7-H3 protein expression has been immunohistologically detected in tumor cell lines (Chapoval, A. *et al.* (2001) “*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN- $\gamma$  Production,*” Nature Immunol. 2:269–274; Saatian, B. *et al.* (2004) “*Expression Of Genes For B7-H3 And Other T Cell Ligands By Nasal Epithelial Cells During Differentiation And Activation,*” Amer. J. Physiol. Lung Cell. Mol. Physiol. 287:L217–L225; Castriconi *et al.* (2004) “*Identification Of 4Ig-B7-H3 As A Neuroblastoma-Associated Molecule That Exerts A Protective Role From An NK Cell-Mediated Lysis,*” Proc. Natl. Acad. Sci. (U.S.A.) 101(34):12640-12645); Sun, M. *et al.* (2002) “*Characterization of Mouse and Human B7-H3 Genes,*” J. Immunol. 168:6294-6297). mRNA expression of B7-H3 has been found in heart, kidney, testes, lung, liver, pancreas, prostate, colon, and osteoblast cells (Collins, M. *et al.* (2005) “*The B7 Family Of Immune-Regulatory Ligands,*” Genome Biol. 6:223.1-223.7). At the protein level, B7-H3 is found in human liver, lung, bladder, testis, prostate, breast, placenta, and lymphoid organs (Hofmeyer, K. *et al.* (2008) “*The Contrasting Role Of B7-H3,*” Proc. Natl. Acad. Sci. (U.S.A.) 105(30):10277-10278).

## II. CD3

**[0012]** CD3 is a T cell co-receptor composed of four distinct chains (Wucherpfennig, K.W. *et al.* (2010) “*Structural Biology Of The T cell Receptor: Insights Into Receptor Assembly, Ligand Recognition, And Initiation Of Signaling,*” Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14; Chetty, R. *et al.* (1994) “*CD3: Structure, Function, And Role Of Immunostaining In Clinical Practice,*” J. Pathol. 173(4):303-307; Guy, C.S. *et al.* (2009) “*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex,*” Immunol. Rev. 232(1):7-21).

**[0013]** In mammals, the complex contains a CD3 $\gamma$  chain, a CD3 $\delta$  chain, and two CD3 $\epsilon$  chains. These chains associate with a molecule known as the T cell receptor (TCR) in order to generate an activation signal in T lymphocytes (Smith-Garvin, J.E. *et al.* (2009) “*T Cell Activation*,” *Annu. Rev. Immunol.* 27:591-619). In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) “*Molecular Immunology Lessons From Therapeutic T cell Receptor Gene Transfer*,” *Immunology* 129(2):170–177). CD3 is found bound to the membranes of all mature T cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: *IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE*,” 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) “*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The CD3 $\epsilon$ : $\gamma$  Heterodimer*,” *Cell* 105(7):913-923; Kuhns, M.S. *et al.* (2006) “*Deconstructing The Form And Function Of The TCR/CD3 Complex*,” *Immunity*. 2006 Feb;24(2):133-139).

**[0014]** The invariant CD3 $\epsilon$  signaling component of the T cell receptor (TCR) complex on T cells has been used as a target to force the formation of an immunological synapse between T cells and tumor cells. Co-engagement of CD3 and the tumor antigen activates the T cells, triggering lysis of tumor cells expressing the tumor antigen (Baeuerle *et al.* (2011) “*Bispecific T Cell Engager For Cancer Therapy*,” In: *BISPECIFIC ANTIBODIES*, Kontermann, R.E. (Ed.) Springer-Verlag; 2011:273-287). This approach allows bispecific antibodies to interact globally with the T cell compartment with high specificity for tumor cells and is widely applicable to a broad array of cell-surface tumor antigens.

### III. Antibodies and Other Binding Molecules

**[0015]** **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 term encompasses not only intact polyclonal or monoclonal antibodies, but also mutants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an epitope-binding site of the required specificity, humanized antibodies, and chimeric antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an epitope-binding site of the required specificity.

**[0016]** The ability of an intact, unmodified antibody (*e.g.*, an IgG antibody) to bind an epitope of an antigen depends upon the presence of Variable Domains on the immunoglobulin

“light” and “heavy” polypeptide chains (*i.e.*, the VL and VH Domains, respectively, and collectively, the “Variable Region”). Each VH and VL is composed of three Complementarity Determining Region (CDR) Domains and four FR Domains arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. 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, each present on a separate polypeptide, forms one of the epitope binding sites of the antibody. In contrast, an scFv construct comprises a VL and VH Domain of an antibody contained in a single polypeptide chain, wherein the Domains are separated by a flexible linker of sufficient length to allow self-assembly of the two Domains into a functional epitope binding site. Where self-assembly of the VL and VH Domains is rendered impossible due to a linker of insufficient length (*e.g.*, a linker of less than about 12 amino acid residues), two scFv molecules can interact with one another other to form a bivalent “diabody” molecule in which the VL of one molecule associates with the VH of the other (reviewed in Marvin *et al.* (2005) “*Recombinant Approaches To IgG-Like Bispecific Antibodies*,” *Acta Pharmacol. Sin.* 26:649-658).

**[0017]** Natural antibodies are capable of binding to only one epitope species (*i.e.*, monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bi-valency or multi-valency). A wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further 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 Publications 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 Publications 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 Publications 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 tri-valent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv domains. PCT Publications 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 Publications Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional Binding Domains or functional groups to an antibody or an antibody portion (e.g., adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

**[0018]** The art has additionally noted the capability to produce **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).

**[0019]** In particular, stable, covalently bonded heterodimeric non-monospecific diabodies have been described (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 Fcgamma 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 Patents No. 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 Publications No. 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 Publications No. 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).

**[0020]** Notwithstanding such success, 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 B7-H3 and CD3.

## Summary Of The Invention

**[0021]** The present invention is directed to bispecific monovalent diabodies that possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 (*i.e.*, a “B7-H3 x CD3 bispecific monovalent diabody”). Most preferably, such B7-H3 x CD3 bispecific monovalent diabodies are composed of three polypeptide chains and possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 and additionally comprise an immunoglobulin Fc Domain (*i.e.*, a “B7-H3 x CD3 bispecific monovalent Fc diabody”). The bispecific monovalent Fc diabodies of the present invention are capable of simultaneous binding to B7-H3 and CD3. The invention is directed to pharmaceutical compositions that contain such bispecific monovalent Fc diabodies. The invention is additionally directed to methods for the use of such diabodies in the treatment of cancer and other diseases and conditions.

**[0022]** The present invention is particularly directed to B7-H3 x CD3 bispecific monovalent Fc diabodies. The B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention comprise polypeptide chains that associate with one another in a heterodimeric manner to form one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3. The B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention are thus monovalent in that they are capable of binding to only one copy of an epitope of B7-H3 and to only one copy of an epitope of CD3, but bispecific in that a single diabody is able to bind simultaneously to the epitope of B7-H3 and to the epitope of CD3.

**[0023]** The preferred B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention 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.

**[0024]** In detail, the invention provides a B7-H3 x CD3 bispecific monovalent Fc diabody, wherein the bispecific monovalent Fc diabody is capable of specific binding to an epitope of B7-H3 and to an epitope of CD3, and possesses an IgG Fc Domain, wherein the bispecific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a 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, and wherein:

I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

A. a Domain IA, comprising:

- (1) a sub-Domain (IA1), which comprises a VL Domain capable of binding to either B7-H3 (VL<sub>B7-H3</sub>) or CD3 (VL<sub>CD3</sub>); and
- (2) a sub-Domain (IA2), which comprises a VH Domain capable of binding to either B7-H3 (VH<sub>B7-H3</sub>) or CD3 (VH<sub>CD3</sub>);

wherein the sub-Domains IA1 and IA2 are separated from one another by a polypeptide linker of 12 or less amino acid residues, and are coordinately selected, such that:

- (a) the sub-Domain IA1 comprises the VL Domain capable of binding to B7-H3 (VL<sub>B7-H3</sub>) and the sub-Domain IA2 is selected to comprise the VH Domain capable of binding to CD3 (VH<sub>CD3</sub>); or
- (b) the sub-Domain IA1 comprises the VL Domain capable of binding to CD3 (VL<sub>CD3</sub>) and the sub-Domain IA2 is selected to comprise the VH Domain capable of binding to B7-H3 (VH<sub>B7-H3</sub>); and

B. an optionally present Domain IB, comprising a polypeptide linker linked to a Heterodimer-Promoting Domain;

C. a Domain IC, comprising a polypeptide linker linked to a CH2-CH3 Domain of an antibody;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

A. a Domain IIA, comprising:

- (1) a sub-Domain (IIA1), which comprises a VL Domain capable of binding to either B7-H3 (VL<sub>B7-H3</sub>) or CD3 (VL<sub>CD3</sub>); and
- (2) a sub-Domain (IIA2), which comprises a VH Domain capable of binding to either B7-H3 (VH<sub>B7-H3</sub>) or CD3 (VH<sub>CD3</sub>);

wherein the sub-Domains IIA1 and IIA2 are separated from one another by a polypeptide linker of 12 or less amino acid residues, and are coordinately selected, such that:

- (a) when the sub-Domain IIA1 comprises the VL Domain capable of binding to B7-H3 (VL<sub>B7-H3</sub>), the sub-Domain IIA1 is selected to comprise the VL Domain capable of binding to CD3 (VL<sub>CD3</sub>)

and the sub-Domain IIA2 is selected to comprise the VH Domain capable of binding to B7-H3 ( $\text{VH}_{\text{B7-H3}}$ ); and

- (b) when the sub-Domain IA1 comprises the VL Domain capable of binding to CD3 ( $\text{VL}_{\text{CD3}}$ ), then sub-Domain IIA1 is selected to comprise the VL Domain capable of binding to B7-H3 ( $\text{VL}_{\text{B7-H3}}$ ) and the sub-Domain IIA2 is selected to comprise the VH Domain capable of binding to CD3 ( $\text{VH}_{\text{CD3}}$ );

B. an optionally present Domain IIB, comprising a polypeptide linker linked to a Heterodimer-Promoting Domain;

III. the third polypeptide chain comprises, in the N-terminal to C-terminal direction a Domain IIIC that comprises a polypeptide linker linked to a CH2-CH3 Domain of an antibody;

wherein:

- (A) (1) at least one of the optionally present Domain IB and the optionally present Domain IIB is present, and wherein the Domain IB or IIB that is present has a positive or negative charge; or
- (2) both the optionally present Domain IB and the optionally present Domain IIB are present, wherein:
  - (i) one of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain having a positive charge, and the other of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain having a negative charge; or
  - (ii) one of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain that comprises the amino acid sequence GVEPKSC (**SEQ ID NO:6**) or VEPKSC (**SEQ ID NO:7**), and the other of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain that comprises the amino acid sequence GFNRGEC (**SEQ ID NO:8**) or FNRGEC (**SEQ ID NO:9**);

(B) the  $\text{VL}_{\text{B7-H3}}$  and the  $\text{VH}_{\text{B7-H3}}$  interact to form an epitope-binding domain capable of binding an epitope of B7-H3, and the  $\text{VL}_{\text{CD3}}$  and  $\text{VH}_{\text{CD3}}$  form an epitope-binding domain capable of binding an epitope of CD3; and

(C) the CH2-CH3 Domains of the first and third polypeptide chains form an Fc Domain capable of binding to an Fc receptor.

[0025] The invention further concerns the embodiments of such B7-H3 x CD3 bispecific monovalent Fc diabodies, which are capable of cross-reacting with both human and primate B7-H3 and CD3.

[0026] The invention particularly concerns the embodiments of such B7-H3 x CD3 bispecific monovalent Fc diabodies wherein:

- (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:53**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:55**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or
- (B) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:59**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:60** and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or
- (C) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:61**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:62**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**
- (D) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:63**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:64**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

[0027] The B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present invention are preferably capable of mediating redirected killing of target tumor cells using human T cells in an assay employing a target human tumor cell line selected from the group consisting of: A498 (kidney cancer), JIMT-1/Luc (breast cancer), A375 (melanoma); 22Rv1 (prostate cancer), Detroit562 (pharyngeal cancer), DU145 (prostate cancer); BxPC3 (pancreatic cancer), SKMES-1 (lung cancer), and U87 (glioblastoma), and using purified human primary T cells as effector cells at an Effector cell to T cell ratio of 1:1, 5:1, or 10:1. In such an assay, target tumor cell killing is measured using a lactate dehydrogenase (LDH) release assay in which the enzymatic activity of LDH released from cells upon cell death is quantitatively measured, or by a luciferase assay in which luciferase relative light unit (RLU) is the read-out to indicate relative viability of target cells, which have been engineered to express both the green

fluorescent protein (GFP) and luciferase reporter genes. The observed EC<sub>50</sub> of such redirected killing is about 1.5 µg/mL or less, about 1.0 µg/mL or less, about 500 ng/mL or less, about 300 ng/mL or less, about 200 ng/mL or less, about 100 ng/mL or less, about 50 ng/mL or less.

**[0028]** The B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present invention are preferably capable of mediating the inhibition of human tumor growth in a co-mix xenograft in which such molecules are introduced into NOD/SCID mice along with 22Rv1 (human prostate cancer) or A498 (human kidney cancer) tumor cells and activated human T cells at a ratio of 5:1. Additionally, or alternatively the B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present invention are capable of mediating the inhibition of human tumor growth and/or exhibiting anti-tumor activity in a in an xenograft model in female NSG B2m - / - mice:

- (A) implanted with human PBMCs ( $1 \times 10^7$ ) by intraperitoneal (IP) injection on Day -1 and Detroit (human pharyngeal cancer tumor cells ( $5 \times 10^6$ )) intradermally (ID) on Day 0, and administration of diabody on or Days 20, 22, 23, 26, 28, 30, 33, 35, and 37; or
- (B) implanted with A498 (human kidney cancer tumor cells ( $5 \times 10^6$ )) intradermally (ID) on Day 0, and human PBMCs ( $1 \times 10^7$ ) by intraperitoneal (IP) injection on Day 13 and administration of diabody on Days 33, 35, 36, 39, 41, 43, 46, 48, and 50.

**[0029]** Preferably, the B7-H3 x CD3 bi-specific monovalent Fc diabodies of the present invention are capable of inhibiting tumor growth in such xenograft models when provided at a concentration of greater than about 1.0 mg/kg, at a concentration of about 1 mg/kg, at a concentration of about 0.5 mg/kg, at a concentration of about 0.25 mg/kg, at a concentration of about 0.1 mg/kg, at a concentration of about 0.05 mg/kg, at a concentration of about 0.02 mg/kg, at a concentration of about 0.01 mg/kg, or at a concentration of about 0.005 mg/kg, or at a concentration less than 0.005 mg/kg.

**[0030]** The invention additionally provides any of the above-described B7-H3 x CD3 bi-specific monovalent Fc diabodies for use as a pharmaceutical.

**[0031]** The invention additionally provides any of the above-described B7-H3 x CD3 bi-specific monovalent Fc diabodies for use in the treatment of a disease or condition associated with or characterized by the expression of B7-H3, or in a method of treating a disease or

condition characterized by the expression of B7-H3, particularly wherein the disease or condition associated with or characterized by the expression of B7-H3 is cancer, and more particularly, 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 phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, 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.

### **Brief Description Of The Drawings**

**[0032]** **Figure 1** illustrates the structure of a covalently associated bispecific monovalent diabody composed of two polypeptide chains, which does not comprise an Fc Region. The polypeptide chains are covalently associated to one another via disulfide bonds that form between cysteine ("C") residues.

**[0033]** **Figures 2A and 2B** illustrate the structures of two versions of the first, second and third polypeptide chains of a three chain bispecific monovalent Fc diabody of the present invention (Version 1, **Figure 2A**; Version 2, **Figure 2B**). The polypeptide chains are

covalently associated to one another via disulfide bonds that form between cysteine (“C”) residues

**[0034]** **Figures 3A-3J** show FACS histograms of A498 (kidney cancer) (**Figure 3A**), JIMT-1/Luc (breast cancer) (**Figure 3B**), A375 (melanoma) (**Figure 3C**), 22Rv1 (prostate cancer) (**Figure 1D**), Detroit562 (pharyngeal cancer) (**Figure 1E**), DU145 (prostate cancer) (**Figure 3F**), BxPC-3 (pancreatic cancer) (**Figure 3G**), SKMES-1 (lung cancer) (**Figure 3H**), U87 (glioblastoma) (**Figure 3I**), and Raji (B-lymphoma) (**Figure 3J**) cell lines. Dashed lines represent cells stained with an isotype control PE-labeled antibody and solid lines represent cells stained with anti-B7-H3-PE antibody.

**[0035]** **Figures 4A-4E** show FACS histograms of anti-EK-coil antibody fluorescence on B7-H3-expressing target cancer cell lines (**Figures 4A-4D**) or human primary T cells (**Figure 4E**). DART-A at a concentration of 10 µg/mL was added to B7-H3-expressing cancer cell lines (A498 (**Figure 4A**), JIMT-1/Luc (**Figure 4B**), Detroit562 (**Figure 4C**), or 22Rv1 (**Figure 4D**)) or human primary T cells (**Figure 4E**) and incubated for 30 minutes. Cells were analyzed by FACS for DART-A cell surface binding after further incubation with biotin-conjugated anti-EK-coil antibody mixed with APC-streptavidin. Solid lines represent DART-A binding profile on cells. Non-specific staining on cells from biotin-conjugated anti-EK-coil antibody is shown by the dashed lines.

**[0036]** **Figures 5A-5L** show dose-response curves for DART-A-mediated cytotoxicity on B7-H3-expressing cell lines (A498 (**Figure 5A**), JIMT-1/Luc (**Figures 5B-5C**), A375 (**Figure 5D**), U87 (**Figure 5E**), DU145 (**Figure 5F**), BxPC-3 (**Figure 5G**), SKMES-1 (**Figure 5H**), Detroit562 (**Figure 5I**) and 22Rv1 (**Figure 5J**)) and B7-H3-negative cell lines (CHO (**Figure 5K**) and Raji (**Figure 5L**)). DART-A or control DART was incubated *in vitro* with the different tumor cell lines and primary human T cells at an effector cell:target cell (E:T) ratio of 5:1 for about 24 hours. Percent cytotoxicity was evaluated using the LDH release assay for all cell lines (**Figures 5A-5B** and **5D-5L**). In addition, cytotoxicity was measured using the LUM assay for the JIMT-1/Luc cell line (**Figure 5C**). Representative data are shown from multiple experiments using T cells from multiple donors. **DART-A: ●; Control DART: ■.**

**[0037]** **Figures 6A-6F** show DART-A-mediated redirected killing of A498 cells (**Figures 6A, 6C and 6E**) and A375 cells (**Figures 6B, 6D and 6F**) at E:T ratios of 10:1 (**Figures 6A**

and 6B), 5:1 (Figures 6C and 6D) and 1:1 (Figures 6E and 6F). Cytotoxicity was determined by LDH assay. **DART-A: ●; Control DART: ■.**

[0038] **Figures 7A-7E** show dose-response curves of DART-A-mediated redirected target cell killing (**Figure 7A**) and induction of T cell activation markers CD25 (**Figures 7B and 7C**) and CD69 (**Figures 7D and 7E**) on CD4+ (**Figures 7B and 7D**) and CD8+ (**Figures 7C and 7E**) T cells following incubation with purified T cells as effector cells and A498 target cells at an E:T cell ratio of 10:1 for 24 hours. **DART-A: ●; Control DART: ■. T cells + A498 cells: ▼; T cells alone ▲.**

[0039] **Figures 8A-8B** show DART-A-mediated T cell proliferation in the presence of B7-H3-positive target cells. Proliferation of human primary T cells was evaluated by FACS analysis after co-culturing of CFSE-labeled human primary T cells with A498 target cells at an E:T ratio of 10:1 in the presence of **DART-A** (heavy line) or **Control DART** (thin line filled) at 10  $\mu$ g/mL for 72 hours (**Figure 8A**) or 96 hours (**Figure 8B**).

[0040] **Figure 9A-9D** show DART-A efficiently binds to human (**Figure 9A**) and cynomolgus monkey (**Figure 9B**) B7-H3-expressing CHO cells and mediates redirected killing of the human (**Figure 9C**) and cynomolgus monkey (**Figure 9D**) B7-H3-expressing CHO cells following incubation with purified human primary T cells as effector cells and B7-H3-expressing CHO target cells at an E:T cell ratio of 5:1 for 24 hours. Cytotoxicity was measured using the LDH assay. **DART-A: ●; Control DART: ■.**

[0041] **Figures 10A-10B** show that DART-A is capable of binding to cynomolgus monkey and human primary T cells. DART-A at 10  $\mu$ g/mL was added to cynomolgus monkey (**Figure 10A**) or human (**Figure 10B**) PBMCs and cells were incubated for 30 minutes at 4°C followed by a second incubation with biotin-conjugated anti-EK-coil antibody mixed with APC-streptavidin. Cells were analyzed by FACS for DART-A T cell surface binding (thick lines) on gated total combined CD4+ and CD8+ cells. Non-specific staining on cells from biotin-conjugated anti-EK-coil secondary antibody is shown by the thin line/thin line with shading.

[0042] **Figures 11A-11C** show DART-A-mediated redirected killing of B7-H3-positive target cell lines JIMT-1/Luc (**Figures 11A and 11B**) and A498 (**Figure 11C**) using cynomolgus monkey PBMCs at an E:T ratio of 30:1. Cytotoxicity was measured using the LUM assay (**Figure 11A**) or the LDH assay (**Figures 11B and 11C**). **DART-A: ●; Control DART: ■.**

**[0043]** **Figure 12** shows the inhibition of tumor growth by DART-A in mice implanted with 22Rv1 tumor cells in the presence of activated human T cells. Female NOD/SCID mice (n = 8/group) were implanted SC with 22Rv1 tumor cells ( $5 \times 10^6$ ) co-mixed with activated human T cells ( $1 \times 10^6$ ) on Day 0 followed by treatment with vehicle control (●), Control DART (○), or DART-A at 0.5 mg/kg (■), 0.1 mg/kg (▲), 0.02 mg/kg (▼) or 0.004 mg/kg (◆) on Days 0, 1, 2 and 3 for a total of 4 doses administered IV. Tumor volume is shown as group mean  $\pm$  SEM.

**[0044]** **Figure 13** shows the inhibition of tumor growth by DART-A in mice implanted with A498 tumor cells in the presence of activated human T cells. Female NOD/SCID mice (n = 8/group) were implanted SC with A498 tumor cells ( $5 \times 10^6$ ) co-mixed with activated human T cells ( $1 \times 10^6$ ) on Day 0 followed by treatment with vehicle control (●), Control DART at 0.5 mg/kg (○), or DART-A at 0.5 mg/kg (■), 0.1 mg/kg (▲), 0.02 mg/kg (▼) or 0.004 mg/kg (◆) on Days 0, 1, 2 and 3 for a total of 4 doses administered IV. Tumor volume is shown as group mean  $\pm$  SEM.

**[0045]** **Figure 14** shows the anti-tumor activity of DART-A in NSG B2m-/- mice implanted with A498 tumor cells and reconstituted with human effector cells. Female NSG B2m-/- mice (n = 7/group) were implanted ID with A498 tumor cells ( $5 \times 10^6$  cells) on Day 0 followed by human PBMCs ( $1 \times 10^7$ ) implanted IP on Day 13. The groups were then treated with vehicle control (●), Control DART at 0.5 mg/kg (○), or DART-A at 1 mg/kg (■), 0.1 mg/kg (▲), 0.01 mg/kg (▼) or 0.001 mg/kg (◆) on Days 33, 35, 36, 39, 41, 43, 46, 48, and 50. Tumor volume is shown as group mean  $\pm$  SEM.

**[0046]** **Figure 15** shows the anti-tumor activity of DART-A in NSG B2m-/- mice implanted with Detroit562 tumor cells and reconstituted with human effector cells. Female NSG B2m-/- mice (n = 8/group) were implanted ID with Detroit562 tumor cells ( $5 \times 10^6$  cells) on Day 0, while human PBMCs ( $1 \times 10^7$ ) were implanted IP on Day -1. Subsequently the groups were treated with vehicle control (●), Control DART at 0.5 mg/kg (○) or DART-A at 1 mg/kg (■), 0.5 mg/kg (▲), 0.25 mg/kg (▼) or 0.1 mg/kg (◆) for a total of 9 doses administered IV on Days 20, 22, 23, 26, 28, 30, 33, 35, and 37. Tumor volume is shown as group mean  $\pm$  SEM.

**[0047]** **Figures 16A-16B** show the anti-tumor activity of DART-A in NSG MHC11 -/- mice implanted with Detroit562 tumor cells and reconstituted with human effector cells. On Day 0

MHC11  $^{-/-}$  mice (n = 7/vehicle control; 7/Group I; and 5/Group II) were implanted ID with Detroit562 tumor cells ( $5 \times 10^6$  cells) and were implanted IP with human PBMCs ( $1 \times 10^7$  cells). Subsequently the groups were treated with vehicle control (●), or DART-A at 0.5 mg/kg (▲). Group I (**Figure 16A**) received 5 doses administered IV on Days 15, 22, 29, 36 and 43. Group II (**Figure 16B**) received 3 doses administered IV on Days 15, 29 and 43. Tumor volume is shown as the group mean  $\pm$  SEM.

**[0048]** **Figure 17** shows the pharmacokinetic profiles of DART-A and DART-B in cynomolgus monkeys. Groups of cynomolgus monkeys (n = 2/group; 1M/1F) were administered one dose of 0.5 mg/kg then three weekly doses of 1.0 mg/kg of DART-A or DART-B for a total of four doses administered IV. The serum concentration of **DART-A** (solid lines) and **DART-B** (dashed lines) for each of the four test animals over the course of the study are plotted.

#### **Detailed Description of the Invention:**

**[0049]** The present invention is directed to bispecific monovalent diabodies that possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 (*i.e.*, a “B7-H3  $\times$  CD3 bispecific monovalent diabody”). Most preferably, such B7-H3  $\times$  CD3 bispecific monovalent diabodies are composed of three polypeptide chains and possess one binding site specific for an epitope of B7-H3 and one binding site specific for an epitope of CD3 and additionally comprise an immunoglobulin Fc Domain (*i.e.*, a “B7-H3  $\times$  CD3 bispecific monovalent Fc diabody”). The bispecific monovalent Fc diabodies of the present invention are capable of simultaneous binding to B7-H3 and CD3. The invention is directed to pharmaceutical compositions that contain such bispecific monovalent Fc diabodies. The invention is additionally directed to methods for the use of such diabodies in the treatment of cancer and other diseases and conditions.

### **I. Antibodies and Other Binding Molecules**

#### **A. Antibodies**

**[0050]** As used herein, the term “**antibodies**” encompasses any molecule possessing an immunoglobulin Variable Domain capable of immunospecifically binding to an epitope (an “an epitope-binding site”). The term thus encompasses not only intact polyclonal or monoclonal antibodies, but also mutants thereof, naturally occurring variants, fusion proteins comprising such epitope-binding site, humanized antibodies and chimeric antibodies, and any

other modified configuration of the immunoglobulin molecule capable of immunospecifically binding to an epitope. Throughout this application, the numbering of amino acid residues of the constant regions of the light and heavy chains of antibodies is according to the EU index as in Kabat *et al.* (1992) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242. 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')<sub>2</sub> Fv), and single chain (scFv), as well as the epitope-binding domain of a diabody.

**[0051]** The term “**monoclonal antibody**” refers to a homogeneous antibody population capable of immunospecifically binding to an epitope. The term “monoclonal antibody” 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, production in transgenic animals, *etc.*). 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. 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 bispecific molecules of the invention as well as a chimeric antibody, a humanized antibody, 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. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415).

**[0052]** 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.

#### **B. Bispecific Antibodies, Multi-Specific Diabodies and DART® Diabodies**

**[0053]** The provision of non-mono-specific “**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 bivalence, 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.

**[0054]** 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 $\gamma$  receptors (Fc $\gamma$ 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 $\gamma$ 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).

**[0055]** 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 mono-specific 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).

**[0056]** 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).

**[0057]** In the face of this challenge, the art has however succeeded in developing stable, covalently bonded heterodimeric non-mono-specific diabodies, termed **DART® (Dual-Affinity Re-Targeting Reagents)** 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 Fc gamma 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 Patents No. 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 Publications No. 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 Publications No. 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.*

**[0058]** 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. They are able to promote redirected T cell mediated killing of cells expressing target antigens. In one embodiment, the third Domains of the first and second polypeptide chains each contain a

cysteine (“C”) 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 Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538).

**[0059]** The preferred Fc-bearing DART® diabodies of the present invention comprise three polypeptide chains, and are depicted in **Figures 2A-2B**. 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 of such DART® 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 of such DART® diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such DART® 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 DART® diabodies of the present invention are B7-H3 x CD3 bispecific monovalent diabodies that are capable of binding to the “first epitope,” which may be either CD3 or B7-H3, and the “second epitope,” which is B7-H3 when the first epitope is CD3, and is CD3 when the first epitope is B7-H3. 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 DARTs® diabodies of the present invention may have either of two orientations (**Table 1**):

**Table 1**

First Orientation	3 <sup>rd</sup> Chain	NH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> -COOH
	1 <sup>st</sup> Chain	NH <sub>2</sub> -VL1-VH2-Heterodimer-Promoting Domain-CH <sub>2</sub> -CH <sub>3</sub> -COOH
	2 <sup>nd</sup> Chain	NH <sub>2</sub> -VL2-VH1-Heterodimer-Promoting Domain-COOH
Second Orientation	3 <sup>rd</sup> Chain	NH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> -COOH
	1 <sup>st</sup> Chain	NH <sub>2</sub> -CH <sub>2</sub> -CH <sub>3</sub> -VL1-VH2-Heterodimer-Promoting Domain-COOH
	2 <sup>nd</sup> Chain	NH <sub>2</sub> -VL2-VH1-Heterodimer-Promoting Domain-COOH

## II. Preferred B7-H3 x CD3 Bispecific Monovalent Diabodies Of The Present Invention

**[0060]** The present invention is particularly directed to such Fc-bearing DARTs® diabodies that are capable of simultaneous binding to B7-H3 and CD3, and are thus B7-H3 x CD3 bispecific monovalent DART® diabodies, and to the uses of such molecules in the treatment of cancer and other diseases and conditions. Although non-optimized B7-H3 x CD3 bispecific monovalent diabodies 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 B7-H3 x CD3 bispecific monovalent diabodies by modifying or refining their sequences.

**[0061]** Preferably, as shown in **Figure 2A**, the first 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 an epitope of a “first” antigen (**VL1**) (either CD3 or B7-H3), a Heavy Chain Variable Domain (**VH**) capable of binding to an epitope of a “second” antigen (**VH2**) (B7-H3, if the first antigen was CD3; CD3, if the first antigen was B7-H3), 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 (**VL2**) is linked to a Heterodimer-Promoting Domain by an intervening linker peptide (**Linker 2**). In a preferred B7-H3 x CD3 bispecific monovalent Fc diabody embodiment, the C-terminus of the Heterodimer-Promoting Domain is linked to the CH<sub>2</sub>-CH<sub>3</sub> domains of an Fc Region (“**Fc Domain**”) by an intervening linker peptide (**Linker 3**) or by an intervening spacer-linker peptide (**Spacer-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 – Spacer-Linker 3 – Fc Domain.

**[0062]** Alternatively, as shown in **Figure 2B**, the first of such three polypeptide chains will contain, in the N-terminal to C-terminal direction, an N-terminus, Linker 3, the CH2-CH3 domains of an Fc Region (“**Fc Domain**”), an intervening spacer peptide (**Linker 4**), having, for example the amino acid sequence: APSSS (**SEQ ID NO:51**) or the amino acid sequence APSSSPME (**SEQ ID NO:52**), a Light Chain Variable Domain (**VL**) capable of binding to an epitope of a “first” antigen (**VL1**) (either CD3 or B7-H3), a Heavy Chain Variable Domain (**VH**) capable of binding to an epitope of a “second” antigen (**VH2**) (B7-H3, if the first antigen was CD3; CD3, if the first antigen was B7-H3), 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 (**VH2**) 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.

**[0063]** Preferably, for either of such two preferred orientations, 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 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: VL1 – Linker 1 – VH2 – Linker 2 – Heterodimer-Promoting Domain.

**[0064]** Preferably, for either of such two preferred orientations, the third of such three polypeptide chains will contain the linker peptide (**Linker 3**) and the CH2-CH3 domains of an Fc region (“**Fc Domain**”). As the third chain polypeptide chain does not comprise a VL Domain or a VH Domain the third polypeptide chain may be identical between two or more different B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention.

**[0065]** 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 B7-H3 or CD3). 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 B7-H3 or CD3). 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 B7-H3 and CD3.

#### A. Preferred Linkers

**[0066]** 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:1**): GGGSGGGG.

**[0067]** 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:2**), 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:3**), which is derived from the IgG CH1 domain. Since the **Linker 2**, ASTKG (**SEQ ID NO:3**) 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:12** or the K-coil of **SEQ ID NO:13** (see below).

**[0068]** The purpose of **Linker 3** is to separate the Heterodimer-Promoting Domain of a polypeptide chain from the Fc Domain of that polypeptide chain. 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:4**). A preferred sequence for **Spacer-Linker 3** has the amino acid sequence: GGGDKTHTCPPCP (**SEQ ID NO:5**).

### B. Preferred Heterodimer-Promoting Domains

**[0069]** The formation of heterodimers of the first and second polypeptide chains can be driven by the inclusion of “**Heterodimer-Promoting Domains**.” Such domains include GVEPKSC (**SEQ ID NO:6**) or VEPKSC (**SEQ ID NO:7**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:8**) or FNRGEC (**SEQ ID NO:9**) on the other polypeptide chain (US2007/0004909).

**[0070]** More preferably, however, the Heterodimer-Promoting Domains of the present invention are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) “*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*,” Biomacromolecules 9:3173–3180; Arndt, K.M. *et al.* (2001) “*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*,” J. Molec. Biol. 312:221-228; Arndt, K.M. *et al.* (2002) “*Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils*,” Structure 10:1235-1248; Boucher, C. *et al.* (2010) “*Protein Detection By Western Blot Via Coiled-Coil Interactions*,” Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) “*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*,” J. Mol. Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) “*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*,” Biochemistry 42:1754-1763; Fernandez-Rodriquez, J. *et al.* (2012) “*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*,” Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) “*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*,” Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) “*Structural Specificity In Coiled-Coil Interactions*,” Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002)

“Designing Heterodimeric Two-Stranded  $\alpha$ -Helical Coiled-Coils: The Effects Of Hydrophobicity And  $\alpha$ -Helical Propensity On Protein Folding, Stability, And Specificity,” J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) “The  $d'$ -- $d$ -- $d'$  Vertical Triad is Less Discriminating Than the  $a'$ -- $a$ -- $a'$  Vertical Triad in the Antiparallel Coiled-coil Dimer Motif,” J. Amer. Chem. Soc. 134(5):2626–2633; Straussman, R. *et al.* (2007) “Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface,” J. Molec. Biol. 366:1232-1242; Tripet, B. *et al.* (2002) “Kinetic Analysis of the Interactions between Tropomodulin C and the C-terminal Tropomodulin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance,” J. Molec. Biol. 323:345–362; Woolfson, D.N. (2005) “The Design Of Coiled-Coil Structures And Assemblies,” Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) “A Ligand-Pseudoreceptor System Based On *de novo* Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism,” J. Gene Med. 10:355-367).

**[0071]** Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimer-Promoting Domain of one polypeptide chain may comprise a sequence of negatively charged amino acid residues and the Heterodimer-Promoting Domain of the other polypeptide chain may comprise a sequence of negatively charged amino acid residues. In particular, embodiments the coil domains comprise eight negatively charged amino acid residues or eight positively charged residues. It is immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain. However, a preferred B7-H3 x CD3 bispecific monovalent Fc diabody of the present invention has a first polypeptide chain having a negatively charged coil. The positively charged amino acid of a positively charged coil domain may be lysine, arginine, histidine, *etc.*, and is preferably lysine. The negatively charged amino acid of a negatively charged coil may be glutamic acid, aspartic acid, *etc.*, and is preferably glutamic acid.

**[0072]** The B7-H3 x CD3 bispecific monovalent DART® diabodies of the present invention may possess only a single Heterodimer-Promoting Domain (*i.e.*, either the first polypeptide chain or the second polypeptide chain, but not both, will contain a Heterodimer-Promoting Domain. The presence of such single Heterodimer-Promoting Domain promotes heterodimerization by impeding the formation of diabodies that are homodimers (such molecules either lacking any Heterodimer-Promoting Domain, or possessing two repelling (like-charged) Heterodimer-Promoting Domains). However, it is preferred for both the first

and second polypeptide chains of the diabodies of the present invention to contain Heterodimer-Promoting Domains.

**[0073]** In a preferred embodiment, one of the Heterodimer-Promoting Domains will comprise four tandem “E-coil” helical domains (**SEQ ID NO:10**: EVAALEEK-EVAALEEK-EVAALEEK-EVAALEEK), 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:11**: 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:10** has been modified to contain a cysteine residue: EVAACEK-EVAALEEK-EVAALEEK-EVAALEEK (**SEQ ID NO:12**) 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:11** has been modified to contain a cysteine residue: KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:13**) is utilized.

### C. Covalent Bonding of the Polypeptide Chains

**[0074]** The B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention are engineered so that their first and second polypeptide chains covalently bond to one another via one or more cysteine residues positioned along their length. Such cysteine residues may be introduced into the intervening linker that separates the VL and VH domains of the polypeptides. Alternatively, **Linker 2** may contain a cysteine residue. Additionally, or alternatively, **Linker 3** may contain a cysteine residue, as in **SEQ ID NO:4** or **SEQ ID NO:5**. Most preferably, one or more coil domains of the Heterodimer-Promoting Domain will be substituted to contain a cysteine residue as in **SEQ ID NO:12** or **SEQ ID NO:13**.

### D. Preferred Fc Domains

**[0075]** The Fc Domain of the preferred B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention may be either a complete Fc region (*e.g.*, a complete IgG Fc region) or only a fragment of a complete Fc region. Although the Fc Domain of the preferred bispecific monovalent Fc diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, Fc $\gamma$ R(s)), more preferably such Fc Domain will have been modified to cause reduced binding to Fc $\gamma$ RIA (CD64), Fc $\gamma$ RIIA (CD32A), Fc $\gamma$ RIIB (CD32B), Fc $\gamma$ RIIIA

(CD16a) or Fc $\gamma$ RIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will have been modified to have substantially eliminated the ability of such Fc Domain to bind to such receptor(s). The Fc Domain of the preferred bispecific monovalent Fc diabodies 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 monovalent 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.*).

**[0076]** In a preferred embodiment the first and third polypeptide chains of the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention each comprise CH2-CH3 domains that complex together to form an immunoglobulin (IgG) Fc Domain. The amino acid sequence of an exemplary CH2-CH3 domain of human IgG1 is (**SEQ ID NO:14**):

231	240	250	260	270	280
APELLGGPSV	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	
290	300	310	320	330	
GVEVHNAKTK	PREEQYNSTY	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	
340	350	360	370	380	
PIEKTISKAK	GQPREPQVYT	LPPSREEMTK	NQVSLTCLVK	GFYPSDIAVE	
390	400	410	420	430	
WESNGQOPENN	YKTPPPVLDs	DGSFFLYSKL	TVDKSRWQQG	NVFSCSVMHE	
440	447				
ALHNHYTQKS	LSLSPGK				

**[0077]** The numbering of the residues in the constant regions of an IgG heavy chain is that of the EU index as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5<sup>th</sup> Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by references. The “EU index as in Kabat” refers to the numbering of the human IgG1 EU antibody. 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 B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention may be 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 B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention. Exemplary B7-H3 x CD3 bispecific monovalent Fc diabodies comprising the C-terminal residue of **SEQ ID NO:14** are provided below. Also specifically encompassed by the instant invention are such constructs that lack the C-terminal lysine residue of **SEQ ID NO:14**.

**[0078]** The CH2 and/or CH3 Domains of the first and third polypeptide chains may both be composed of **SEQ ID NO:14**, or a variant thereof.

**[0079]** In particular, it is preferred for the CH2-CH3 domains of the first and third polypeptide chains of the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention to exhibit decreased (or substantially no) binding to Fc $\gamma$ RIA (CD64), Fc $\gamma$ RIIA (CD32A), Fc $\gamma$ RIIB (CD32B), Fc $\gamma$ RIIIA (CD16a) or Fc $\gamma$ RIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region (**SEQ ID NO:14**)). Fc variants and mutant forms capable of mediating such altered binding are well known in the art and include amino acid substitutions at positions 234 and 235, a substitution at position 265 or a substitution at position 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 a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of the B7-H3 x CD3 bispecific monovalent Fc diabodies 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.

**[0080]** The CH2 and/or CH3 Domains of the first and third polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a “knob,” e.g., tryptophan) can be

introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, a “hole” (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the bispecific monovalent Fc 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). Preferably 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 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 monovalent Fc diabody comprising the first, second 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 bispecific monovalent Fc diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

**[0081]** A preferred sequence for the CH2 and CH3 Domains of the first polypeptide chain of the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention will have the “knob-bearing” sequence (**SEQ ID NO:15**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD  
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA  
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE  
WESNGQPENN YKTPPVVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE  
ALHNHYTQKS LSLSPGK

**[0082]** A preferred sequence for the CH2 and CH3 Domains of the third polypeptide chain of the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention will have the “hole-bearing” sequence (**SEQ ID NO:16**):

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

**[0083]** As will be noted, the CH2-CH3 Domains of **SEQ ID NO:15** and **SEQ ID NO:16** 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 Fc $\gamma$ RIA (CD64), Fc $\gamma$ RIIA (CD32A), Fc $\gamma$ RIIB (CD32B), Fc $\gamma$ RIIIA (CD16a) or Fc $\gamma$ RIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc region (**SEQ ID NO:14**)). Furthermore, specifically encompassed by the instant invention are B7-H3 x CD3 bispecific monovalent Fc diabodies constructs lacking the C-terminal lysine residue of **SEQ ID NO:14**, **SEQ ID NO:15**, and/or **SEQ ID NO:16**.

**[0084]** It is preferred that the first polypeptide chain will have a “knob-bearing” CH2-CH3 sequence, such as that of **SEQ ID NO:15**. However, as will be recognized, a “hole-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:16**) could be employed in the first polypeptide chain, in which case, a “knob-bearing” CH2-CH3 Domain (*e.g.*, **SEQ ID NO:15**) would be employed in the third polypeptide chain.

#### E. Preferred B7-H3 Variable Domains

**[0085]** The Antigen-Binding Domain of any anti-B7-H3 antibody may be used in accordance with the present invention. Exemplary antibodies that are immunospecific for human B7-H7 (designated “**B7-H3 mAb A**,” “**B7-H3 mAb B**,” and “**B7-H3 mAb C**”) are provided below.

## 1. B7-H3 mAb A

[0086] The amino acid sequence of the VL Domain of B7-H3 mAb A (**SEQ ID NO:17**) is shown below (CDR<sub>L</sub> residues are shown underlined):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS  
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFFGQ  
 GTKLEIK

CDR<sub>L1</sub> of B7-H3 mAb A: (**SEQ ID NO:18**) KASQNVDTNVA

CDR<sub>L2</sub> of B7-H3 mAb A: (**SEQ ID NO:19**) SASYRYS

CDR<sub>L3</sub> of B7-H3 mAb A: (**SEQ ID NO:20**) QQYNNYPFT

[0087] The amino acid sequence of the VH Domain of B7-H3 mAb A (**SEQ ID NO:21**) is shown below (CDR<sub>H</sub> residues are shown underlined):

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

CDR<sub>H1</sub> of B7-H3 mAb A: (**SEQ ID NO:22**) SFGMH

CDR<sub>H2</sub> of B7-H3 mAb A: (**SEQ ID NO:23**) YISSDSSAIYYADTVKG

CDR<sub>H3</sub> of B7-H3 mAb A: (**SEQ ID NO:24**) GRENIYYGSRLDY

## 2. B7-H3 mAb B

[0088] The amino acid sequence of the VL Domain of B7-H3 mAb B (**SEQ ID NO:25**) is shown below (CDR<sub>H</sub> residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY  
TSRLHSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPPTFGG  
 GTKLEIK

CDR<sub>L1</sub> of B7-H3 mAb B: (**SEQ ID NO:26**) RASQDISNYLN

CDR<sub>L2</sub> of B7-H3 mAb B: (**SEQ ID NO:27**) YTSRLHS

CDR<sub>L3</sub> of B7-H3 mAb B: (**SEQ ID NO:28**) QQGNTLPPT

[0089] The amino acid sequence of the VH Domain of B7-H3 mAb B (**SEQ ID NO:29**) is shown below (CDR<sub>H</sub> residues are shown underlined):

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

CDR<sub>H1</sub> of B7-H3 mAb B: (**SEQ ID NO:30**) SYWMQ

CDR<sub>H2</sub> of B7-H3 mAb B: (**SEQ ID NO:31**) TIYPGDGDTYTQKFKG

CDR<sub>H3</sub> of B7-H3 mAb B: (**SEQ ID NO:32**) RGIPRLWYFDV

### 3. B7-H3 mAb C

[0090] The amino acid sequence of the VL Domain of B7-H3 mAb C (**SEQ ID NO:33**) is shown below (CDR<sub>L</sub> residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYY  
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG  
 GTKLEIK

CDR<sub>L1</sub> of B7-H3 mAb C: (**SEQ ID NO:34**) RASQSISSYLN

CDR<sub>L2</sub> of B7-H3 mAb C: (**SEQ ID NO:35**) YTSRLQS

CDR<sub>L3</sub> of B7-H3 mAb C: (**SEQ ID NO:36**) QQGNTLPPT

[0091] The amino acid sequence of the VH Domain of B7-H3 mAb C (**SEQ ID NO:37**) is shown below (CDR<sub>H</sub> residues are shown underlined):

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

CDR<sub>H1</sub> of B7-H3 mAb C: (**SEQ ID NO:38**) SYWMQ

CDR<sub>H2</sub> of B7-H3 mAb C: (**SEQ ID NO:39**) TIYPGGGDTRYTQKFQG

CDR<sub>H3</sub> of B7-H3 mAb C: (**SEQ ID NO:40**) RGIPRLWYFDV

### F. Preferred CD3 Variable Domains

[0092] The Antigen-Binding Domain of any anti-CD3 antibody may be used in accordance with the present invention. An exemplary antibody that is immunospecific for human CD3 (designated “**CD3 mAb A**”) is provided below.

[0093] The amino acid sequence of the VL Domain of CD3 mAb A (**SEQ ID NO:41**) is shown below (CDR<sub>L</sub> residues are shown underlined):

QAVVTQEPLS TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI  
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF  
 GGGTKLTVLG

CDR<sub>L1</sub> of CD3 mAb A: (**SEQ ID NO:42**) RSSTGAVTTSNYAN

CDR<sub>L2</sub> of CD3 mAb A: (**SEQ ID NO:43**) GTNKRAP

CDR<sub>L3</sub> of CD3 mAb A: (**SEQ ID NO:44**) ALWYSNLWV

**[0094]** The amino acid sequence of the VH Domain of CD3 mAb A (**SEQ ID NO:45**) is shown below (CDR<sub>H</sub> residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWVRQA PGKGLEWVGR  
IRSKYNNYAT YYADSVKDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR  
HGNFGNSYVS WFAYWGQGTL VTVSS

CDR<sub>H1</sub> of CD3 mAb A: (**SEQ ID NO:46**) TYAMN

CDR<sub>H2</sub> of CD3 mAb A: (**SEQ ID NO:47**) RIRSKYNNYATYYADSVKD

CDR<sub>H3</sub> of CD3 mAb A: (**SEQ ID NO:48**) HGNFGNSYVSWFAY

**[0095]** In certain embodiments, the VH Domain of CD3 mAb A comprises an aspartate to glycine substitution at Kabat position 65 (**D65G** substitution, corresponds to residue 68 of SEQ ID NO:45), such that the amino acid sequence of CDR<sub>H2</sub> is: RIRSKYNNYATYYADSVK**G** (**SEQ ID NO:49**). The amino acid sequence of the VH Domain of CD3 mAb A having the **D65G** substitution (**SEQ ID NO:50**) is shown below (the substituted residue is shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS TYAMNWRQA PGKGLEWVGR  
 IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR  
 HGNFGNSYVS WFAYWGQGTL VTVSS

### III. Exemplary B7-H3 x CD3 Bispecific Monovalent Fc Diabodies

**[0096]** The invention provides B7-H3 x CD3 bispecific monovalent Fc diabodies capable of simultaneously and specifically binding to B7-H3 and to CD3. As indicated above, the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention comprise three polypeptide chains. The polypeptide chains of four exemplary B7-H3 x CD3 bispecific monovalent Fc diabodies capable that binding to B7-H3 and to CD3 (designated “**DART-A**,” “**DART-B**,” “**DART-C**,” and “**DART-D**”) are provided below.

#### A. **DART-A**

**[0097]** The first polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to B7-H3 (VL<sub>B7-H3</sub> B7-H3 mAb A) (**SEQ ID NO:17**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to CD3 (VH<sub>CD3</sub> CD3 mAb A) (**SEQ ID NO:45**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (E-coil) Domain (EVAAEK-EVAAEK-EVAAEK-EVAAEK (**SEQ ID NO:10**)), an intervening linker peptide (**Spacer-**

**Linker 3;** GGGDKTHTCPPCP (**SEQ ID NO:5**)), a “knob-bearing” Fc Domain (**SEQ ID NO:15**), and a C-terminus.

**[0098]** Thus, the first polypeptide chain of **DART-A** is composed of: **SEQ ID NO:17 – SEQ ID NO:1 – SEQ ID NO:45 – SEQ ID NO:2 – SEQ ID NO:10 – SEQ ID NO:5 – SEQ ID NO:15**.

**[0099]** The amino acid sequence of the first polypeptide of **DART-A** is (**SEQ ID NO:53**):

```
DIQLTQSPLS LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPVCPAPE AAGGPSVFLF
PPPKPKDTLMI SRTPEVTCVV VDVSHEDEPV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK
```

**[00100]** An exemplary polynucleotide encoding such a polypeptide is (**SEQ ID NO:54**):

```
gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggccctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaaggcgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttacccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact accctttcac cttcggccag
ggcaccaagc tggaaatcaa gggaggcgga tccggcggcg gaggcgaggt
gcagctggtg gagtctgggg gaggtctggt ccagcctgga gggtcctga
gactctcctg tgcagcctct ggattcacct tcagcacata cgctatgaat
tgggtccggcc aggctccagg gaaggggctg gagtgggtg gaaggatcag
gtccaagtac aacaattatg caacctacta tgccgactct gtgaaggata
gattcaccat ctcaagagat gattcaaaga actcaactgt tctgcaaatg
aacagcctga aaaccggagga cacggccgtg tattactgtg tgagacacgg
taacttcggc aattcttacg tgtcttgggt tgcttattgg ggacagggga
cactgggtgac tgtgtcttcc ggaggatgtg gcgggtggaga agtggccgca
ctggagaaag aggttgcgtc tttggagaag gaggtcgctg cacttggaaa
ggaggtcgca gcccctggaga aaggcggcgg ggacaaaact cacacatgcc
caccgtgccc agcacctgaa gccgcgggg gaccgtcagt cttcctcttc
cccccaaaac ccaaggacac cctcatgatc tcccgaccc ctgaggtcac
atgcgtggtg gtggacgtga gcccacgaaga ccctgagggtc aagttcaact
ggtacgtgga cggcgtggag gtgcataatg ccaagacaaa gccgcgggag
gagcagtaca acagcacgta ccgtgtggtc agcgtcctca ccgtcctgca
ccaggactgg ctgaatggca aggagtacaa gtgcaagggtc tccaaacaaag
ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc
cgagaaccac aggtgtacac cctgccccca tcccgagg agatgaccaa
gaaccaggtc agcctgtggt gcctggtaa aggcttctat cccagcgaca
```

tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc  
 acgcctcccg tgctggactc cgacggctcc ttcttcctct acagcaagct  
 caccgtggac aagagcaggt ggcagcaggg gaacgtctc tcatgtccg  
 tcatgtcatga ggctctgcac aaccactaca cgcagaagag cctctccctg  
 tctccggta aa

**[00101]** The second polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD3 (VL<sub>CD3</sub> CD3 mAb A) (**SEQ ID NO:41**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to B7-H3 (VH<sub>B7-H3</sub> B7-H3 mAb A) (**SEQ ID NO:21**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:11**), and a C-terminus.

**[00102]** Thus, the second polypeptide of **DART-A** is composed of: **SEQ ID NO:41** – **SEQ ID NO:1** – **SEQ ID NO:21** – **SEQ ID NO:2** – **SEQ ID NO:11**.

**[00103]** The amino acid sequence of the second polypeptide of **DART-A** is (**SEQ ID NO:55**):

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

**[00104]** An exemplary polynucleotide encoding such a polypeptide has the sequence (**SEQ ID NO:56**):

caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggAAC  
 tgtgaccctg acatgcagat ccagcacagg cgcagtgacc acatctaact  
 acgccaattg ggtgcagcag aagccaggac aggacccaag gggcctgatc  
 ggggttacaa acaaaagggc tccctggacc cctgcacggg tttcttggaa  
 tctgctggc ggaaaggccg ctctgactat taccggggca caggccgagg  
 acgaagccgaa ttactattgt gctctgtggt atagcaatct gtgggtgttc  
 ggggttggca caaaaactgac tgtgctgggaa ggggttggat ccggccggcgg  
 aggcgaggtg cagctggtcg agtctggcgg aggactggg cagcctggcg  
 gctccctgag actgtcttcg gccgcctccg gcttcacctt ctccagcttc  
 ggcatgcact gggccggca ggctccaggc aaggactgg aatgggtggc  
 ctacatctcc tccgactcct ccgcacatcta ctacccgac accgtgaagg  
 gcagggttacacatctccgg gacaacgcca agaactccct gtacctgcag  
 atgaactccc tgcgggacga ggacaccgccc gtgtactact gcggcagagg  
 ccgggagaat atctactacg gctcccgct ggattattgg ggccagggca  
 ccaccgtgac cgtgtcctcc ggaggatgtg gcgggtggaaa agtggccgca

ctgaaggaga aagttgctgc tttgaaagag aaggtcgccg cacttaagga  
aaaggctcgca gccctgaaag ag

**[00105]** The third polypeptide chain of **DART-A** comprises, in the N-terminal to C-terminal direction, an N-terminus, a peptide (**Linker 3**; DKTHTCPPCP (**SEQ ID NO:4**)), a “hole-bearing” Fc Domain (**SEQ ID NO:16**), and a C-terminus.

**[00106]** Thus, the third polypeptide of **DART-A** is composed of: **SEQ ID NO:4 – SEQ ID NO:16**.

**[00107]** The amino acid sequence of the third polypeptide of **DART-A** is (**SEQ ID NO:57**):

DKTHTCPPCP APEAAGGPSV FLFPPPKD<sup>T</sup> LMISRTPEVT CVVVDVSHED  
PEVKFNWYVD GVEVHN<sup>A</sup>TKT PREEQYNSTY RVVSVLTVLH QDWLNGKEYK  
CKVSNKALPA PIEKTISKAK GQP<sup>R</sup>PQVYT LPPSREEMTK NQVSLSCAVK  
GFYPSDIAVE WESNGQPENN YKTPPVLD<sup>S</sup> DGSFFLVSKL TVDKSRWQ<sup>Q</sup>G  
NVFSCSVMHE ALHNRYTQKS LSLS<sup>P</sup>KGK

**[00108]** A preferred polynucleotide that encodes such a polypeptide has the sequence (**SEQ ID NO:58**):

gacaaaactc acacatgccc accgtgccca gcacctgaag ccgcgggggg  
accgtcagtc ttcc<sup>T</sup>cttcc cccaaaacc caaggacacc ctcatgatct  
cccgacccc tgaggtcaca tgcgtggtg tggacgtgag ccacgaagac  
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc  
caagacaaag ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca  
gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag  
tgcaaggtct ccaacaaagc cctcc<sup>T</sup>agcc cccatcgaga aaaccatctc  
caaagccaaa gggcagcccc gagaaccaca ggtgtacacc ctgccccat  
cccg<sup>T</sup>ggagga gatgaccaag aaccagg<sup>T</sup>ca gcctgagttg cgcagtcaaa  
ggcttctatc ccagcgacat cgccgtggag tggagagca atgggcagcc  
ggagaacaac tacaagacca cgcctcccg<sup>T</sup> gctggactcc gacggctcct  
tcttcctcg<sup>T</sup> cagcaagctc accgtggaca agagcagg<sup>T</sup>g gcagcagg<sup>T</sup>gg  
aacgttctc catgctccgt gatgc<sup>T</sup>atgag gctctgcaca accgctacac  
gcagaagagc ctctccctgt ctccggtaa a

## B. DART-B

**[00109]** The first polypeptide chain of **DART-B** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to B7-H3 (VL<sub>B7-H3</sub> B7-H3 mAb B) (**SEQ ID NO:25**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to CD3 (VH<sub>CD3</sub> CD3 mAb A) (**SEQ ID NO:45**), an intervening linker peptide (**Linker 2**; GGC<sup>T</sup>GGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (E-coil) Domain (EVA<sup>A</sup>LEEK-EVA<sup>A</sup>LEEK-EVA<sup>A</sup>LEEK (**SEQ ID NO:10**)), an intervening linker peptide (**Spacer-**

**Linker 3;** GGGDKTHTCPPCP (**SEQ ID NO:5**)), a “knob-bearing” Fc Domain (**SEQ ID NO:15**), and a C-terminus.

[00110] Thus, the first polypeptide chain of **DART-B** is composed of: **SEQ ID NO:25 – SEQ ID NO:1 – SEQ ID NO:45 – SEQ ID NO:2 – SEQ ID NO:10 – SEQ ID NO:5 – SEQ ID NO:15**.

[00111] The amino acid sequence of the first polypeptide of **DART-B** is (**SEQ ID NO:59**):

```
DIQMTQSPSS LSASVGDRVT ITCRASQDIS NYLNWYQQKP GKAPKLLIYY
TSRLHSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKDRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPPCPAPE AAGGPSVFLF
PPPKPKDTLMI SRTPEVTCVV VDVSHEDEPV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK
```

[00112] The second polypeptide chain of **DART-B** comprises, comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD3 (VL<sub>CD3</sub> CD3 mAb A) (**SEQ ID NO:41**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to B7-H3 (VH<sub>B7-H3</sub> B7-H3 mAb B) (**SEQ ID NO:29**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (K-coil) Domain (KVAALKE–KVAALKE–KVAALKE–KVAALKE (**SEQ ID NO:11**), and a C-terminus.

[00113] Thus, the second polypeptide of **DART-B** is composed of: **SEQ ID NO:41 – SEQ ID NO:1 – SEQ ID NO:29 – SEQ ID NO:2 – SEQ ID NO:11**.

[00114] The amino acid sequence of the second polypeptide of **DART-B** is (**SEQ ID NO:60**):

```
QAVVTQEPLS TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTSY
WMQWVRQAPG QGLEWMGTIY PGDGDTRYTQ KFKGRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARRGIP RLWYFDVWQQ GTTVTVSSGG CGGGKVAALK
EKVAALKEKV AALKEKVAAL KE
```

[00115] The third polypeptide chain of **DART-B** comprises, in the N-terminal to C-terminal direction, an N-terminus, a peptide (Linker 3; DKTHTCPPCP (**SEQ ID NO:4**)), a “hole-bearing” Fc Domain (**SEQ ID NO:16**), and a C-terminus.

[00116] Thus, the third polypeptide of **DART-B** is composed of: **SEQ ID NO:4 – SEQ ID NO:16** and has the same amino acid sequence as the third polypeptide of **DART-A** (**SEQ ID NO:57**) provided above.

### C. DART-C

[00117] The first 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 B7-H3 (VL<sub>B7-H3</sub> B7-H3 mAb C) (**SEQ ID NO:33**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to CD3 (VH<sub>CD3</sub> CD3 mAb A having a **D65G** substitution) (**SEQ ID NO:50**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:10**)), an intervening linker peptide (**Spacer-Linker 3**; GGGDKTHTCPPCP (**SEQ ID NO:5**)), a “knob-bearing” Fc Domain (**SEQ ID NO:15**), and a C-terminus.

[00118] Thus, the first polypeptide chain of **DART-C** is composed of: **SEQ ID NO:33 – SEQ ID NO:1 – SEQ ID NO:50 – SEQ ID NO:2 – SEQ ID NO:10 – SEQ ID NO:5 – SEQ ID NO:15**.

[00119] The amino acid sequence of the first polypeptide of **DART-C** is (**SEQ ID NO:61**):

```

DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYY
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLTVSS GGCGGGEVAA
LEKEVAALEK EVAALEKEVA ALEKGGGDKT HTCPCPAPE AAGGPSVFLF
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
REPQVYTLPP SREEMTKNQV SLWCLVKGY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
SPGK

```

[00120] The second 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 CD3 (VL<sub>CD3</sub> CD3 mAb C) (**SEQ ID NO:41**), an intervening linker peptide (**Linker**

1; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to B7-H3 (VH<sub>B7-H3</sub> B7-H3 mAb B) (**SEQ ID NO:37**), an intervening linker peptide (**Linker 2**; GGCGGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:11**), and a C-terminus.

[00121] Thus, the second polypeptide of **DART-C** is composed of: **SEQ ID NO:41** – **SEQ ID NO:1** – **SEQ ID NO:37** – **SEQ ID NO:2** – **SEQ ID NO:11**.

[00122] The amino acid sequence of the second polypeptide of **DART-C** is (**SEQ ID NO:62**):

QAVVTQEPLS TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI  
 GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF  
 GGGTKLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTSY  
 WMQWVRQAPG QGLEWMGTIY PGGGDTRYTQ KFQGRVTITA DKSTSTAYME  
 LSSLRSEDTA VYYCARRGIP RLWYFDVWGQ GTTVTVSSGG CGGGKVAALK  
 EKVAALKEKV AALKEKVAAL KE

[00123] The third polypeptide chain of **DART-C** comprises, in the N-terminal to C-terminal direction, an N-terminus, a peptide (**Linker 3**; DKTHTCPPCP (**SEQ ID NO:4**)), a “hole-bearing” Fc Domain (**SEQ ID NO:16**), and a C-terminus.

[00124] Thus, the third polypeptide of **DART-C** is composed of: **SEQ ID NO:4** – **SEQ ID NO:16** and has the same amino acid sequence as the third polypeptide of **DART-A** (**SEQ ID NO:57**) provided above.

#### D. DART-D

[00125] The general structure of the first and second polypeptide chains of exemplary **DART-D** are the same as that of **DART-A**, **DART-B**, and **DART-C** except that **DART-D** comprises an alternative **Linker 2**, which lacks a cysteine residue, and comprises cysteine-containing Heterodimer-Promoting Domains. The first 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 to B7-H3 (VL<sub>B7-H3</sub> B7-H3 mAb C) (**SEQ ID NO:33**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to CD3 (VH<sub>CD3</sub> CD3 mAb A having a **D65G** substitution) (**SEQ ID NO:50**), an intervening linker peptide (**Linker 2**; ASTKG (**SEQ ID NO:3**)), a Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:12**)), an intervening linker peptide (**Spacer-Linker 3**;

GGGDKTHTCPPCP (**SEQ ID NO:5**)), a “knob-bearing” Fc Domain (**SEQ ID NO:15**), and a C-terminus.

**[00126]** Thus, the first polypeptide chain of **DART-D** is composed of: **SEQ ID NO:33 – SEQ ID NO:1 – SEQ ID NO:50 – SEQ ID NO:3 – SEQ ID NO:12 – SEQ ID NO:5 – SEQ ID NO:15**.

**[00127]** The amino acid sequence of the first polypeptide of **DART-D** is (**SEQ ID NO:63**):

```
DIQMTQSPSS LSASVGDRVT ITCRASQSI SYLNWYQQKP GKAPKLLIYY
TSRLQSGVPS RFSGSGSGTD FTLTISSLQP EDIATYYCQQ GNTLPPTFGG
GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN
WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISRD DSKNSLYLQM
NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLTVSS ASTKGEVAAC
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSL
PGK
```

**[00128]** The second polypeptide chain of **DART-D** comprises, comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD3 (VL<sub>CD3</sub> CD3 mAb C) (**SEQ ID NO:41**), an intervening linker peptide (**Linker 1**; **GGGSGGGG (SEQ ID NO:1)**), a VH domain of a monoclonal antibody capable of binding to B7-H3 (VH<sub>B7-H3</sub> B7-H3 mAb B) (**SEQ ID NO:37**), an intervening linker peptide (**Linker 2**; **ASTKG (SEQ ID NO:3)**), a Heterodimer-Promoting (K-coil) Domain (**KVAACKE – KVAALK – KVAALK – KVAALK (SEQ ID NO:13)**), and a C-terminus.

**[00129]** Thus, the second polypeptide of **DART-C** is composed of: **SEQ ID NO:41 – SEQ ID NO:1 – SEQ ID NO:37 – SEQ ID NO:3 – SEQ ID NO:13**.

**[00130]** The amino acid sequence of the second polypeptide of **DART-D** is (**SEQ ID NO:64**):

```
QAVVTQEPLS TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTLTVLG GGGSGGGGQV QLVQSGAEVK KPGASVKVSC KASGYTFTSY
WMQWVRQAPG QGLEWMGTIY PGGGDTRYTQ KFQGRVTITA DKSTSTAYME
LSSLRSEDTA VYYCARRGIP RLWYFDVWGQ GTTFTVSSAS TKGKVAACKE
KVAALKVVA ALKEKVAALK E
```

**[00131]** While the first and second polypeptide chains incorporate different linkers and Heterodimer-Promoting Domains, the third polypeptide chain of **DART-D** comprises, in the

N-terminal to C-terminal direction, an N-terminus, a peptide (**Linker 3**; DKTHTCPPCP (**SEQ ID NO:4**)), a “hole-bearing” Fc Domain (**SEQ ID NO:16**), and a C-terminus.

**[00132]** Thus, the third polypeptide of **DART-D** is composed of: **SEQ ID NO:4 – SEQ ID NO:16** and has the same amino acid sequence as the third polypeptide of **DART-A** (**SEQ ID NO:57**) provided above.

#### IV. Control DART® Diabodies

**[00133]** In order to more meaningfully demonstrate the properties of the B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention, a control bispecific monovalent Fc diabody was constructed capable of binding to fluorescein and CD3 (designated “**Control DART**”).

**[00134]** The anti-fluorescein antibody used to form the **Control DART®** diabody was 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 Idiotype Family*,” *J. Biol. Chem.* 264(3): 1565-1569) were used in control diabodies. The amino acid sequences of the variable light and variable heavy Domains of anti-fluorescein antibody 4-4-20 are as follows:

**[00135]** The amino acid sequence of the VL Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:65**) is shown below (CDR<sub>H</sub> residues are shown underlined):

DVVMTQTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK  
 VLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP  
WTFGGGTKLE IK

**[00136]** The amino acid sequence of the VH Domain of anti-fluorescein antibody 4-4-20 (**SEQ ID NO:66**) is shown below (CDR<sub>H</sub> residues are shown underlined):

EVKLDETGGG LVQPGRPMKL SCVASGFTFS DYWMNWVRQS PEKGLEWVAQ  
IRNKPNYET YYSDSVKGRF TISRDDSKSS VYLQMNNLRV EDMGIYYCTG  
SYYGMDYWGQ GTSVTVSS

**[00137]** The first polypeptide chain of **Control DART** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to fluorescein (VL<sub>Fluor</sub> 4-4-20) (**SEQ ID NO:65**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to CD3 (VH<sub>CD3</sub> CD3 mAb A having a **D65G** substitution) (**SEQ ID NO:50**), an intervening

linker peptide (**Linker 2**; GGC<sub>2</sub>GGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:10**)), an intervening linker peptide (**Spacer-Linker 3**; GGGDKTHTCPPCP (**SEQ ID NO:5**)), a “knob-bearing” Fc Domain (**SEQ ID NO:15**), and a C-terminus.

**[00138]** Thus, the first polypeptide chain of **Control DART** is composed of: **SEQ ID NO:65 – SEQ ID NO:1 – SEQ ID NO:50 – SEQ ID NO:2 – SEQ ID NO:10 – SEQ ID NO:5 – SEQ ID NO:15**.

**[00139]** The amino acid sequence of the first polypeptide chain of **Control DART** is (**SEQ ID NO:67**):

```
DVVMQTTPFS LPVSLGDQAS ISCRSSQSLV HSNGNTYLRW YLQKPGQSPK
VLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCQSSTHVP
WTFGGGKTLE IKGGGSGGGG EVQLVESGGG LVQPGGSLRL SCAASGFTFS
TYAMNWVRQA PGKGLEWVGR IRSKYNNYAT YYADSVKGRF TISRDDSKNS
LYLQMNSLKT EDTAVYYCVR HGNFGNSYVS WFAYWGQGTL VTVSSGGCGG
GEVAALEKEV AALEKEVAAL EKEVAALEKG GGDKTHTCPP CPAPEAAGGP
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK
TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK
AKGQPREPQV YTLPPSREEM TKNQVSLWCL VKGFYPSDIA VEWESNGQPE
NNYKTTPPVLDSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ
KSLSLSPGK
```

**[00140]** The second polypeptide chain of **Control DART** comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL domain of a monoclonal antibody capable of binding to CD3 (VL<sub>CD3</sub> CD3 mAb A) (**SEQ ID NO:37**), an intervening linker peptide (**Linker 1**; GGGSGGGG (**SEQ ID NO:1**)), a VH domain of a monoclonal antibody capable of binding to fluorescein (VH<sub>fluor</sub> 4-4-20) (**SEQ ID NO:65**), an intervening linker peptide (**Linker 2**; GGC<sub>2</sub>GGG (**SEQ ID NO:2**)), a Heterodimer-Promoting (K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:11**), and a C-terminus.

**[00141]** Thus, the second polypeptide chain of **Control DART** is composed of: **SEQ ID NO:37 – SEQ ID NO:1 – SEQ ID NO:65 – SEQ ID NO:2 – SEQ ID NO:11**.

**[00142]** The amino acid sequence of the second polypeptide chain of **Control DART** is (**SEQ ID NO:68**):

```
QAVVTQEPESL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGGEV KLDETGGGLV QPGRPMKLSC VASGFTFSDY
WMNWVRQSPE KGLEWVAQIR NKPYNYETYY SDSVKGRFTI SRDDSKSSVY
```

LQMNRLVED MGIYYCTGSY YGMDYWGQGT SVTVSSGGCG GGKVAALKEK  
VAALKEKVAAC LKEKVAALKE

**[00143]** The third polypeptide chain of **Control DART** comprises, in the N-terminal to C-terminal direction, an N-terminus, a peptide (**Linker 3**; DKTHTCPPCP (**SEQ ID NO:4**)), a “hole-bearing” Fc Domain (**SEQ ID NO:16**), and a C-terminus.

**[00144]** Thus, the third polypeptide chain of **Control DART** is composed of: **SEQ ID NO:4** – **SEQ ID NO:16** and has the same amino acid sequence as the third polypeptide of **DART-A** (**SEQ ID NO:57**) provided above.

## V. Pharmaceutical Compositions

**[00145]** 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 B7-H3 x CD3 bispecific monovalent Fc diabody 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 B7-H3 x CD3 bispecific monovalent Fc diabody of the invention and a pharmaceutically acceptable carrier.

**[00146]** The invention also encompasses pharmaceutical compositions comprising a B7-H3 x CD3 bispecific monovalent Fc diabody 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 cancer antigen (e.g., tumor specific monoclonal antibody or diabody) that is specific for at least one particular cancer antigen, and a pharmaceutically acceptable carrier. As used herein, the term “cancer antigen” denotes an antigen that is characteristically expressed on the surface of a tumor cell. Examples of cancer antigens include: **A33** (a colorectal carcinoma antigen; Almqvist, Y. 2006, *Nucl Med Biol.* Nov;33(8):991-998); **B1** (Egloff, A.M. *et al.* 2006, *Cancer Res.* 66(1):6-9); **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); **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); **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); **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); **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); **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); **CTLA4** (Peggs, K.S. *et al.* 2006 *Curr Opin Immunol.* 18(2):206-13); **EGF-R** (epidermal growth factor receptor; Adenis, A. *et al.* 2003 *Bull Cancer.* 90 Spec No:S228-32); **Erb** (ErbB1; ErbB3; ErbB4; Zhou, H. *et al.* 2002 *Oncogene* 21(57):8732-40; Rimon, E. *et al.* 2004 *Int J Oncol.* 24(5):1325-38); **GAGE** (GAGE-1; GAGE-2; Akcakanat, A. *et al.* 2006 *Int J Cancer.* 118(1):123-8); **GD2/GD3/GM2** (Livingston, P.O. *et al.* 2005 *Cancer Immunol Immunother.* 54(10):1018-25); **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 papillomavirus-E6/human papillomavirus-E7** (DiMaio, D. *et al.* 2006 *Adv Virus Res.* 66:125-59; **KSA** (17-1A) (Ragupathi, G. 2005 *Cancer Treat Res.* 123:157-80); **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; **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); **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); **sTn** (Holmberg, L.A. 2001 *Expert Opin Biol Ther.* 1(5):881-91); **TNF-receptor** (TNF- $\alpha$  receptor, TNF- $\beta$  receptor; or TNF- $\gamma$  receptor; van Horssen, R. *et al.* 2006 *Oncologist.* 11(4):397-408; Gardnerova, M. *et al.* 2000 *Curr Drug Targets.* 1(4):327-64); **VEGF receptor** (O'Dwyer, P.J. 2006 *Oncologist.* 11(9):992-8); **ADAM-9** (United States Patent Publication No. 2006/0172350; PCT Publication No. WO 06/084075); **ALCAM** (PCT Publication No. WO 03/093443); **Carboxypeptidase M** (United States Patent Publication No. 2006/0166291); **CD46** (United States Patent No. 7,148,038; PCT Publication No. WO 03/032814); **Cytokeratin 8** (PCT Publication No. WO 03/024191); **Ephrin receptors** (and in particular EphA2 (United States Patent No. 7,569,672; PCT Publication No. WO 06/084226);

**Integrin Alpha-V-Beta-6** (PCT Publication No. WO 03/087340); **JAM-3** (PCT Publication No. WO 06/084078); **KID3** (PCT Publication No. WO 05/028498); **KID31** (PCT Publication No. WO 06/076584); **LUCA-2** (United States Patent Publication No. 2006/0172349; PCT Publication No. WO 06/083852); **Oncostatin M** (Oncostatin Receptor Beta) (United States Patent No. 7,572,896; PCT Publication No. WO 06/084092); **PIPA** (United States Patent No. 7,405,061; PCT Publication No. WO 04/043239); **ROR1** (United States Patent No. 5,843,749); and the **Transferrin Receptor** (United States Patent No. 7,572,895; PCT Publication No. WO 05/121179).

**[00147]** 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, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Aqueous carriers, such as saline solutions, aqueous dextrose and glycerol solutions are preferred when the pharmaceutical composition is administered intravenously. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain a minor amount of a wetting or emulsifying agent, or a pH buffering agent. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

**[00148]** 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.

**[00149]** The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

**[00150]** The invention also provides a pharmaceutical pack or kit comprising one or more containers containing a B7-H3 x CD3 bispecific monovalent Fc diabody 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.

**[00151]** A kit can comprise a B7-H3 x CD3 bispecific monovalent Fc diabody 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 cancer antigens. 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.

## **VI. Methods of Administration**

**[00152]** 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.

**[00153]** 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.*

**[00154]** 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 B7-H3 x CD3 bispecific monovalent Fc diabodies 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. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

**[00155]** The invention also provides that the B7-H3 x CD3 bispecific monovalent Fc diabodies 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 B7-H3 x CD3 bispecific monovalent Fc diabodies 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 B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

**[00156]** The lyophilized B7-H3 x CD3 bispecific monovalent Fc diabodies 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, B7-H3 x CD3

bispecific monovalent Fc diabodies 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 B7-H3 x CD3 bispecific monovalent Fc diabodies of the invention are supplied in a hermetically sealed container.

**[00157]** 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.

**[00158]** 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.

**[00159]** 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.

**[00160]** For the B7-H3 x CD3 bispecific monovalent Fc diabodies 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 at least about 0.01  $\mu\text{g}/\text{kg}$ , at least about 0.05  $\mu\text{g}/\text{kg}$ , at least about 0.1  $\mu\text{g}/\text{kg}$ , at least about 0.2  $\mu\text{g}/\text{kg}$ , at least about 0.5  $\mu\text{g}/\text{kg}$ , at least about 1  $\mu\text{g}/\text{kg}$ , at least about 2  $\mu\text{g}/\text{kg}$ , at least about 3  $\mu\text{g}/\text{kg}$ , at least about 5  $\mu\text{g}/\text{kg}$ , at least about 10  $\mu\text{g}/\text{kg}$ , at least about 20  $\mu\text{g}/\text{kg}$ , at least about 30  $\mu\text{g}/\text{kg}$ , at least about 50  $\mu\text{g}/\text{kg}$ , at least about 0.1  $\text{mg}/\text{kg}$ , at least about 0.15  $\text{mg}/\text{kg}$ , at least about 0.2  $\text{mg}/\text{kg}$ , at least about 0.5  $\text{mg}/\text{kg}$ , at least about 1.0  $\text{mg}/\text{kg}$ , or more of the subject's body weight.

**[00161]** Treatment of a subject with a therapeutically or prophylactically effective amount of a B7-H3 x CD3 bispecific monovalent Fc diabody of the invention can comprise a single treatment or, preferably, a series of treatments that may involve the same or differing dosages. For example, a subject may be treated with a B7-H3 x CD3 bispecific monovalent Fc diabody of the invention once a week or once every two weeks for between about 2 to about 120 weeks, or more than 120 weeks. It will be appreciated that the effective dosage of the B7-H3 x CD3 bispecific monovalent Fc diabody used for treatment may increase or decrease over the course of a particular treatment.

**[00162]** Preferably the B7-H3 x CD3 bispecific monovalent Fc diabody is administered using a course of treatment regimen comprising one or more doses (which may remain unchanged, or may increase or decrease in response to a subject's response to the treatment, wherein the treatment regimen is administered over 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 or more than 8 weeks. Typically, there are 1, 2, 3, 4, 5 or more than 5 courses of treatment. Each course of treatment may be the same or different from any prior regimen.

**[00163]** In certain embodiments, a dosage regimen comprises a first 6-week cycle in which a B7-H3 x CD3 bispecific monovalent Fc diabody is administered to a subject bi-weekly (*i.e.*, once every other week), followed by one or more 8 week cycles in which the B7-H3 x CD3 bispecific monovalent Fc diabody is administered to a subject bi-weekly. In certain embodiments, a first 6 week cycle is followed by one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more than fourteen 8 week cycles.

**[00164]** In particular embodiments, the dosage of a B7-H3 x CD3 bispecific monovalent Fc diabody, administered to a subject is at least about 0.1  $\mu\text{g}/\text{kg}$ , 0.3  $\mu\text{g}/\text{kg}$ , 1.3  $\mu\text{g}/\text{kg}$ , 3  $\mu\text{g}/\text{kg}$ , 10  $\mu\text{g}/\text{kg}$ , 30  $\mu\text{g}/\text{kg}$ , or 100  $\mu\text{g}/\text{kg}$  of the subject's body weight. The calculated dose will be

administered based on the patient's body weight at baseline. However, a significant ( $\geq 10\%$ ) change in body weight from baseline or established plateau weight should prompt recalculation of the administered dose.

**[00165]** The dosage and frequency of administration of the B7-H3  $\times$  CD3 bispecific monovalent Fc diabodies of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the B7-H3  $\times$  CD3 bispecific monovalent Fc diabodies by modifications such as, for example, lipidation.

**[00166]** The dosage of the B7-H3  $\times$  CD3 bispecific monovalent Fc diabodies of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the B7-H3  $\times$  CD3 bispecific monovalent Fc diabodies 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.

**[00167]** The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

**[00168]** The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) "New Methods Of Drug Delivery," Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 3 17-327).

**[00169]** The compositions of the invention can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more B7-H3  $\times$  CD3 bispecific monovalent Fc diabodies of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) "Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) "Antibody Mediated Lung Targeting Of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology

50:372-397; Cleek *et al.* (1997) "Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) "Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (See Langer, *supra*; Sefton, (1987) "Implantable Pumps," CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) "Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis," Surgery 88:507-516; and Saudek *et al.* (1989) "A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery," N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled-release of the molecules (see e.g., MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) "Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate," Science 228:190-192; During *et al.* (1989) "Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization," Ann. Neurol. 25:351-356; Howard *et al.* (1989) "Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits," J. Neurosurg. 71(1):105-112; U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. A controlled-release system can be placed in proximity of the therapeutic target (e.g., the lungs), thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). Polymeric compositions useful as controlled-release implants can be used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. A non-polymeric sustained delivery system can be used, whereby a non-polymeric implant in

the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

**[00170]** Controlled-release systems are discussed in the review by Langer (1990, "New Methods Of Drug Delivery," Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, e.g., U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) "Intratumoral Radioimmunotherapy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel," Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) "Antibody Mediated Lung Targeting Of Long-Circulating Emulsions," PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) "Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) "Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which documents is incorporated herein by reference in its entirety.

**[00171]** Where the composition of the invention is a nucleic acid encoding a B7-H3 x CD3 bispecific monovalent Fc diabody of the invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded B7-H3 x CD3 bispecific monovalent Fc diabody, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (See U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biostatic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See e.g., Joliot *et al.* (1991) "Antennapedia Homeobox Peptide Regulates Neural Morphogenesis," Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

**[00172]** Treatment of a subject with a therapeutically or prophylactically effective amount of a B7-H3 x CD3 bispecific monovalent Fc diabody 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.

## **VII. Uses of the Compositions of the Invention**

**[00173]** The B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention have the ability to co-localize T cells to B7-H3-expressing cells, and thus may be used to treat any disease or condition associated with or characterized by the expression of B7-H3. Thus, without limitation, pharmaceutical compositions comprising such molecules may be employed in the diagnosis or treatment of cancers including cancers characterized by the presence of a cancer cell, including but not limited to a 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 phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious 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.

**[00174]** In particular, B7-H3 x CD3 bispecific monovalent Fc diabodies 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 B7-H3.

**[00175]** The B7-H3 x CD3 bispecific monovalent Fc diabodies of the present invention may additionally be used in the manufacture of medicaments for the treatment of the above-described conditions.

**[00176]** Having now generally described the invention, the same will be more readily understood through reference to the following Embodiments, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

### **Embodiments of the Invention**

**[00177]** Provided hereafter are non-limiting examples of certain embodiments of the invention.

**[00178]** Embodiment 1:

A B7-H3 x CD3 bispecific monovalent Fc diabody, wherein the bispecific monovalent Fc diabody is capable of specific binding to an epitope of B7-H3 and to an epitope of CD3, and possesses an IgG Fc Domain, wherein the bispecific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a 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, and wherein:

- I. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:
  - A. a Domain IA, comprising:

(1) a sub-Domain (IA1), which comprises a VL Domain capable of binding to either B7-H3 (VL<sub>B7-H3</sub>) or CD3 (VL<sub>CD3</sub>); and

(2) a sub-Domain (IA2), which comprises a VH Domain capable of binding to either B7-H3 (VH<sub>B7-H3</sub>) or CD3 (VH<sub>CD3</sub>);

wherein the sub-Domains IA1 and IA2 are separated from one another by a polypeptide linker of 12 or less amino acid residues, and are coordinately selected, such that:

(a) the sub-Domain IA1 comprises the VL Domain capable of binding to B7-H3 (VL<sub>B7-H3</sub>) and the sub-Domain IA2 is selected to comprise the VH Domain capable of binding to CD3 (VH<sub>CD3</sub>); or

(b) the sub-Domain IA1 comprises the VL Domain capable of binding to CD3 (VL<sub>CD3</sub>) and the sub-Domain IA2 is selected to comprise the VH Domain capable of binding to B7-H3 (VH<sub>B7-H3</sub>); and

B. an optionally present Domain IB, comprising a polypeptide linker linked to a Heterodimer-Promoting Domain;

C. a Domain IC, comprising a polypeptide linker linked to a CH2-CH3 Domain of an antibody;

II. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

A. a Domain IIA, comprising:

(1) a sub-Domain (IIA1), which comprises a VL Domain capable of binding to either B7-H3 (VL<sub>B7-H3</sub>) or CD3 (VL<sub>CD3</sub>); and

(2) a sub-Domain (IIA2), which comprises a VH Domain capable of binding to either B7-H3 (VH<sub>B7-H3</sub>) or CD3 (VH<sub>CD3</sub>);

wherein the sub-Domains IIA1 and IIA2 are separated from one another by a polypeptide linker of 12 or less amino acid residues, and are coordinately selected, such that:

(a) when the sub-Domain IA1 comprises the VL Domain capable of binding to B7-H3 (VL<sub>B7-H3</sub>), the sub-Domain IIA1 is selected to comprise the VL Domain capable of binding to

CD3 (VL<sub>CD3</sub>) and the sub-Domain IIA2 is selected to comprise the VH Domain capable of binding to B7-H3 (VH<sub>B7-H3</sub>); and

(b) when the sub-Domain IA1 comprises the VL Domain capable of binding to CD3 (VL<sub>CD3</sub>), then sub-Domain IIA1 is selected to comprise the VL Domain capable of binding to B7-H3 (VL<sub>B7-H3</sub>) and the sub-Domain IIA2 is selected to comprise the VH Domain capable of binding to CD3 (VH<sub>CD3</sub>);

B. an optionally present Domain IIB, comprising a polypeptide linker linked to a Heterodimer-Promoting Domain;

III. the third polypeptide chain comprises, in the N-terminal to C-terminal direction a Domain IIIC that comprises a polypeptide linker linked to a CH2-CH3 Domain of an antibody;

wherein:

(A) (1) at least one of the optionally present Domain IB and the optionally present Domain IIB is present, and wherein the Domain IB or IIB that is present has a positive or negative charge; or

(2) both the optionally present Domain IB and the optionally present Domain IIB are present, wherein:

(i) one of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain having a positive charge, and the other of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain having a negative charge; or

(ii) one of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain that comprises the amino acid sequence GVEPKSC (**SEQ ID NO:6**) or VEPKSC (**SEQ ID NO:7**), and the other of the Domain 1B and the Domain IIB has a Heterodimer-Promoting Domain that comprises the amino acid sequence GFNRGEC (**SEQ ID NO:8**) or FNRGEC (**SEQ ID NO:9**);

- (B) the  $VL_{B7-H3}$  and the  $VH_{B7-H3}$  interact to form an epitope-binding domain capable of binding an epitope of B7-H3, and the  $VL_{CD3}$  and  $VH_{CD3}$  form an epitope-binding domain capable of binding an epitope of CD3; and
- (C) the CH2-CH3 Domains of the first and third polypeptide chains form an Fc Domain capable of binding to an Fc receptor.

**[00179]** Embodiment 2:

The B7-H3 x CD3 bispecific monovalent Fc diabody of embodiment 1, which is capable of cross-reacting with both human and primate B7-H3 and CD3.

**[00180]** Embodiment 3:

The B7-H3 x CD3 bispecific monovalent Fc diabody of embodiment 1 or 2, wherein:

- (A) (1) the Domains IB and IIB each comprise a cysteine residue that covalently bond the first polypeptide chain to the second polypeptide chain via a disulfide bond; and
- (2) the Domains IC and IIIC each comprise a cysteine residue that covalently bonds the first polypeptide chain to the third polypeptide chain via a disulfide bond; or
- (B) (1) the polypeptide linkers separating the Domains IA2 and IB and the Domains IIA2 and IIB each comprise a cysteine residue that covalently bonds the first polypeptide chain to the second polypeptide chain via a disulfide bond ; and
- (2) the Domains IC and IIIC each comprise a cysteine residue that covalently bonds the first polypeptide chain to the third polypeptide chain via a disulfide bond.

**[00181]** Embodiment 4:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-3, wherein:

- (A) the  $VL_{B7-H3}$  has the amino acid sequence of **SEQ ID NO:17** and the  $VH_{B7-H3}$  has the amino acid sequence of **SEQ ID NO:21**; or
- (B) the  $VL_{B7-H3}$  has the amino acid sequence of **SEQ ID NO:25** and the  $VH_{B7-H3}$  has the amino acid sequence of **SEQ ID NO:29**; or

(C) the VL<sub>B7-H3</sub> has the amino acid sequence of **SEQ ID NO:33** and the VH<sub>B7-H3</sub> has the amino acid sequence of **SEQ ID NO:37**.

**[00182]** Embodiment 5:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-4, wherein the VL<sub>CD3</sub> has the amino acid sequence of **SEQ ID NO:41** and the VH<sub>CD3</sub> has the amino acid sequence of **SEQ ID NO:45** or **50**.

**[00183]** Embodiment 6:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-5, wherein:

(A) the CH2-CH3 Domain of the Domain IC has the amino acid sequence of **SEQ ID NO:15** and the CH2-CH3 Domain of the Domain IIIC has the amino acid sequence of **SEQ ID NO:16**; or

(B) the CH2-CH3 Domain of the Domain IC has the amino acid sequence of **SEQ ID NO:16** and the CH2-CH3 Domain of the Domain IIIC has the amino acid sequence of **SEQ ID NO:15**.

**[00184]** Embodiment 7:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-6, wherein:

(A) the polypeptide linker separating the sub-Domains IA1 and IA2 has the amino acid sequence of **SEQ ID NO:1**; and/or

(B) the polypeptide linker separating the sub-Domains IIA1 and IIA2 has the amino acid sequence of **SEQ ID NO:1**.

**[00185]** Embodiment 8:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-7, wherein:

(A) the Heterodimer-Promoting Domain of the Domain IB has the amino acid sequence of **SEQ ID NO:10** and the Heterodimer-Promoting Domain of the Domain IIB has the amino acid sequence of **SEQ ID NO:11**; or

(B) the Heterodimer-Promoting Domain of the Domain IB has the amino acid sequence of **SEQ ID NO:11** and the Heterodimer-Promoting Domain of the Domain IIB has the amino acid sequence of **SEQ ID NO:10**; or

- (C) the Heterodimer-Promoting Domain of the Domain IB has the amino acid sequence of **SEQ ID NO:12** and the Heterodimer-Promoting Domain of the Domain IIB has the amino acid sequence of **SEQ ID NO:13**; or
- (D) the Heterodimer-Promoting Domain of the Domain IB has the amino acid sequence of **SEQ ID NO:13** and the Heterodimer-Promoting Domain of the Domain IIB has the amino acid sequence of **SEQ ID NO:12**.

**[00186]** Embodiment 9:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-8, wherein:

- (A) the polypeptide linker separating the Domains IB and IA has the amino acid sequence of **SEQ ID NO:2** or **3**; and/or
- (B) the polypeptide linker separating the sub-Domains IIB and IIA has the amino acid sequence of **SEQ ID NO:2** or **SEQ ID NO:3**.

**[00187]** Embodiment 10:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-9, wherein the Domain IC further comprises a polypeptide linker that separates the CH2-CH3 Domain from the Domain IB, and wherein the Domain IIIC further comprises a polypeptide linker N-terminal to the CH2-CH3 Domain.

**[00188]** Embodiment 11:

The B7-H3 x CD3 bispecific monovalent Fc diabody of embodiment 10, wherein the polypeptide linkers of Domains IC and IIIC have the amino acid sequence of **SEQ ID NO:4** or **5**.

**[00189]** Embodiment 12:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-11, wherein:

- (A) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:53**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:55**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or
- (B) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:59**, the second polypeptide chain has the amino acid sequence of **SEQ ID**

**NO:60** and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or

- (C) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:61**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:62**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**; or
- (D) the first polypeptide chain has the amino acid sequence of **SEQ ID NO:63**, the second polypeptide chain has the amino acid sequence of **SEQ ID NO:64**, and the third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

**[00190]** Embodiment 13:

The B7-H3 x CD3 bi-specific monovalent Fc diabody of any one of embodiments 1-12, which is capable of mediating redirected killing of target tumor cells using human T cells in an assay employing a target human tumor cell line selected from the group consisting of: A498 (kidney cancer), JIMT-1/Luc (breast cancer), A375 (melanoma); 22Rv1 (prostate cancer), Detroit562 (pharyngeal cancer), DU145 (prostate cancer); BxPC3 (pancreatic cancer), SKMES-1 (lung cancer), and U87 (glioblastoma), and using purified human primary T cells as effector cells at an Effector cell to T cell ratio of 1:1, 5:1, or 10:1, wherein the observed EC50 of such redirected killing is about 1.5  $\mu$ g/mL or less, about 1.0  $\mu$ g/mL or less, about 500 ng/mL or less, about 300 ng/mL or less, about 200 ng/mL or less, about 100 ng/mL or less, about 50 ng/mL or less.

**[00191]** Embodiment 14:

The B7-H3 x CD3 bi-specific monovalent Fc diabody of embodiment 13 wherein, target tumor cell killing is measured using a lactate dehydrogenase (LDH) release assay in which the enzymatic activity of LDH released from cells upon cell death is quantitatively measured, or by a luciferase assay in which luciferase relative light unit (RLU) is the read-out to indicate relative viability of Raji/GF target cells, which have been engineered to express both the green fluorescent protein (GFP) and luciferase reporter genes.

**[00192]** Embodiment 15:

The B7-H3 x CD3 bi-specific monovalent Fc diabody of any one of embodiments 1-14, which is capable of mediating the inhibition of human tumor growth in a co-mix xenograft in which such molecules are introduced into NOD/SCID mice along with 22Rv1 (human prostate cancer) or A498 (human kidney cancer) tumor cells and activated human T cells at a ratio of 5:1.

**[00193]** Embodiment 16:

The B7-H3 x CD3 bi-specific monovalent Fc diabody of any one of embodiments 1-15, which is capable of mediating the inhibition of human tumor growth and/or exhibiting anti-tumor activity in a in an xenograft model in female NSG B2m-/- mice:

- (A) implanted with human PBMCs ( $1 \times 10^7$ ) by intraperitoneal (IP) injection on Day -1 and Detroit (human pharyngeal cancer tumor cells ( $5 \times 10^6$ ) intradermally (ID) on Day 0, and administration of diabody on or Days 20, 22, 23, 26, 28, 30, 33, 35, and 37; or
- (B) implanted with A498 (human kidney cancer tumor cells ( $5 \times 10^6$ ) intradermally (ID) on Day 0, and human PBMCs ( $1 \times 10^7$ ) by intraperitoneal (IP) injection on Day 13 and administration of diabody on Days 33, 35, 36, 39, 41, 43, 46, 48, and 50.

**[00194]** Embodiment 17:

The B7-H3 x CD3 bi-specific monovalent Fc diabody of embodiment 15 or 16, wherein the B7-H3 x CD3 bi-specific monovalent Fc diabody inhibits tumor growth and/or exhibits anti-tumor activity when provided at a concentration of greater than about 0.5 mg/kg, at a concentration of about 0.5 mg/kg, at a concentration about 0.2 mg/kg, at a concentration of about 0.1 mg/kg, at a concentration of about 0.05 mg/kg, at a concentration of about 0.02 mg/kg, at a concentration of about 0.01 mg/kg, or at a concentration of about 0.005 mg/kg, or at a concentration less than 0.005 mg/kg.

**[00195]** Embodiment 18:

The B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-17, for use as a pharmaceutical.

**[00196]** Embodiment 19:

A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of any one of embodiments 1-17 and a physiologically acceptable carrier.

**[00197]** Embodiment 20:

Use of the pharmaceutical composition of embodiment 19 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

**[00198]** Embodiment 21:

The use of embodiment 20, wherein the disease or condition associated with or characterized by the expression of B7-H3 is cancer.

**[00199]** Embodiment 22:

The use of embodiment 21, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a 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 phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious 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.

**[00200]** Embodiment 23:

The use of embodiment 22, 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).

**[00201]** Embodiment 24:

The use of any one of embodiments 21-23, wherein the B7-H3 x CD3 bispecific monovalent Fc diabody is administered to a subject bi-weekly (*i.e.*, every other week).

**[00202]** Embodiment 25:

The use of embodiment 24, wherein the B7-H3 x CD3 bispecific monovalent Fc diabody is administered at a dosage of 0.1  $\mu$ g/kg, 0.3  $\mu$ g/kg, 1.3  $\mu$ g/kg, 3  $\mu$ g/kg, 10  $\mu$ g/kg, 30  $\mu$ g/kg, or 100  $\mu$ g/kg of the subject's body weight at baseline.

**[00203]** Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

### Examples

**[00204]** 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 Binding Affinity

**[00205]** The binding affinity of several exemplary B7-H3 x CD3 bispecific monovalent Fc diabodies (**DART-A**, **DART-B**, and **DART-C**) to human or cynomolgus monkey B7-H3, was evaluated by BIACORE<sup>TM</sup> analyses. BIACORE<sup>TM</sup> analyses measure the dissociation off-rate,  $k_d$ . The binding affinity (KD) between an antibody and its target is a function of the kinetic

constants for association (on rate,  $k_a$ ) and dissociation (off-rate,  $k_d$ ) according to the formula:  $KD = [k_d]/[k_a]$ . The BIACORE™ analysis uses surface plasmon resonance to directly measure these kinetic parameters.

**[00206]** The results of binding of DART-A, DART-B, and DART-C to human and cynomolgus monkey B7-H3, analyzed by surface plasmon resonance (SPR) technology (BIACore), are shown in **Table 2**.

<b>Table 2</b>				
<b>DART</b>	<b>Antigens</b>	<b><math>k_a</math> (± SD) (M-1s-1)</b>	<b><math>k_d</math> (± SD) (s-1)</b>	<b><math>KD</math> (± SD) (nM)</b>
DART-A	Human B7-H3	$2.3 \times 10^5$	$5.6 \times 10^{-3}$	24.6
DART-A	Cynomolgus B7-H3	$1.7 \times 10^5$	$5.1 \times 10^{-3}$	30.2
DART-B	Human B7-H3	$2.4 \times 10^6$	$7.6 \times 10^{-3}$	3.2
DART-B	Cynomolgus B7-H3	$21.5 \times 10^6$	$13.3 \times 10^{-3}$	8.9
DART-C	Human B7-H3	$4.6 \times 10^6$	$7.2 \times 10^{-3}$	1.6
DART-C	Cynomolgus B7-H3	n.d.	n.d.	n.d.

n.d. – BIACore analysis not done

**[00207]** The binding affinity of DART-A is similar for human and cynomolgus monkey B7-H3 ( $KD = 24.6$  nM and 30.2 nM, respectively). The binding affinity of DART-B for human B7-H3 is within three fold of the affinity for cynomolgus monkey B7-H3 is ( $KD = 3.2$  nM and 8.9 nM, respectively). However, DART-B has an approximate 8 fold higher affinity for human B7-H3 than DART-A ( $KD = 3.2$  nM and 24.6 nM, respectively). Similarly, DART-C has an approximate 15 fold higher affinity for human B7-H3 than DART-A. DART-D comprises the same B7-H3 binding domains as DART-C, and is expected to have the same binding affinity for B7-H3. The B7-H3 binding domains of DART-C and DART-D were shown to bind to cynomolgus monkey B7-H3 in other studies. The binding affinities ( $KD$ ) for human and cynomolgus monkey CD3 are nearly identical (~14 nM). In view of the similar binding affinities for human and cynomolgus monkey B7-H3 the cynomolgus monkey is a relevant species for toxicology evaluations.

### **Example 2**

#### **FACS Analysis of B7-H3 Expression on Tumor Cell Lines**

**[00208]** B7-H3 expression was evaluated across a panel of tumor cell lines originated from different human tissue tumor types in order to identify suitable target cell lines for evaluating the biological activity of illustrative B7-H3 x CD3 bispecific monovalent Fc diabodies. **Figure 3** shows FACS histograms of anti-B7-H3-PE antibody binding detected on various cancer cell lines. Nine cell lines were confirmed positive for B7-H3 expression and showed a range of B7-

H3 expression levels based on the fluorescence intensity of anti-B7-H3-PE antibody binding. The cell lines with the highest B7-H3 expression were: A498 (kidney cancer) (**Figure 3A**), JIMT-1/Luc (breast cancer) (**Figure 3B**), and A375 (melanoma) (**Figure 3C**); medium B7-H3 expression: 22Rv1 (prostate cancer) (**Figure 3D**), Detroit562 (pharyngeal cancer) (**Figure 3E**), and DU145 (prostate cancer) (**Figure 3F**); and low B7-H3 expression: BxPC3 (pancreatic cancer) (**Figure 1G**), SKMES-1 (lung cancer) (**Figure 3H**), and U87 (glioblastoma) (**Figure 3I**). Raji cells, a B-lymphoma cell line that is known to be negative for B7-H3 expression, did not show any fluorescence with the anti-B7-H3-PE antibody used (**Figure 3J**). The range of B7-H3 expression on the panel of cell lines evaluated provides a basis to characterize the biological activity of B7-H3 X CD3 bispecific monovalent Fc diabodies (e.g., DART-A) on tumor cell lines with various levels of target density and derived from different human tissues.

### **Example 3** **Binding to B7-H3-Positive Cancer Cell Lines and CD3-Expressing T Cells**

**[00209]** The illustrative B7-H3 x CD3 bispecific monovalent Fc diabodies were examined for their bispecific binding capacity. Four B7-H3-expressing tumor cell lines (A948, JIMT-1/Luc, Detroit562, and 22Rv1) and human primary T cells were evaluated for DART-A cell surface binding by FACS analysis. Since DART-A binds with CD3, instead of using CD3 as a marker for T cells, the combination of CD4 and CD8 was used as a T cell marker. Therefore, in this study when primary human leukocytes were used, the combined CD4+ plus CD8+ gated events represent the T cell population.

**[00210]** After incubation with 10 µg/mL of DART-A, cell-bound DART-A on target cancer cell lines and T cells was detected using an anti-EK-coil antibody, which recognizes the E-coil/K-coil (EK) Heterodimer-Promoting Domain of the DART-A protein. DART-A showed binding to both human B7-H3-expressing tumor cells (**Figures 4A-4D**) and CD3-expressing T cells (**Figure 4E**). DART-B, DART-C, and DART-D were also found to have bispecific binding capacity in similar studies.

### **Example 4** **CTL Activity On Multiple Target Cancer Cell Lines With Purified Human T Cells As Effector Cells**

**[00211]** After confirming binding to both CD3-expressing T cells and B7-H3-expressing tumor cells (see **Example 3**), B7-H3 x CD3 bispecific monovalent Fc diabody-mediated redirected T cell killing of B7-H3-expressing target cells was evaluated *in vitro* using 9 human tumor cell lines (A498, JIMT-1/Luc, A375, U87, DU145, BxPC-3, SKMES-1, Detroit562, and

22Rv1) as target cells and normal human T cells as effector cells. Cytotoxicity was determined using the LDH release assay that quantitatively measures the enzymatic activity of LDH, a stable cytosolic enzyme that is released from cells upon cell death. Since the LDH assay measures LDH activity in supernatants from wells containing both target and effector cells, there is a possibility of interference from effector cell death. Therefore, to confirm that the cytotoxicity measured in the LDH release assay was specific to B7-H3 x CD3 bispecific monovalent Fc diabody-mediated redirected killing of target cells, cytotoxicity was also evaluated using the luminescence (LUM) assay. In this assay format, the luciferase activity of target cells transfected and selected to stably express the luciferase gene (JIMT-1/Luc cells) is measured and used to enumerate viable target cells remaining at the end of the assay. An FITC x CD3 bispecific monovalent Fc diabody (designated “**Control DART**”) was used as a control protein in these studies. The FITC x CD3 bispecific monovalent Fc diabody is an anti-fluorescein (FITC) x anti-CD3 diabody protein in which the anti-CD3 binding component is the same as that in the B7-H3 x CD3 bispecific monovalent Fc diabodies, but the anti-FITC component represents an irrelevant binding target. Thus, FITC x CD3 bispecific monovalent Fc diabody will engage CD3 on T cells but is not expected to co-engage them with target cells. EC50 values were determined by curve fitting the data to a 3-parameter sigmoidal dose-response function using GraphPad Prism 6 software.

**[00212]** DART-A mediated potent, specific, redirected killing of B7-H3-expressing target cells using purified human T cells as effector cells (E:T cell ratio = 5:1). DART-A dose-dependent killing of target cells with representative donor T cells is shown in **Figures 5A-5J** and EC50 values and maximum percent cytotoxicity (Emax) are presented in **Table 3**. DART-A EC50 concentrations ranging from 47 to 1275 ng/mL were observed across the 9 target cell lines evaluated, with JIMT-1/Luc being the most sensitive cell line (EC50 = 47 ng/mL). Near complete tumor cell killing was observed using the LUM assay, which specifically measures viable JIMT-1/Luc target cells (see **Figure 5C**). DART-A activity was generally correlated with B7-H3 expression, as the lower EC50 values were observed for the target cell lines with higher B7-H3 expression. At the highest concentration evaluated (10,000 ng/mL), minimal or no activity was observed with the Control DART. No cytotoxicity was observed in the presence of DART-A in B7-H3 negative CHO cells (**Figure 5K**) or Raji cells (**Figure 5L**) confirming the specificity of DART-A activity to B7-H3 expressing target cells.

**Table 3**  
**Representative E<sub>max</sub> and EC<sub>50</sub> Concentrations of DART-A for Redirected Killing of Target Cell Lines at E:T Cell Ratio of 5:1**

Target Cell Line	Tumor type	E <sub>max</sub> (%)	EC <sub>50</sub> (ng/mL)	B7-H3 Expression
JIMT-1/Luc	Breast cancer	78	47	High
A498	Kidney cancer	80	64	High
DU145	Prostate cancer	57	84	Medium
A375	Melanoma	49	97	High
U87	Glioblastoma	79	99	Low
BxPC-3	Pancreatic cancer	54	196	Low
22Rv1	Prostate cancer	34	212	Medium
SKMES-1	Lung cancer	73	319	Low
Detroit562	Pharyngeal cancer	31	1275	Medium
Raji	B-lymphoma	No activity		Negative
CHO	Normal Chinese hamster ovary	No activity		Negative

**[00213]** Similar studies performed with DART-C and DART-D using a number of different target cell lines including A498, and JIMT-1, demonstrated that these two molecules had similar maximum lysis levels (Emax) but were on average 20 times more potent in mediating redirected cell killing than DART-A. DART-B was also tested using a number of different target cell lines including A498, THP-1, and DU145, and was on average 6 times more potent than DART-A.

**Example 5**  
**CTL Activity of DART-A At Different Effector Cell: Target Cell (E:T) Ratios**

**[00214]** To demonstrate the relationship of E:T cell ratio with B7-H3 x CD3 bispecific monovalent Fc diabody-mediated redirected killing, CTL activity of DART-A was further evaluated at E:T cell ratios of 10:1 (**Figures 6A and 6B**), 5:1 (**Figures 6C and 6D**), and 1:1 (**Figures 6E and 6F**) in the LDH assay using A498 (**Figures 6A, 6C and 6E**) and A375 (**Figures 6B, 6D and 6F**) target cells and purified human T cells as effector cells. DART-A showed the highest potency (EC50) and maximum percent cytotoxicity (Emax) at an E:T cell ratio of 10:1 (**Figures 6A and 6B**) and the potency and maximal cytotoxicity decreased with decreasing E:T cell ratios (**Figures 6A-6F and Table 4**). However, specific activity, albeit to a lesser extent, was observed even at the lowest E:T cell ratio evaluated (1:1) (**Figures 6E and 6F and Table 4**).

<b>Table 4</b> <b>E<sub>max</sub> and EC<sub>50</sub> Concentrations of DART-A for Redirected Killing of Target Cell Lines at Different E:T Cell Ratios</b>						
<b>Target Cell Line</b>	<b>E:T = 10:1</b>		<b>E:T = 5:1</b>		<b>E:T = 1:1</b>	
	<b>E<sub>max</sub> (%)</b>	<b>EC<sub>50</sub> (ng/mL)</b>	<b>E<sub>max</sub> (%)</b>	<b>EC<sub>50</sub> (ng/mL)</b>	<b>E<sub>max</sub> (%)</b>	<b>EC<sub>50</sub> (ng/mL)</b>
A498	83.91	27.2	73.56	42.51	36.3	128.5
A375	41.5	165.6	38.1	242.7	11.01	352.5

**Example 6****B7-H3 x CD3 Bispecific Monovalent Fc diabody-Mediated T Cell Activation Depends On Target Cell Engagement**

**[00215]** The level of T cell activation induced by B7-H3 x CD3 bispecific monovalent Fc diabodies was evaluated in human T cells either alone or in the presence of B7-H3-expressing target cells (A498) at an E:T cell ratio of 10:1. The results of these studies using DART-A are shown in **Figure 7**. Flow cytometry analyses revealed upregulation of CD25 (**Figures 7B and 7C**) and CD69 (**Figures 7D and 7E**), T cell activation markers, on CD4+ (**Figures 7B and 7D**) and CD8+ (**Figures 7C and 7E**) T cell subsets in a dose-dependent manner by DART-A in the presence of B7-H3-expressing target cells (**Figures 7B-7D**). DART-A-mediated T cell activation correlated with the cytotoxicity of target cells (**Figure 7A**).

**[00216]** At all concentrations evaluated using either DART-A or the Control DART, no T cell activation was observed with T cells alone in the absence of B7-H3-expressing target cells (**Figures 7B-7E**). These data suggest that T cell activation mediated by B7-H3 x CD3 bispecific monovalent Fc diabodies such as DART-A is dependent upon effector cell-target cell co engagement. Furthermore, no cytotoxicity was observed when T cells alone were incubated with DART-A (**Figures 7B-7E**) or the Control DART in the CTL assay. In contrast, significant DART-A-mediated cytotoxicity was observed in the presence of target cells (**Figure 7A**). Comparable results were found in similar studies performed using DART-B, DART-C or DART-D.

**Example 7****B7-H3 x CD3 Bispecific Monovalent Fc Diabody-Mediated T Cell Proliferation Upon Target Cell Co-Engagement**

**[00217]** T cell expansion associated with CTL activity induced by a bispecific antibody has been previously reported (Klinger M *et al.* (2012) “*Immunopharmacologic Response Of Patients With B-Lineage Acute Lymphoblastic Leukemia To Continuous Infusion Of T Cell-Engaging CD19/CD3-Bispecific BiTE Antibody Blinatumomab*,” *Blood* 119(26):6226-6233).

Therefore, after observing that treatment with B7-H3 x CD3 bispecific monovalent Fc diabodies resulted in dose-dependent depletion of target cells accompanied by an increase in T cell activation markers, the expansion of T cells cultured with target cells and B7-H3 x CD3 bispecific monovalent Fc diabodies was evaluated. To do this, human PBMCs were labeled with CFSE and co-cultured with A498 target cells at an E:T cell ratio of 10:1 in the presence of DART-A or Control DART at a concentration of 10 µg/mL for 72 or 96 hours. As each cell division reduces the level of dye contained in the daughter cells by half, proliferation of CFSE-labeled T cells was monitored by measuring levels of CFSE over time by FACS analysis. **Figure 8A** (72 hours) and **Figure 8B** (96 hours) show the CFSE-staining profiles following incubation after starting the co culture in the presence of DART-A or Control DART and target cells. The presence of DART-A led to proliferation of CFSE-labeled T cells with the percentages of proliferation around 45% after 72 hours (**Figure 8A**) and 73% after 96 hours (**Figure 8B**) incubation. In contrast, no proliferation of CFSE-labeled T cells was observed in the presence of the Control DART (**Figures 8A and 8B**).

#### Example 8

##### Characterization of Binding to Human and Cynomolgus Monkey B7-H3-expressing CHO Cells and Redirected Killing

**[00218]** To confirm the capability of DART-A to cross-react with cynomolgus monkey B7-H3, DART-A binding to CHO cells transfected with human B7-H3 (huB7-H3-CHO) (**Figure 9A**) or cynomolgus monkey B7-H3 (cyno-B7-H3-CHO) (**Figure 9B**) was evaluated by flow cytometry. Cells were treated with 5-fold decreasing concentrations of DART-A or Control DART protein starting from 10 µg/mL and cell-bound DART-A was detected using the anti-EK-coil antibody. **Figures 9A and 9B** shows concentration-dependent binding of DART-A to both human B7-H3-expressing and cynomolgus monkey B7-H3-expressing CHO cells, respectively.

**[00219]** To evaluate B7-H3 x CD3 bispecific monovalent Fc diabody-mediated redirected killing using CHO cells expressing human B7-H3 or cynomolgus monkey B7-H3, cells were incubated with varying concentrations of DART-A in the presence of human T cells at an E:T cell ratio of 5:1. As shown in **Figures 9C and 9D**, efficient DART-A-mediated killing was observed for both cynomolgus monkey (**Figure 9C**) and human (**Figure 9D**) B7-H3-expressing CHO cells.

**Example 9****B7-H3 X CD3 Bispecific Monovalent Fc Diabody-Mediated Redirected Killing of Target Cells with Cynomolgus Monkey PBMCs as Effector Cells**

**[00220]** DART-A binding to cynomolgus monkey T cells was assessed by flow cytometry where the binding of DART-A was profiled in gated CD4+ and CD8+ T cell populations. DART-A binding to cynomolgus monkey T cells (**Figure 10A**) was similar to human T cells (**Figure 10B**).

**[00221]** After confirming the cross-reactivity of DART-A with cynomolgus monkey T cells, DART-A-mediated *ex vivo* CTL activity was evaluated using cynomolgus monkey PBMCs. DART-A or Control DART was added to cynomolgus monkey PBMCs mixed with B7-H3-expressing target cells (JIMT-1/Luc (**Figures 11A** and **11B**) or A498 (**Figure 11C**)) at an E:T cell ratio of 30:1 and incubated for 24 hours. As shown in **Figures 11A-11C**, dose-dependent DART-A-mediated *ex vivo* cytotoxicity was observed using cynomolgus monkey PBMCs as effector cells against human B7-H3-expressing target cell lines.

**Example 10****Co-mix Xenograft Model of 22Rv1 Human Prostate Cancer**

**[00222]** Human T cells were isolated from heparinized whole blood according to the manufacturer's protocol provided in the RosetteSep T cell isolation kit (STEMCELL Technologies, Vancouver, Canada). The purified T cells were subsequently activated by exposing the cells to anti-CD3 (OKT-3; 1 µg/mL) and anti-CD28 (66 µg/mL) antibodies or to anti-CD3/CD28 Dynabeads (1:1 ratio) for a period of 48 hours. Following stimulation, the cells were grown in RPMI 1640 medium with 10% FBS and 1% penicillin/streptomycin in the presence of IL2 (7.6 ng/mL) for up to 3 weeks.

**[00223]** Human T cells ( $1 \times 10^6$ ) and 22Rv1 tumor cells ( $5 \times 10^6$ ) were combined and injected subcutaneously (SC) on Day 0 after being resuspended in 200 µL of Ham's F12 medium. Following 22Rv1 tumor cell and T cell implantation, mice were treated IV with vehicle control (●), Control DART (0.5 mg/kg) (○), or DART-A at 4 different dose levels (0.004 (◆), 0.02 (▼), 0.1 (▲), or 0.5 (■) mg/kg) once daily for 4 days starting on the day of tumor cell implantation (Days 0, 1, 2, and 3).

**[00224]** As shown in **Figure 12**, the growth of 22Rv1 tumor cells was delayed and inhibited following IV treatment with DART-A once daily on Days 0 to 3 at a dose level of 0.1 (▲) or 0.5 (■) mg/kg. While there was partial inhibition in tumor growth at the 0.02 mg/kg (▼)

DART-A dose level, it did not reach significance. No inhibition in tumor growth was noted at the 0.004 mg/kg (♦) DART-A dose level.

**Example 11**  
**Co-mix Xenograft Model of A498 Human Kidney Cancer**

**[00225]** Human T cells were isolated and prepared as above. Human T cells ( $1 \times 10^6$ ) and A498 tumor cells ( $5 \times 10^6$ ) were combined and injected SC on Day 0 after being resuspended in 200  $\mu$ L of Ham's F12 medium. Following A498 tumor cell and T cell implantation, mice were treated IV with vehicle control (●), Control DART (0.5 mg/kg) (○), or DART-A at 4 different dose levels (0.004 (♦), 0.02 (▼), 0.1 (▲), or 0.5 (■) mg/kg) once daily for 4 days starting on the day of tumor cell implantation (Days 0, 1, 2 and 3).

**[00226]** As shown in **Figure 13**, A498 tumor cells injected on Day 0 in the presence of activated human T cells showed some initial tumor shrinkage even in control animals, the result of tumor adaptation to the *in vivo* environment. Brisk tumor growth, however, was noted at later time points in animals receiving vehicle control (●) and Control DART (○) as well as in animals treated once daily on Days 0, 1, 2 and 3 with 0.004 (♦) or 0.02 (▼) mg/kg DART-A, with no inhibition in tumor growth compared to control animals. Tumor growth was delayed and inhibited following IV treatment with DART-A at a dose level of 0.1 (▲) or 0.5 (■) mg/kg.

**Example 12**  
**Established A498 Human Kidney Cancer Model in Human PBMC-Reconstituted Mice**

**[00227]** To evaluate the activity of DART-A on established tumor xenografts in NSG B2m-/- mice, a human effector cell-reconstituted model was employed. This model also provides an environment in which the anti-tumor activity depends on the recruitment of engrafted human T cells by DART-A to the established tumor.

**[00228]** Female NSG B2m-/- mice (Jackson Laboratory) were used for the established tumor studies ( $n = 7$ -8/group). beta-2 microglobulin (B2m) knockout mice with impaired expression of MHC class I were employed in order to delay and minimize the incidence and severity of graft-versus-host disease (GVHD) associated with the engraftment of human peripheral blood mononuclear cells (PBMCs). It should be noted, however, that the lack of B2m expression in these mice is also associated with impaired expression of FcRn (Israel, E.J. *et al.* (1996) "Increased clearance of IgG in mice that lack beta 2-microglobulin: possible protective role of FcRn," *Immunology* 89: 573-578). Hence, the half-life of DART-A in these mice was expected to be short compared to that in wild-type mice or primates. Based on

previous studies in this strain of mice with molecules comprising a human Fc Region, a dosing frequency of once every 2 to 4 days was adopted to ensure adequate exposure in these studies.

**[00229]** Human PBMCs were isolated from heparinized whole blood using Ficoll-Paque according to the manufacturer's protocol. A498 tumor cells ( $5 \times 10^6$  viable cells) were resuspended in 100  $\mu$ L of Ham's F12 and injected intradermally (ID) on Day 0, followed by the intraperitoneal (IP) injection (200  $\mu$ L, saline) of human PBMCs ( $1 \times 10^7$  viable cells) on Day 13. The timing of PBMC inoculation with respect to tumor cell implantation related to the growth rate of the tumor cells and was empirically determined in order to obtain optimal human effector cell reconstitution with tumor sizes of approximately 150-300  $\text{mm}^3$  at the time of randomization and treatment initiation. The treatment period was on Days 33, 35, 36, 39, 41, 43, 46, 48 and 50 for a total of 9 doses administered IV including vehicle control, Control DART (1 mg/kg), or DART-A at 4 different dose levels (0.001, 0.01, 0.1, or 1 mg/kg).

**[00230]** A498 tumors had reached an average approximate volume of 250  $\text{mm}^3$  on Day 32 prior to treatment initiation (**Figure 14**). In the group that received 1 mg/kg (■) DART-A, tumor volume regressed from  $242 \pm 19 \text{ mm}^3$  on Day 32 to  $106 \pm 35 \text{ mm}^3$  by Day 39. In the group that received 0.1 mg/kg (▲) DART-A, there was a smaller reduction in tumor volume ( $249 \pm 25$  to  $181 \pm 87 \text{ mm}^3$ ), while in the 0.01 mg/kg (▼) group there was a period of cytostasis during the same interval (Days 32 to 39). In the 1 mg/kg (■) DART-A group, tumor growth continued to regress in 5/7 animals, but relapsed in the remaining 2 animals by the end of the study (Day 53). By the end of the study in the 0.1 (▲) and 0.01 (▼) mg/kg groups, tumor growth remained regressed in 5/7 and 4/7 animals, respectively. Progression of tumor growth was evident for all animals treated with 0.001 (◆) mg/kg DART-A. No effect on the growth of the tumors was noted with vehicle control (●) or the Control DART (○).

**[00231]** A498 human kidney cancer model in human PBMC-reconstituted mice was also used to evaluate the activity of DART-B dosed at 0.02, 0.1 or 0.5 mg/kg, Control DART (0.5 mg/kg), or vehicle control. Animals treated with DART-B showed substantial inhibition of tumor growth at all doses, while no effect on the growth of the tumors was noted with vehicle control or the Control DART.

**Example 13****Established Detroit562 Human Pharyngeal Cancer Model in Human PBMC-Reconstituted Mice**

**[00232]** Human PBMCs were prepared as above. Detroit562 tumor cells ( $5 \times 10^6$  viable cells) were resuspended in 100  $\mu$ L of Ham's F12 and injected ID on Day 0. Human PBMCs ( $1 \times 10^7$  viable cells) were implanted by IP injection (200  $\mu$ L, saline) on Day -1, one day prior to tumor cell implantation, in NSG B2m-/- mice. The treatment period was on Days 20, 22, 23, 26, 28, 30, 33, 35 and 37 for a total of 9 doses administered IV and included vehicle control, Control DART (0.5 mg/kg), or DART-A at 4 different dose levels (0.1, 0.25, 0.5, or 1 mg/kg).

**[00233]** Detroit562 tumors had reached a tumor volume of approximately 150  $\text{mm}^3$  on Day 19 prior to treatment initiation (**Figure 15**). The tumors in the groups that received DART-A at the 1 (■), 0.5 (▲), and 0.25 (▼) mg/kg dose levels decreased in size during the treatment period with the nadir being reached on Day 27 ( $106 \pm 21 \text{ mm}^3$ ) for the 0.25 (▼) mg/kg group (**Figure 15**). For the 1 (■) and 0.5 (▲) mg/kg groups, the greatest tumor reduction was observed on Day 37, the last day of the study, with mean tumor volumes of  $32 \pm 6$  and  $47 \pm 7 \text{ mm}^3$ , respectively (**Figure 15**). In the 0.1 (◆) mg/kg DART-A group, reduced tumor growth was observed with a maximum tumor volume ( $258 \pm 37 \text{ mm}^3$ ) that was reduced compared with that of the Control DART (○) ( $347 \pm 36 \text{ mm}^3$ ) and vehicle ( $390 \pm 42 \text{ mm}^3$ ) groups at the end of the study (Day 37) (**Figure 15**).

**Example 14****Established Detroit562 Human Pharyngeal Cancer Model in Human PBMC-Reconstituted Mice Dosing Study**

**[00234]** To evaluate the activity of DART-A on established tumor xenografts in MHC11 -/- mice, a human effector cell-reconstituted model was employed. This model also provides an environment in which the anti-tumor activity depends on the recruitment of engrafted human T cells by DART-A to the established tumor. In these studies, weekly and bi-weekly (*i.e.*, every other week) dosing regimens were examined.

**[00235]** Human PBMCs were prepared as described above. Detroit562 tumor cells ( $5 \times 10^6$  viable cells) were re-suspended in 50  $\mu$ L of Ham's F12 medium and combined with 50  $\mu$ L of Matrigel, and then injected intradermally (ID) on Day 0. Human PBMCs ( $1 \times 10^7$  viable cells) were implanted by IP injection (200  $\mu$ L, Ham's F12 medium) on Day 0, in MHC11 -/- mice. The treatment period was initiated on Day 15. Group I mice were administered DART-A (0.5 mg/kg) once per week (Q1W) on days 15, 22, 29, 36 and 43 for a total of 5 doses

administered IV. Group II mice were administered DART-A (0.5 mg/kg) once every two weeks (Q2W) on days 15, 29, and 43 for a total of 3 doses, administered IV. The vehicle control animals were dosed once per week.

**[00236]** Detroit562 tumors ranged from  $200.49 \pm 15.58 \text{ mm}^3$  to  $287.5 \pm 48.79 \text{ mm}^3$  on Day 14 prior to treatment initiation. The tumors in DART-A-treated animals in both Groups I and II (▲) decreased in size during the treatment period as compared to vehicle treated animals (●) (**Figure 16A and 16B**). In Group I, DART-A-treated animals, reduced tumor growth was observed with a [maximum] tumor volume ( $24.3 \pm 9.5 \text{ mm}^3$ ) on Day 45 that was reduced compared with that of the animals treated with vehicle ( $801.9 \pm 155.5 \text{ mm}^3$ ) on Day 31 (**Figure 16A**). In Group II, DART-A-treated animals, reduced tumor growth was observed with a [maximum] tumor volume ( $47.6 \pm 28.5 \text{ mm}^3$ ) that was reduced compared with that of the animals treated with vehicle ( $801.9 \pm 155.5 \text{ mm}^3$ ) on Day 31 (**Figure 16B**).

#### **Example 15** **Toxicokinetic Studies in Cynomolgus Monkeys**

**[00237]** Three non-GLP, pilot, exploratory, dosing studies of DART-A, DART-B, DART-C, or DART-D were performed in cynomolgus monkeys. In one dose-escalation study two groups of cynomolgus monkeys (n = 2/group; 1M/1F) were administered one dose of 0.5 mg/kg then three weekly doses of 1.0 mg/kg of DART-A or DART-B. There were no significant findings for either group, although transient increases in IL-6 levels were observed following infusion. There were no apparent clinical observations associated with increased cytokine levels. The serum concentrations of DART-A and DART-B were examined during the course of the study (**Figure 17**). As shown in **Figure 17** both molecules exhibited acceptable pharmacokinetic profiles. The serum levels of DART-A showed less variability over the course of the study.

**[00238]** In a single dose study two groups of cynomolgus monkeys (n = 2/group; 1M/1F) were administered one dose of 0.5 mg/kg of DART-D or one dose of 0.5 mg/kg then three weekly doses of 1.0 mg/kg of the Control DART. Both animals treated with DART-D at 0.5 mg/kg exhibited abnormal clinical signals. In a further study four groups of cynomolgus monkeys (n = 2/group; 1M/1F) were administered four weekly doses of either 0.075, 0.15 or 0.3 mg/kg of DART-D, or four weekly doses of 0.5 mg/kg of DART-C. In these studies all the doses were tolerated. However, there was an apparent transient effect on platelet levels at 0.15 and 0.3 mg/kg of DART-D, and at 0.5 mg/kg of DART-C.

**[00239]** All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

**What Is Claimed Is:**

Claim 1. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:53**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:55**; and
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

Claim 2. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 1 and a physiologically acceptable carrier.

Claim 3. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 1 or the pharmaceutical composition of claim 2 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

Claim 4. The use of claim 3, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 5. The use of claim 4, wherein said 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 phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious 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.

Claim 6. The use of claim 5, wherein said 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).

Claim 7. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:59**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:60**; and
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

Claim 8. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 7 and a physiologically acceptable carrier.

Claim 9. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 7 or the pharmaceutical composition of claim 8 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

Claim 10. The use of claim 9, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 11. The use of claim 10, wherein said 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 phaeochromocytoma, 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.

Claim 12. The use of claim 11, wherein said 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).

Claim 13. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:61**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:62**; and
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

Claim 14. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 13 and a physiologically acceptable carrier.

Claim 15. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 13 or the pharmaceutical composition of claim 14 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

Claim 16. The use of claim 15, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 17. The use of claim 16, wherein said 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 phaeochromocytoma, 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.

Claim 18. The use of claim 17, wherein said 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).

Claim 19. A B7-H3 x CD3 bispecific monovalent Fc diabody capable of specific binding to B7-H3 and to CD3, wherein the diabody comprises a first, a second and a third polypeptide chain, wherein said polypeptide chains form a covalently bonded complex, and wherein:

- (A) said first polypeptide chain has the amino acid sequence of **SEQ ID NO:63**;
- (B) said second polypeptide chain has the amino acid sequence of **SEQ ID NO:64**; and
- (C) said third polypeptide chain has the amino acid sequence of **SEQ ID NO:57**.

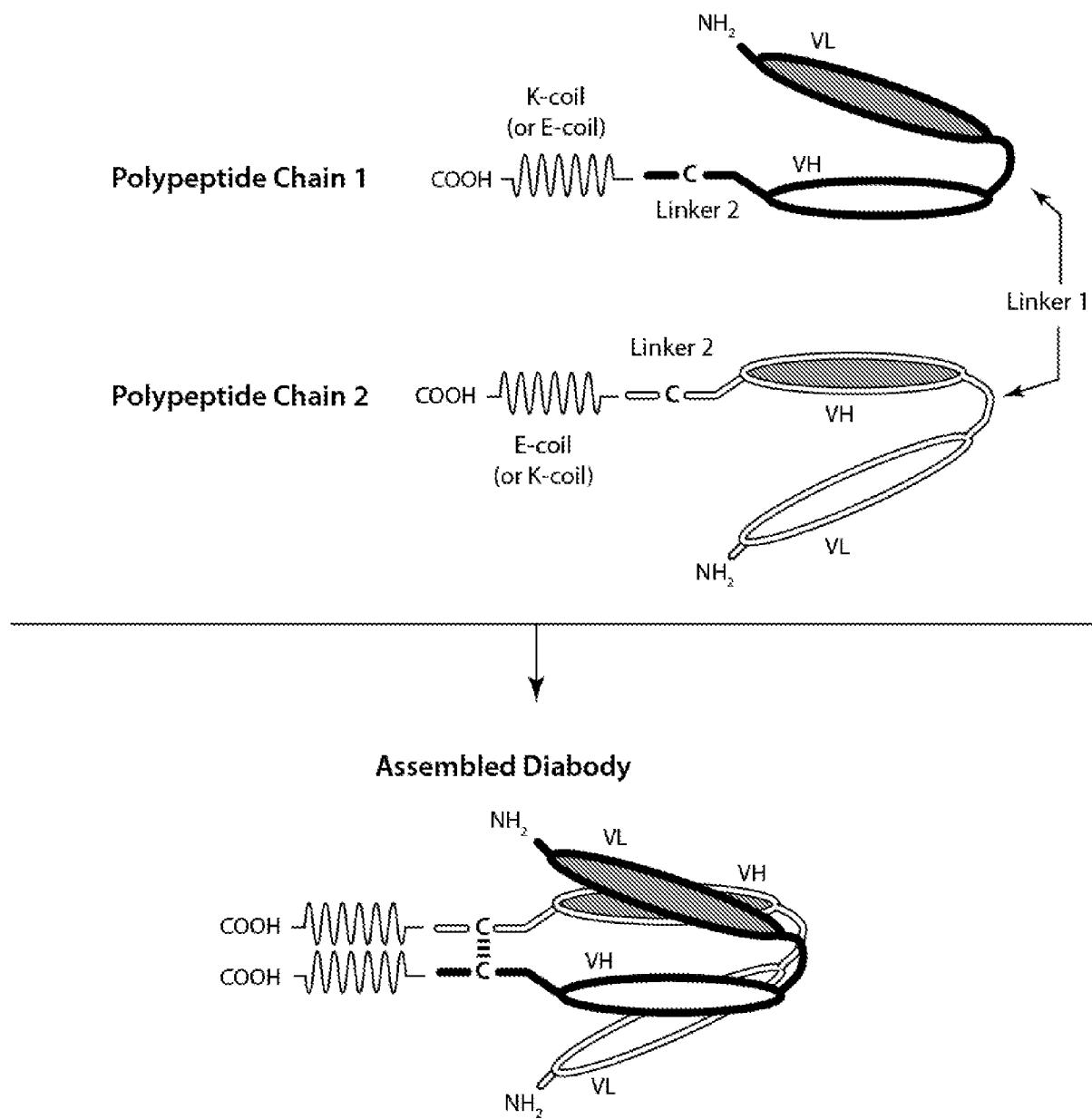
Claim 20. A pharmaceutical composition comprising the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 19 and a physiologically acceptable carrier.

Claim 21. Use of the B7-H3 x CD3 bispecific monovalent Fc diabody of claim 19 or the pharmaceutical composition of claim 20 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

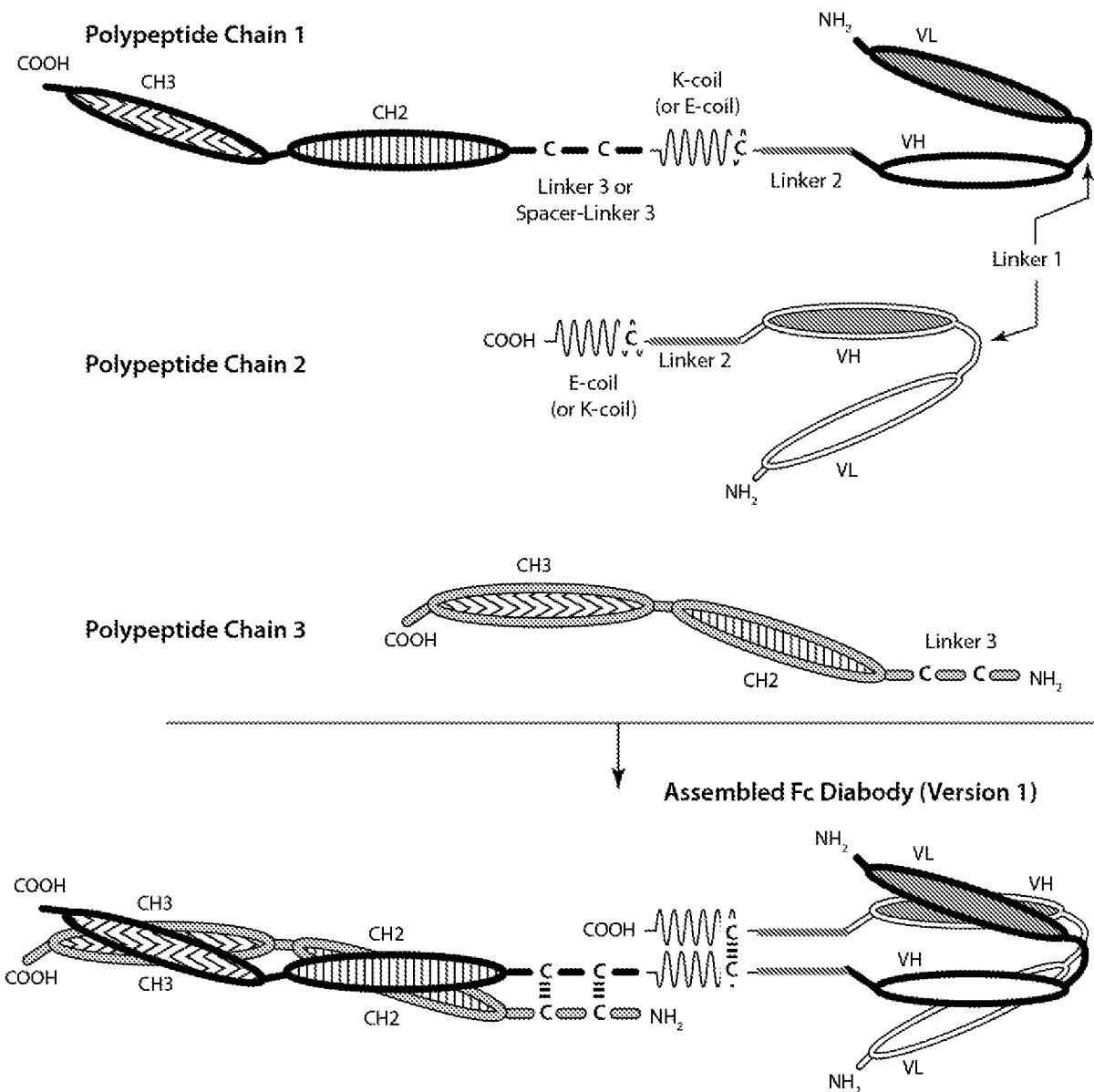
Claim 22. The use of claim 21, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

Claim 23. The use of claim 22, wherein said 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 phaeochromocytoma, 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.

Claim 24. The use of claim 23, wherein said 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).

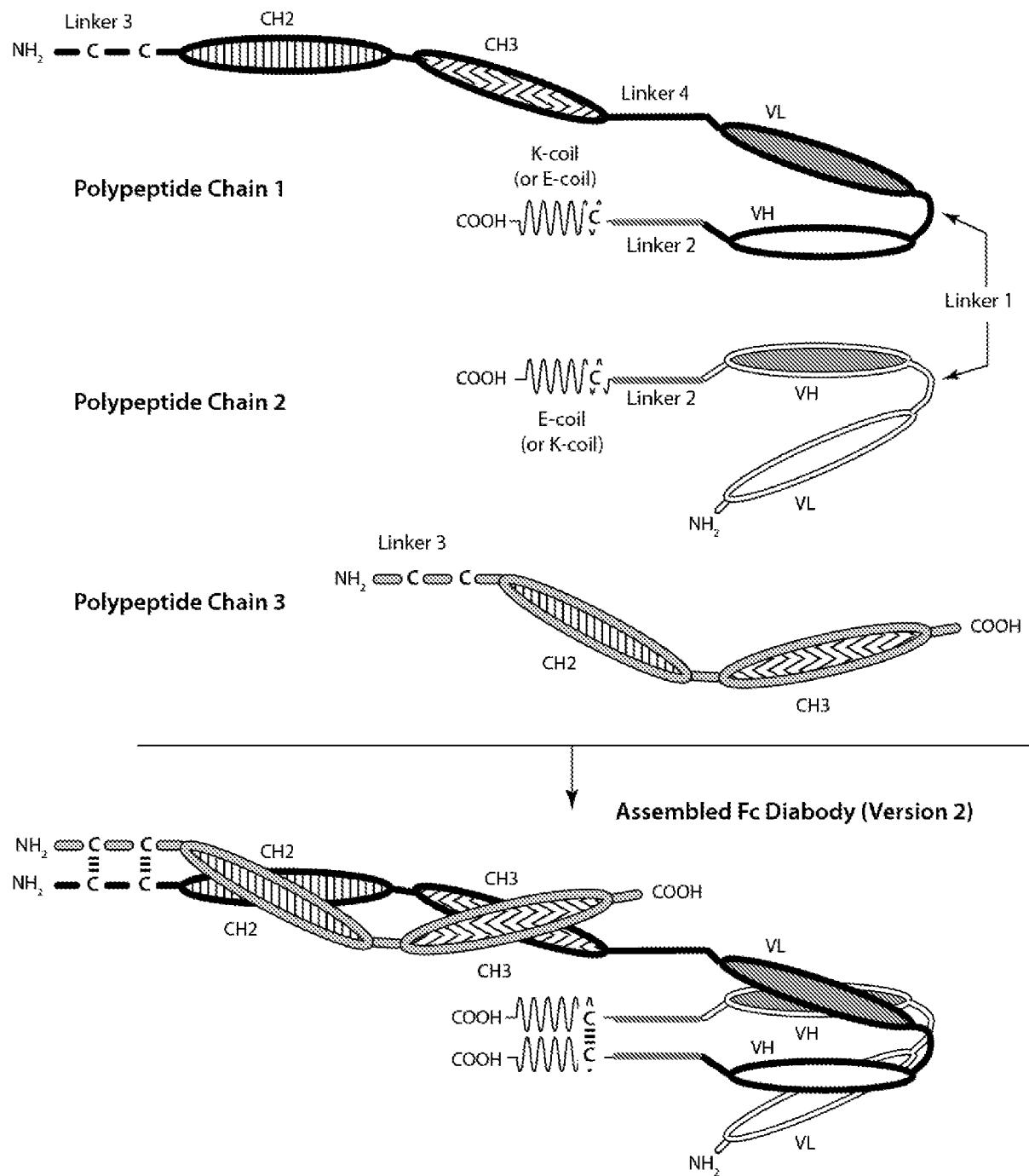


**Figure 1**

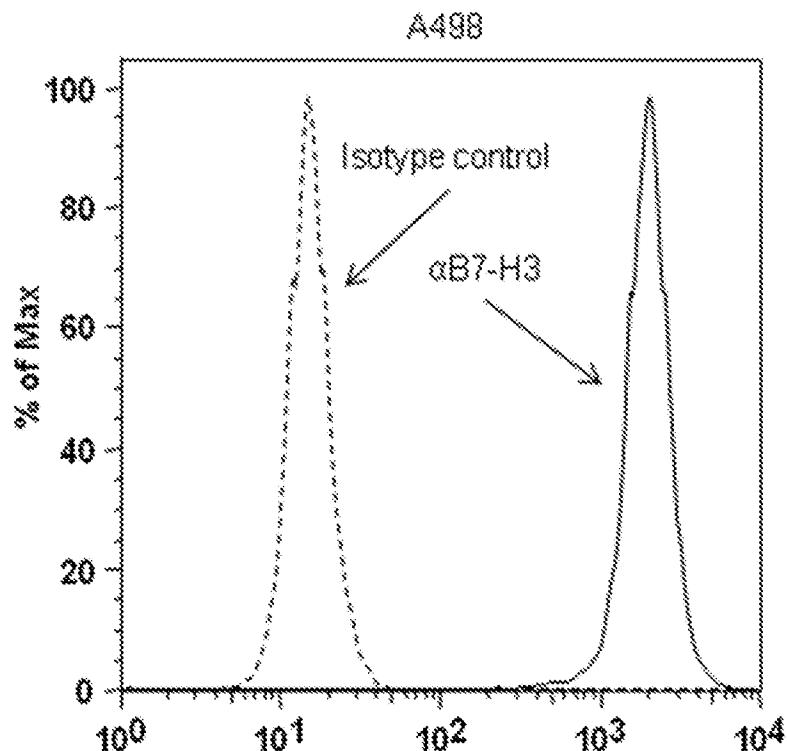
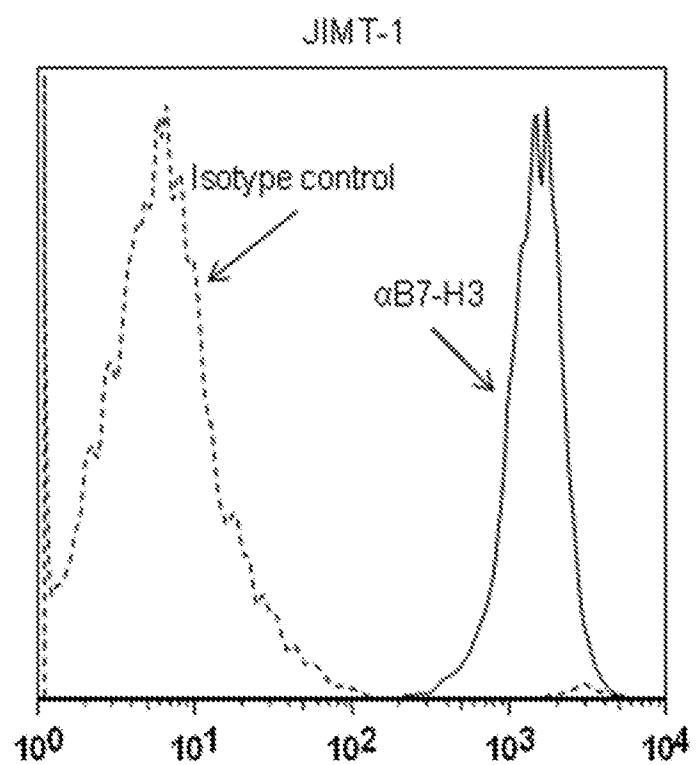


**Figure 2A**

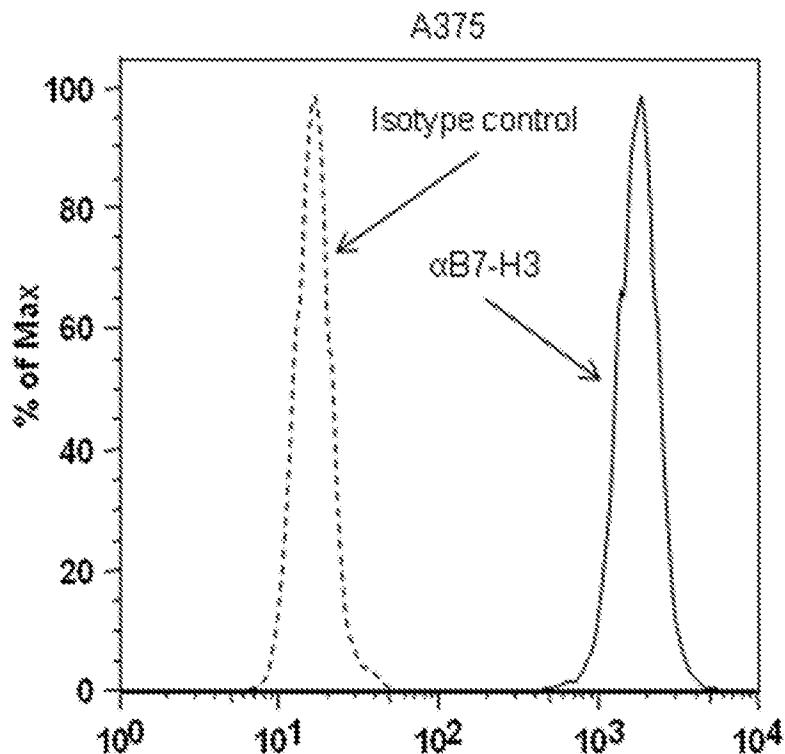
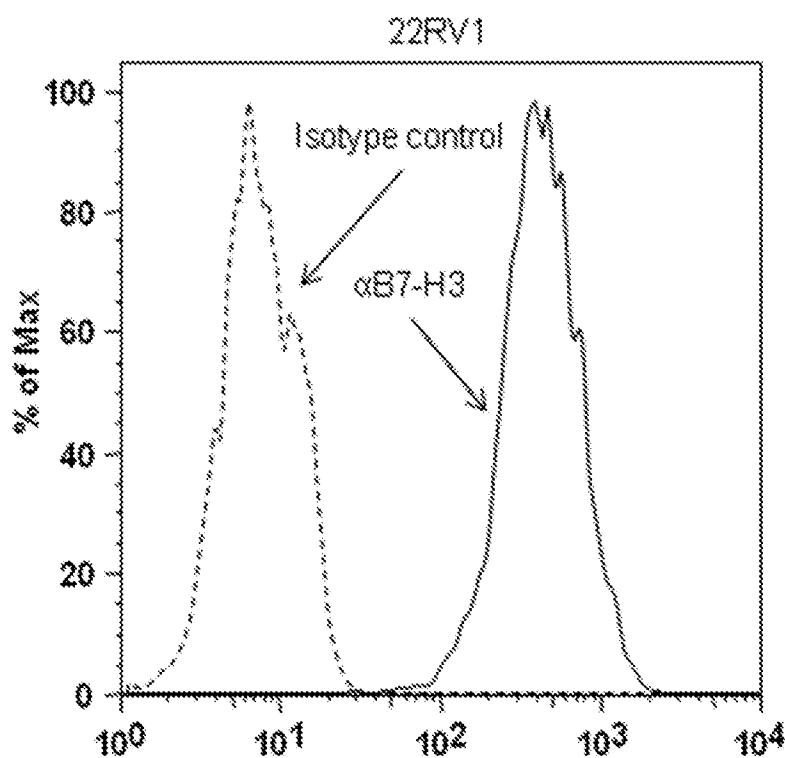
3/50

**Figure 2B**

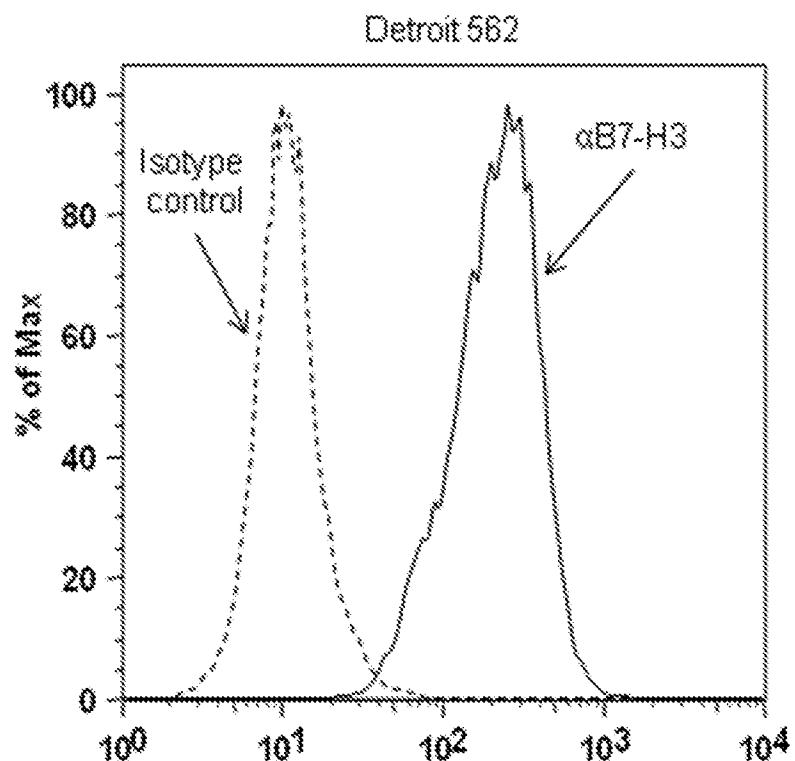
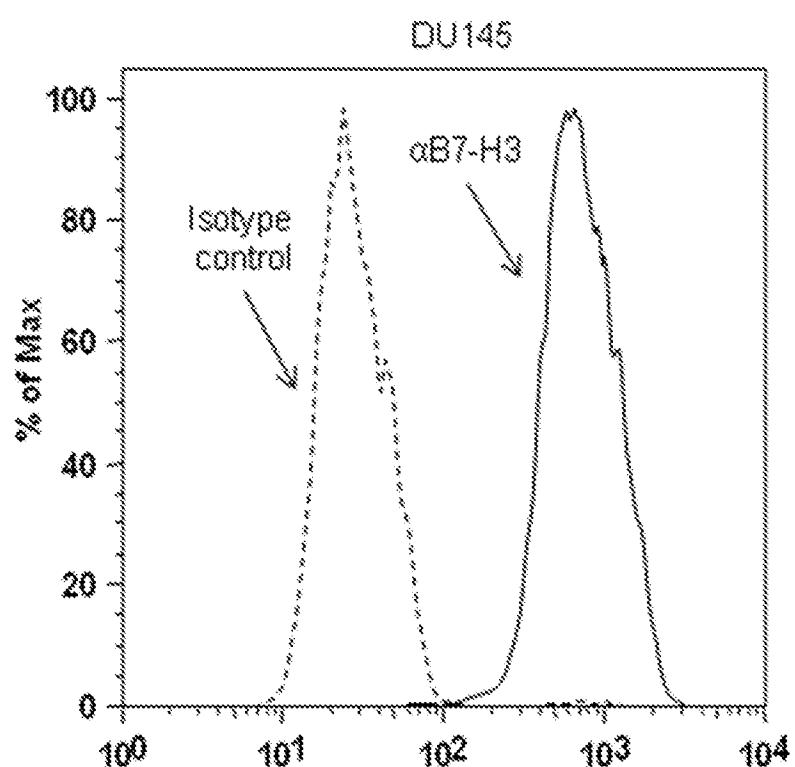
4/50

**Figure 3A****Figure 3B**

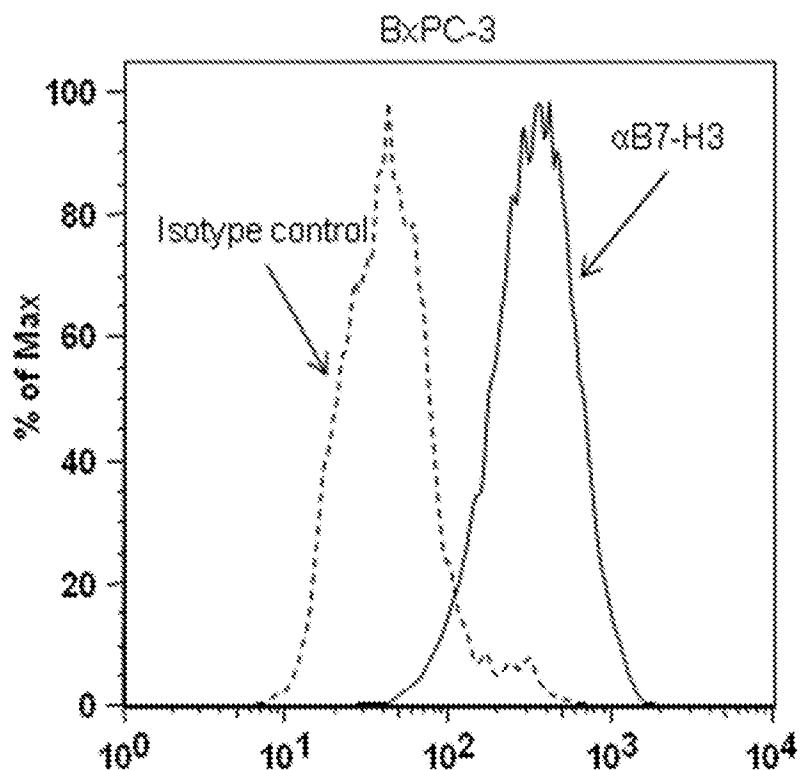
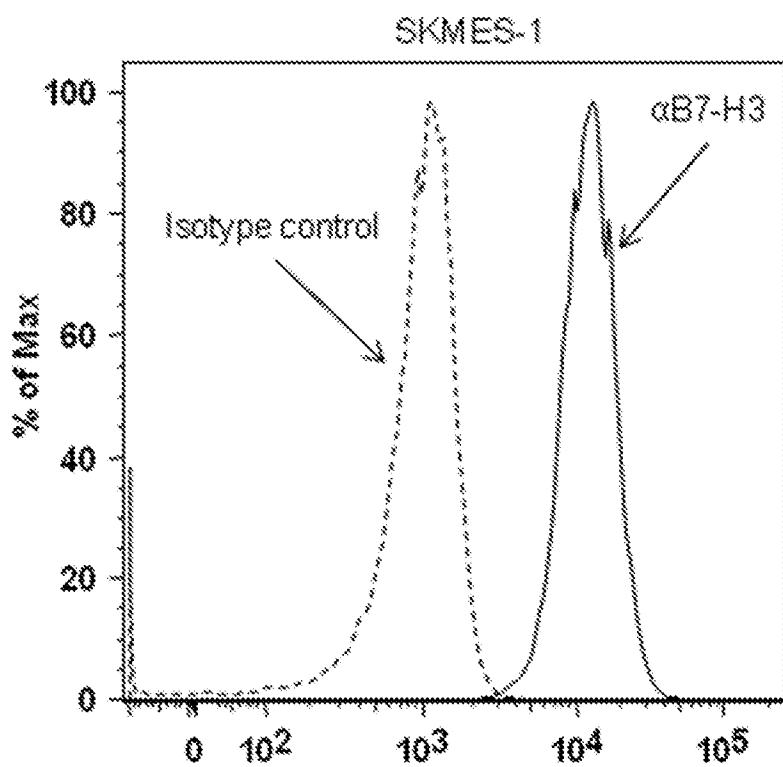
5/50

**Figure 3C****Figure 3D**

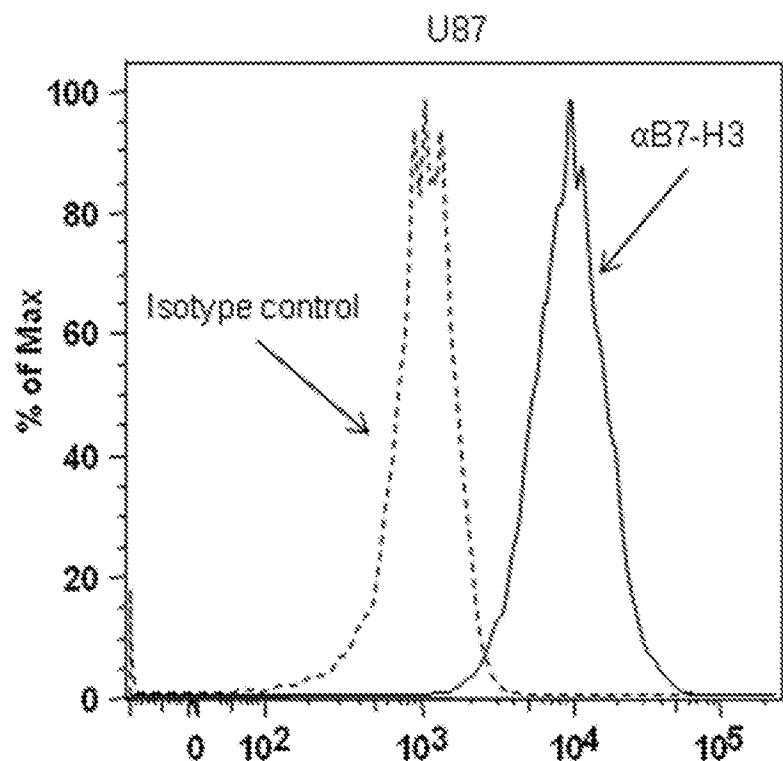
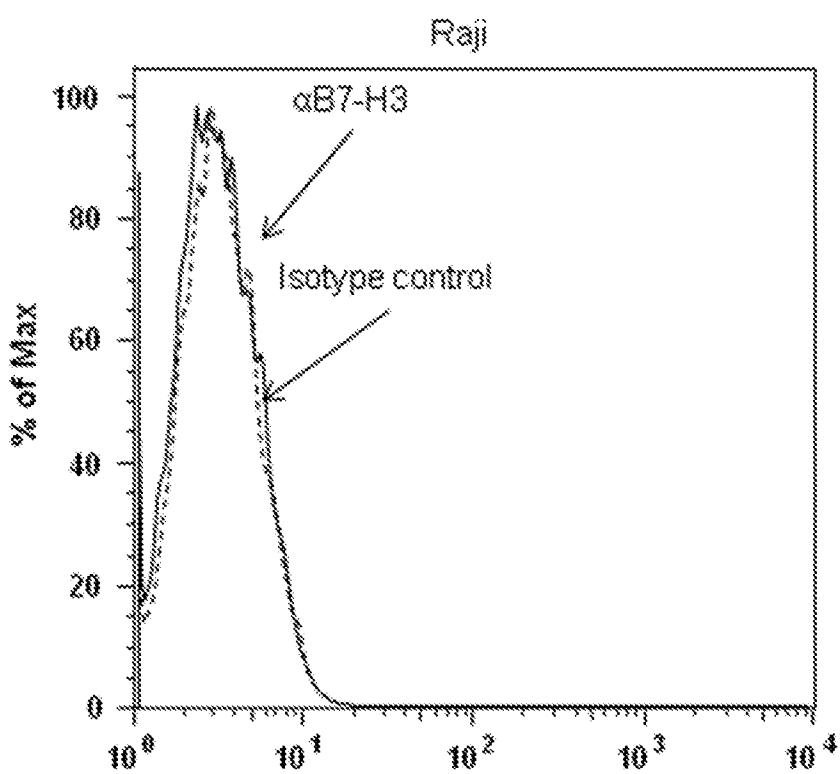
6/50

**Figure 3E****Figure 3F**

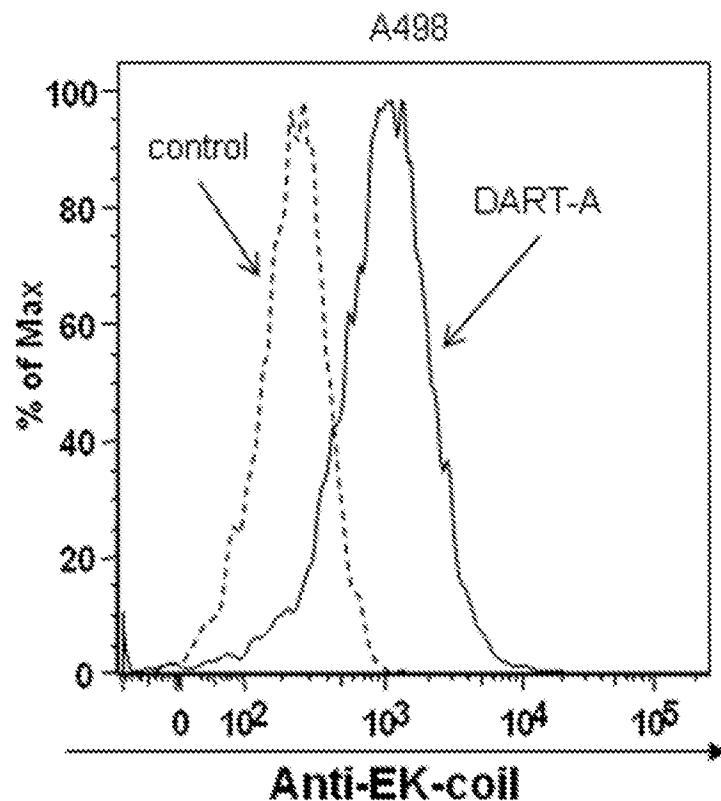
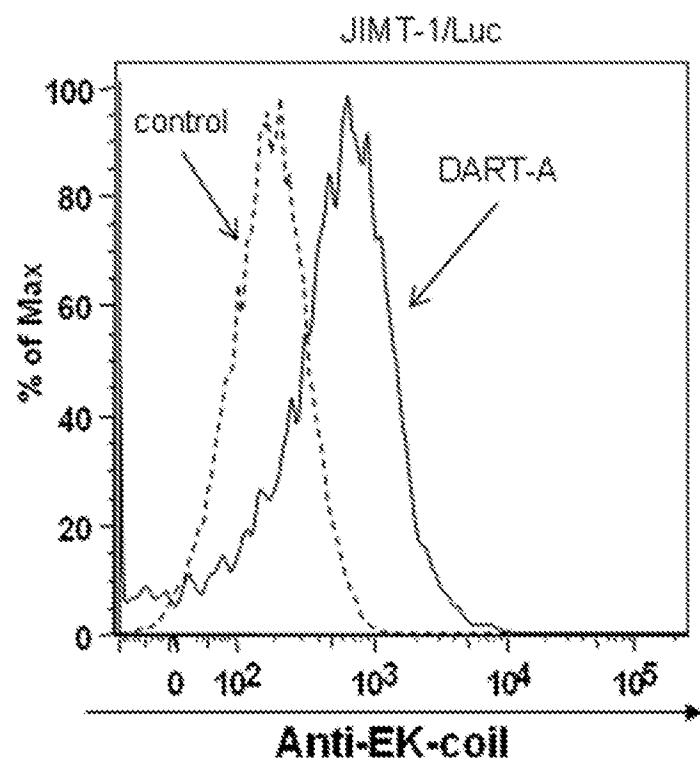
7/50

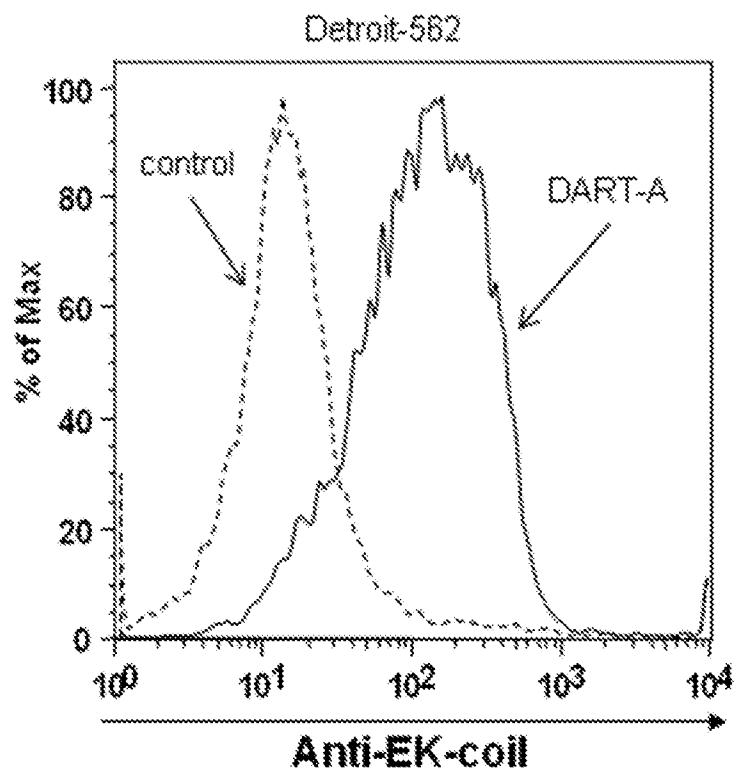
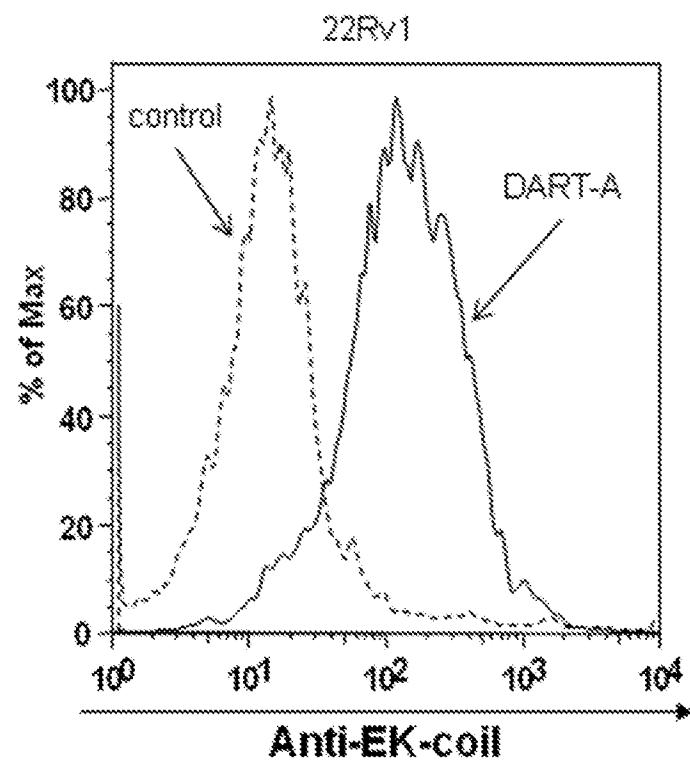
**Figure 3G****Figure 3H**

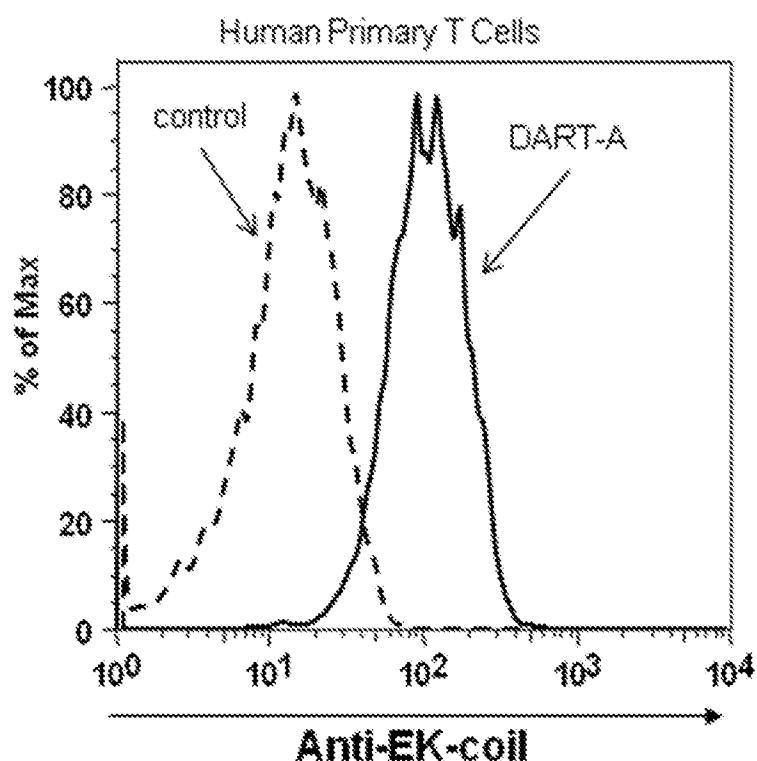
8/50

**Figure 3I****Figure 3J**

9/50

**Figure 4A****Figure 4B**

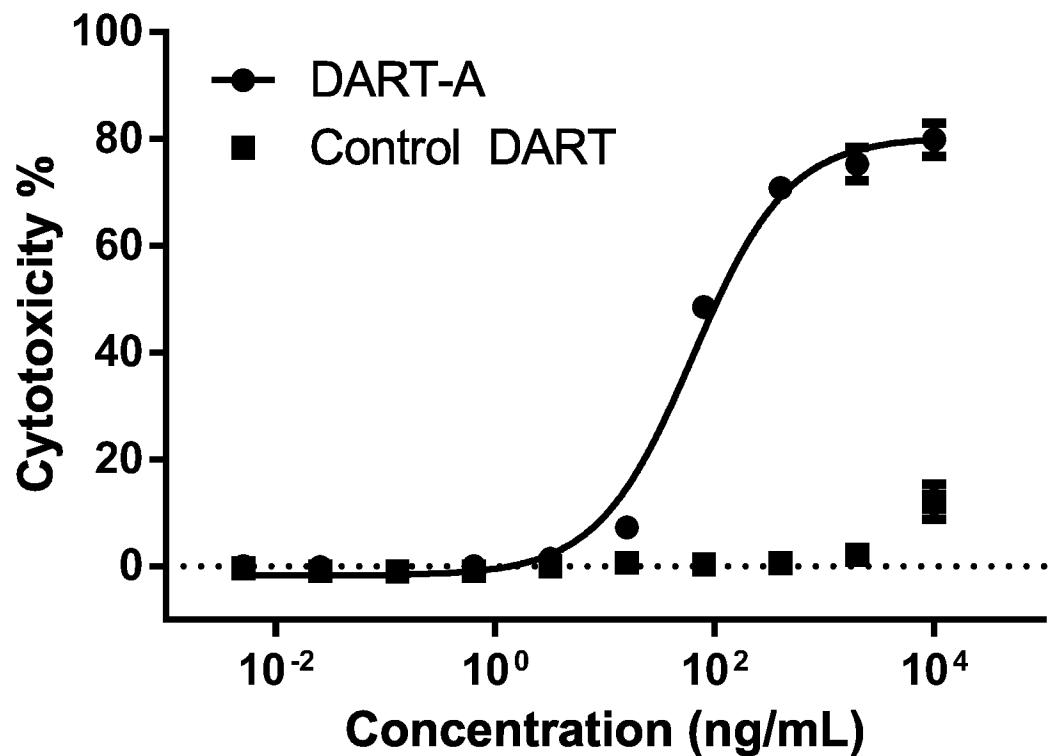
**10/50****Figure 4C****Figure 4D**



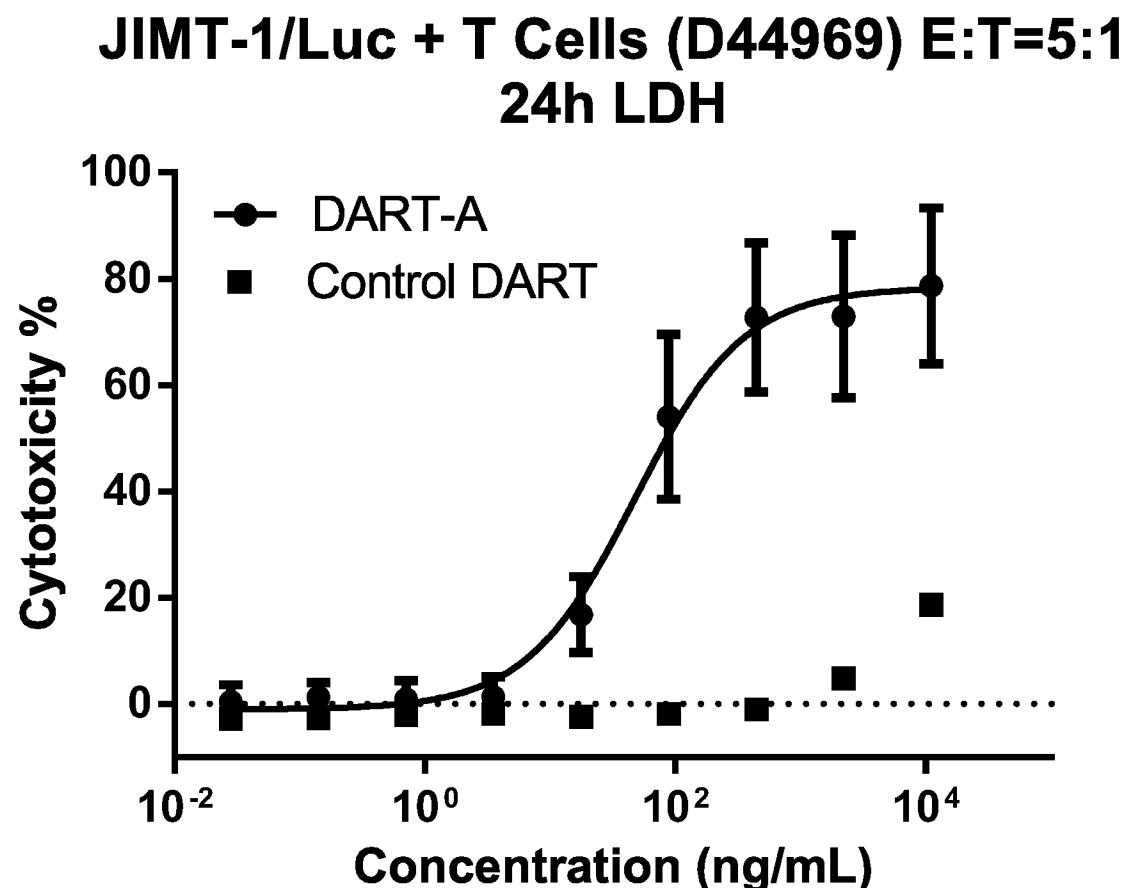
**Figure 4E**

12/50

**A498 + T Cells (D56767) E:T=5:1  
24h LDH**



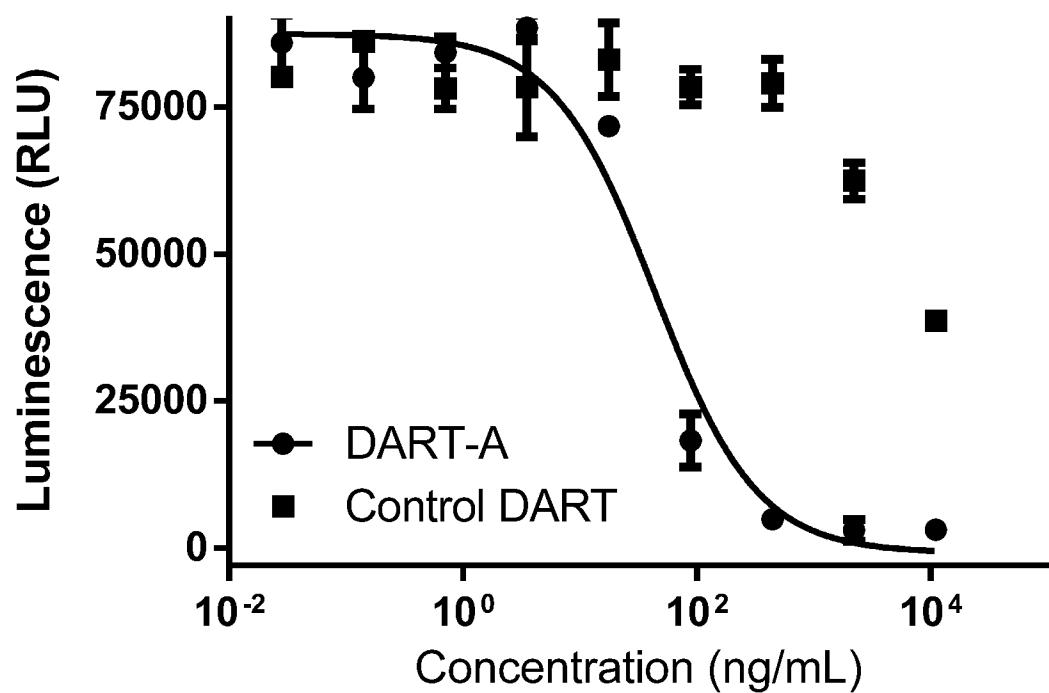
**Figure 5A**



**Figure 5B**

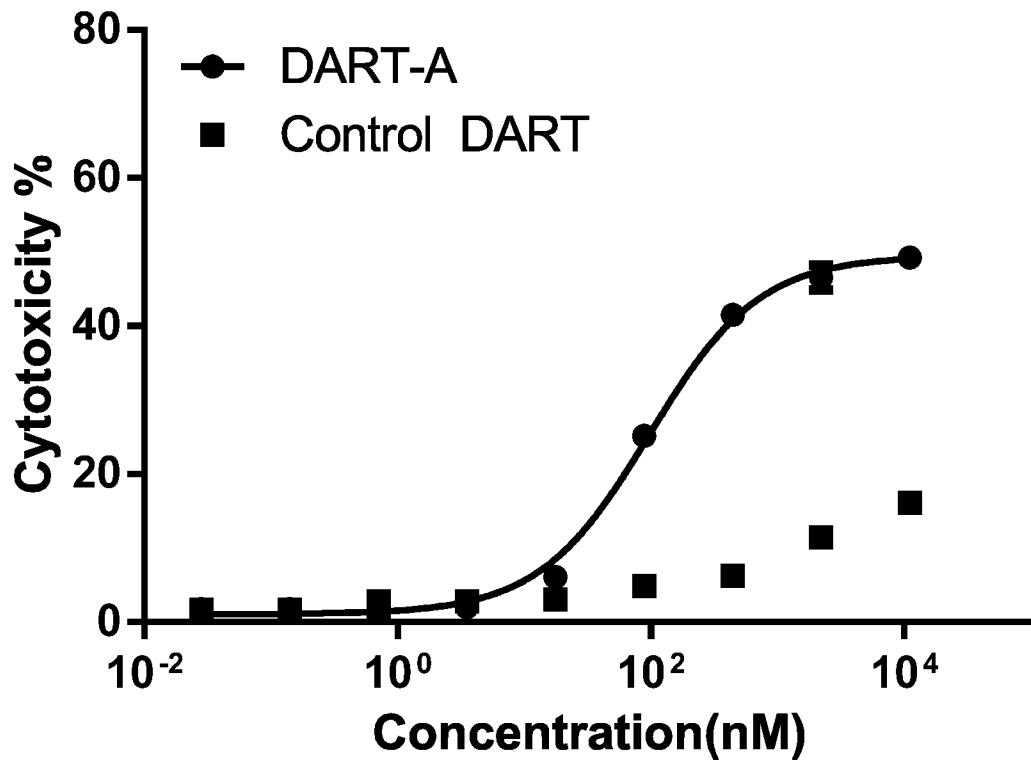
14/50

**JIMT-1/Luc + T Cells (D44969) E:T=5:1  
24h LUM**



**Figure 5C**

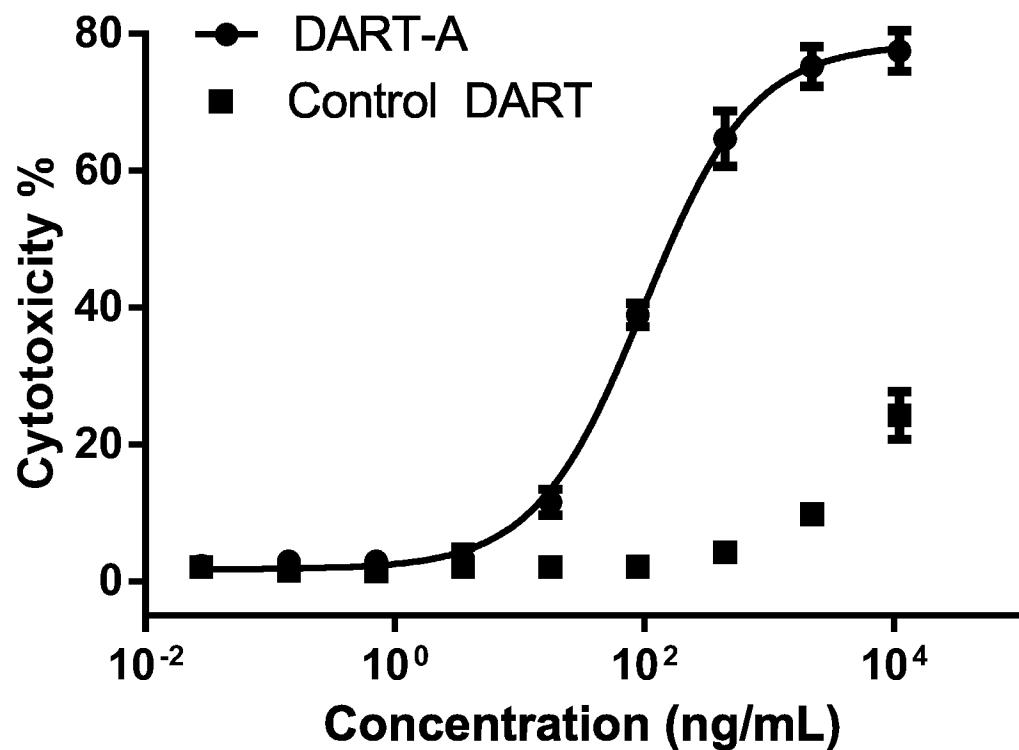
**A375 + T Cells (D39162) E:T=5:1  
24h LDH**



**Figure 5D**

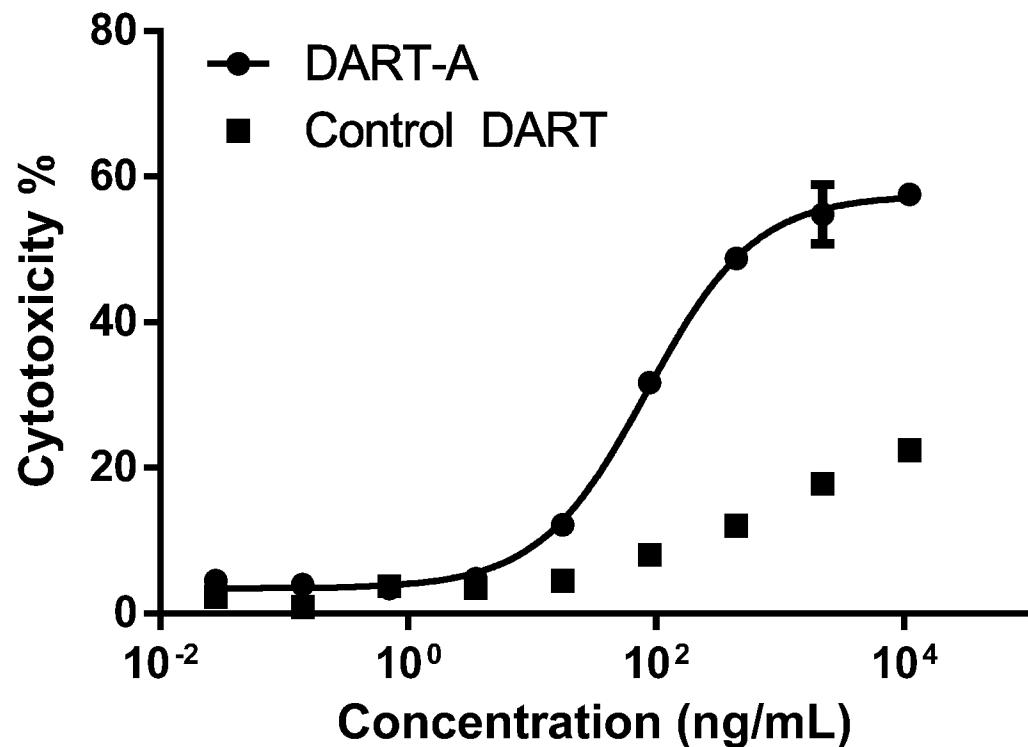
16/50

**U87 + T Cells (D45421) E:T=5:1  
24h LDH**



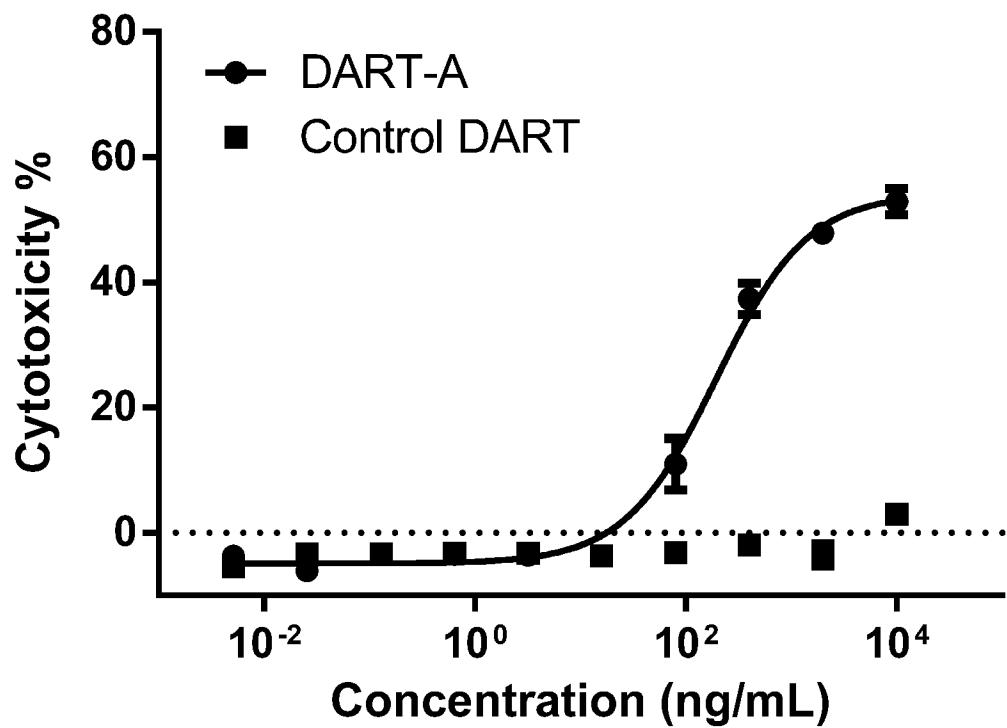
**Figure 5E**

**DU145 + T Cells (D39162) E:T=5:1  
24h LDH**



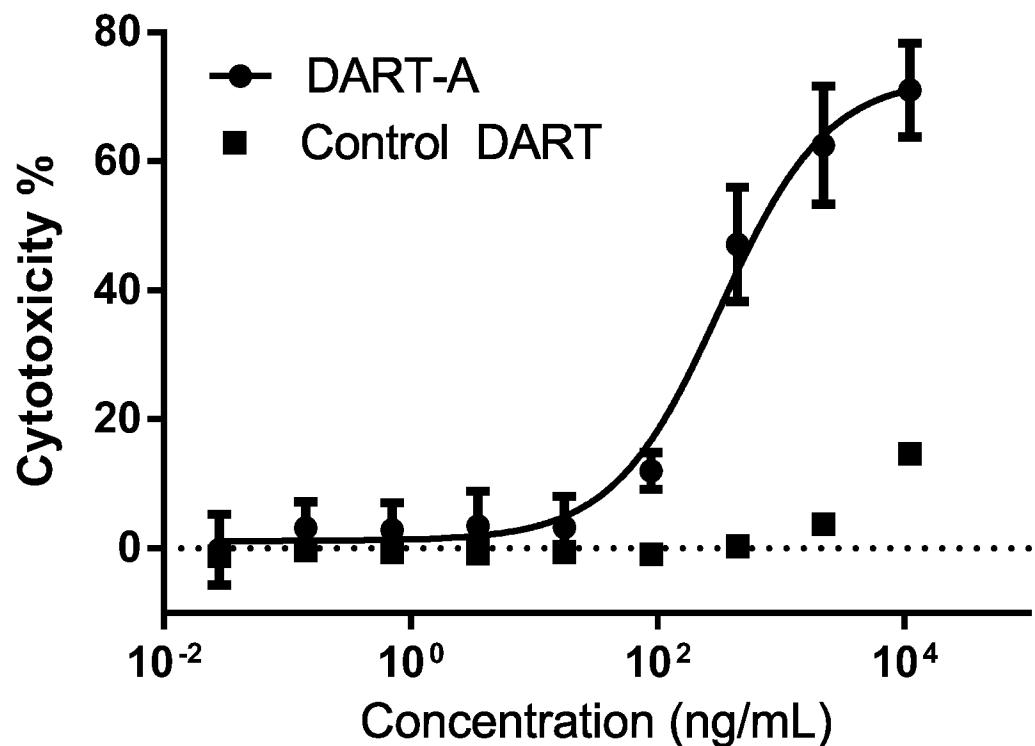
**Figure 5F**

**BxPC-3 + T Cells (D40717) E:T=5:1  
24h LDH**



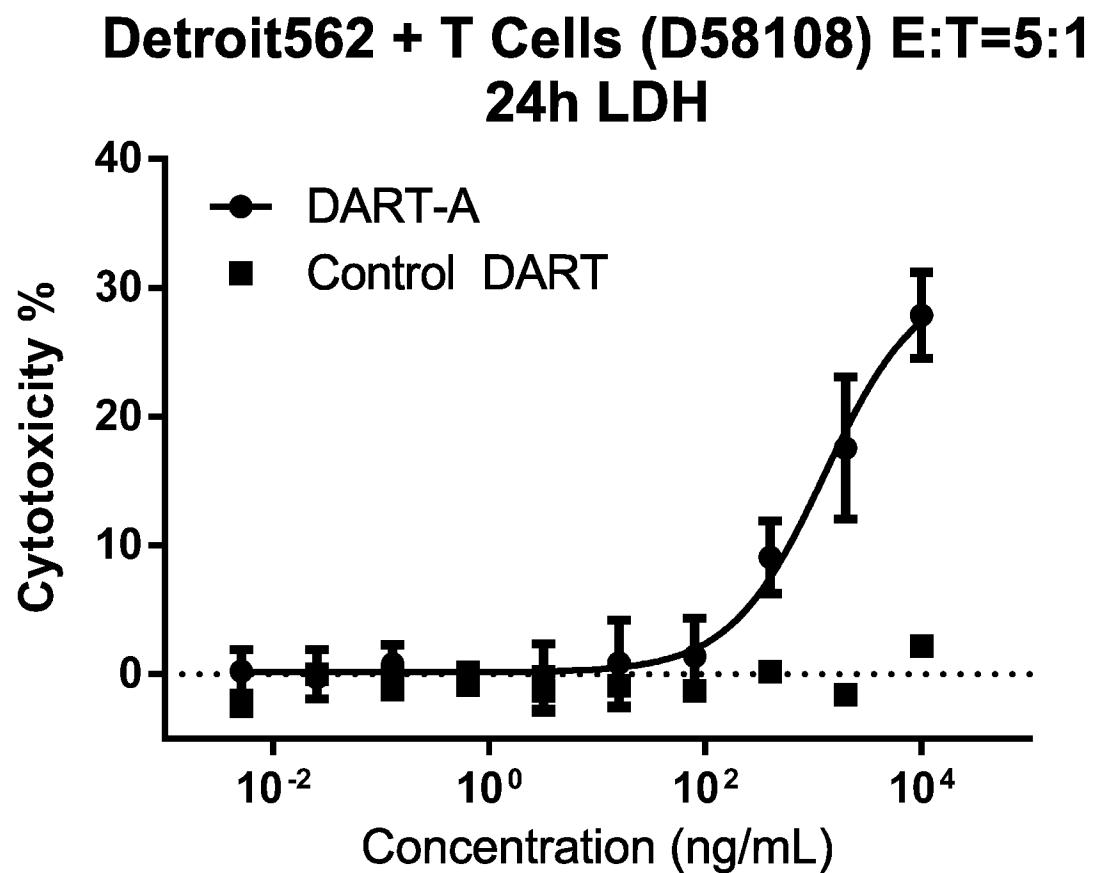
**Figure 5G**

**SKMES-1 + T Cells (D44969) E:T=5:1  
24h LDH**



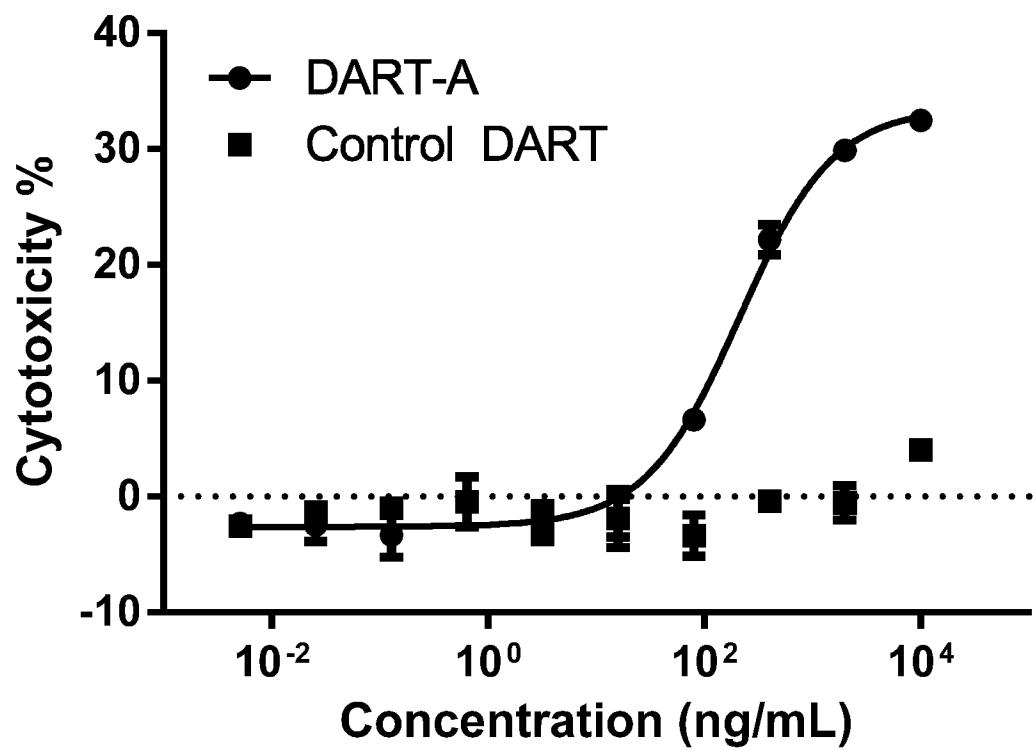
**Figure 5H**

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**Figure 5l**

21/50

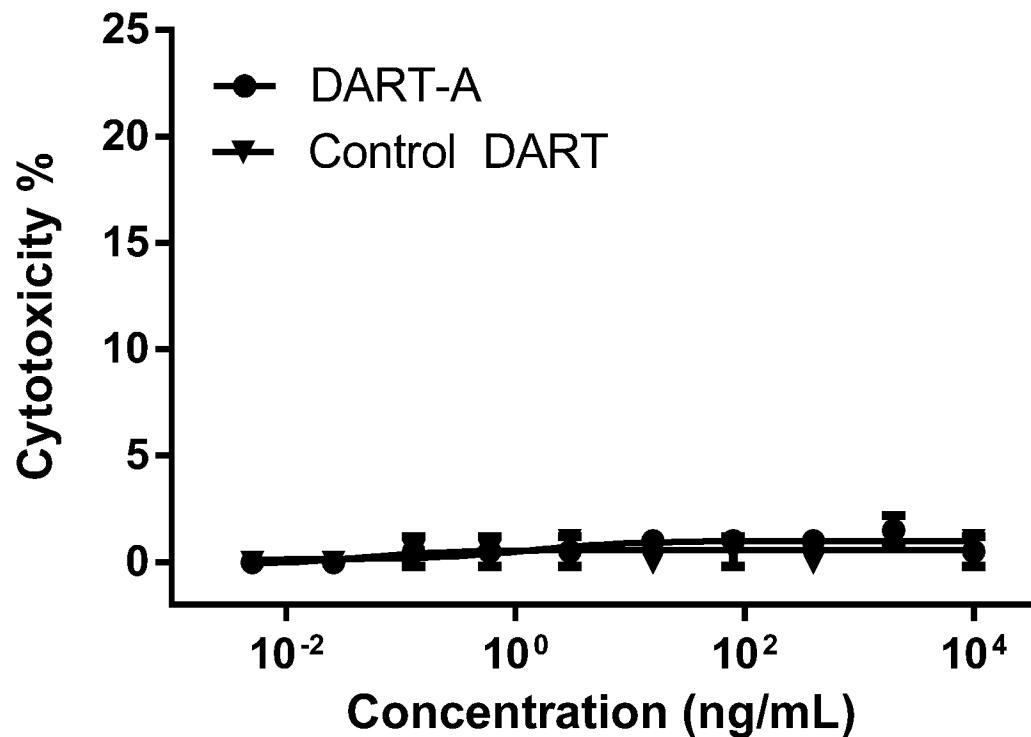
**22Rv1 + T Cells (D58108) E:T=5:1  
24h LDH**



**Figure 5J**

22/50

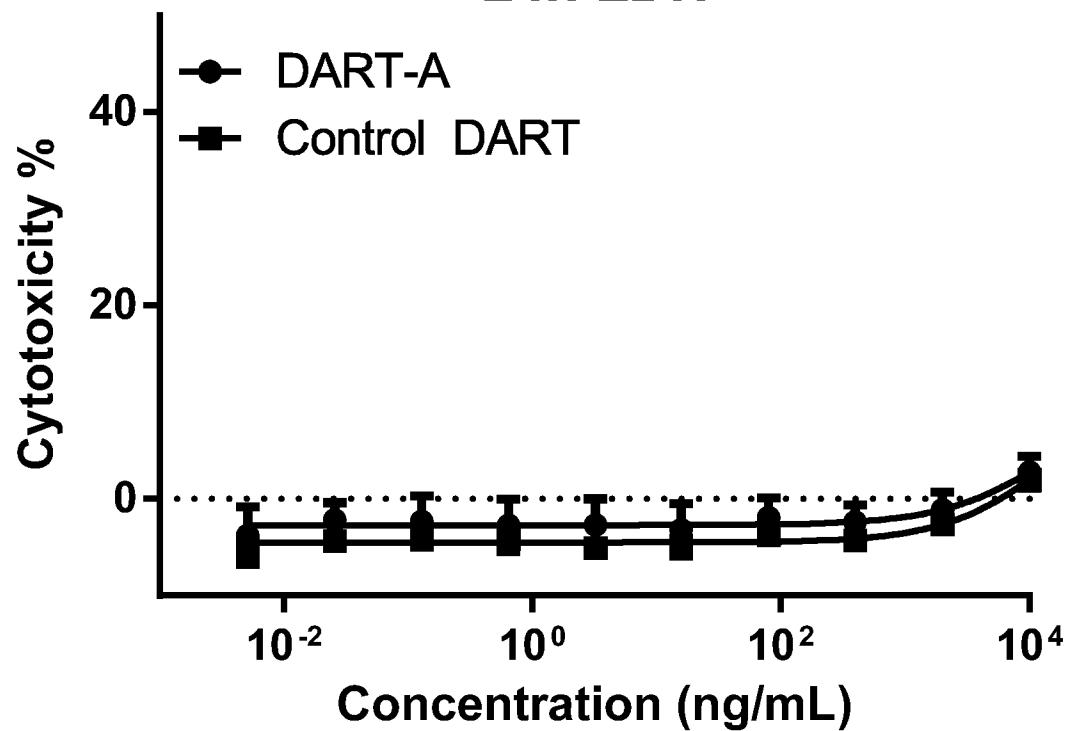
**CHO + T Cells (D55778) E:T=5:1  
24h LDH**



**Figure 5K**

23/50

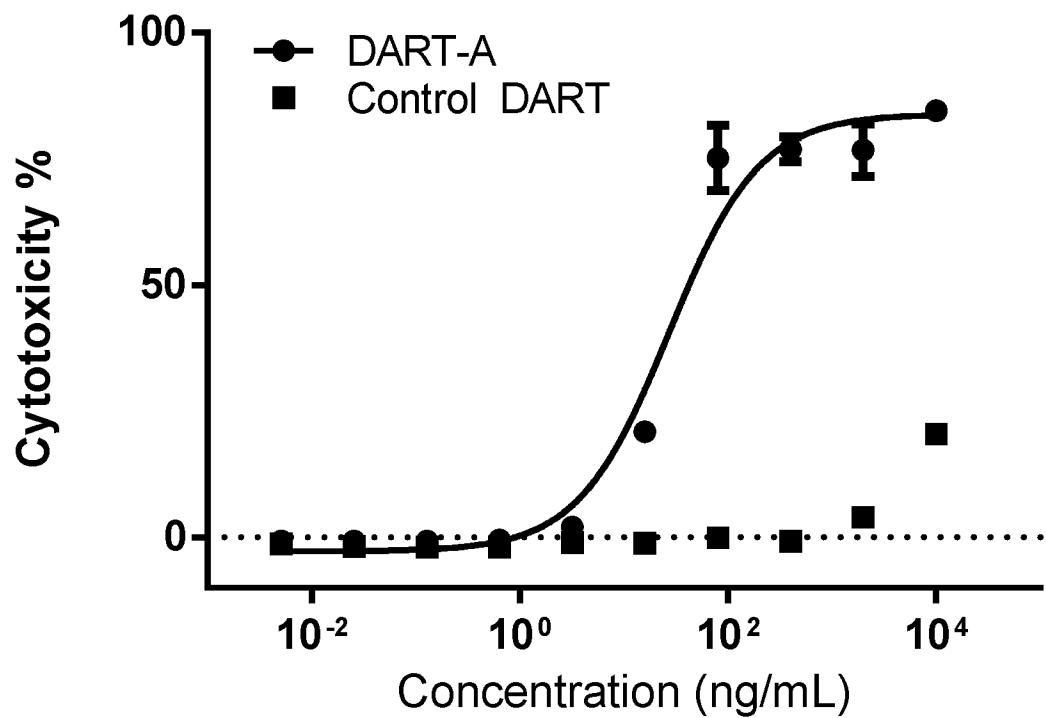
**Raji + T Cells (D43480) E:T=5:1  
24h LDH**



**Figure 5L**

24/50

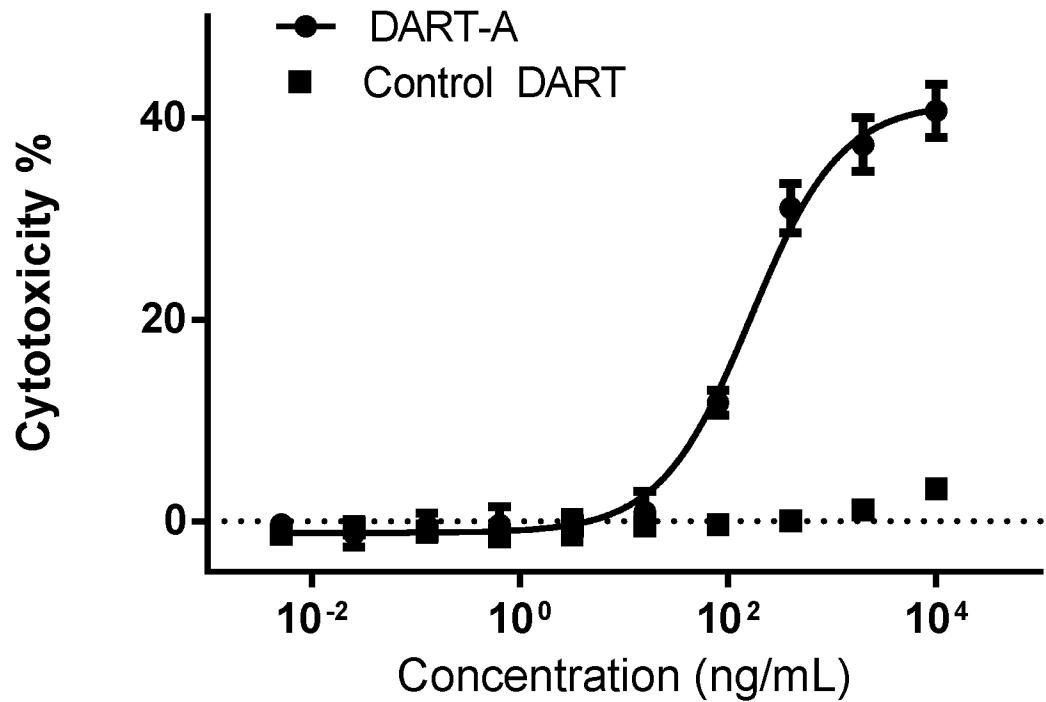
**A498 + T Cells (D59346) E:T=10:1  
24h LDH**



**Figure 6A**

25/50

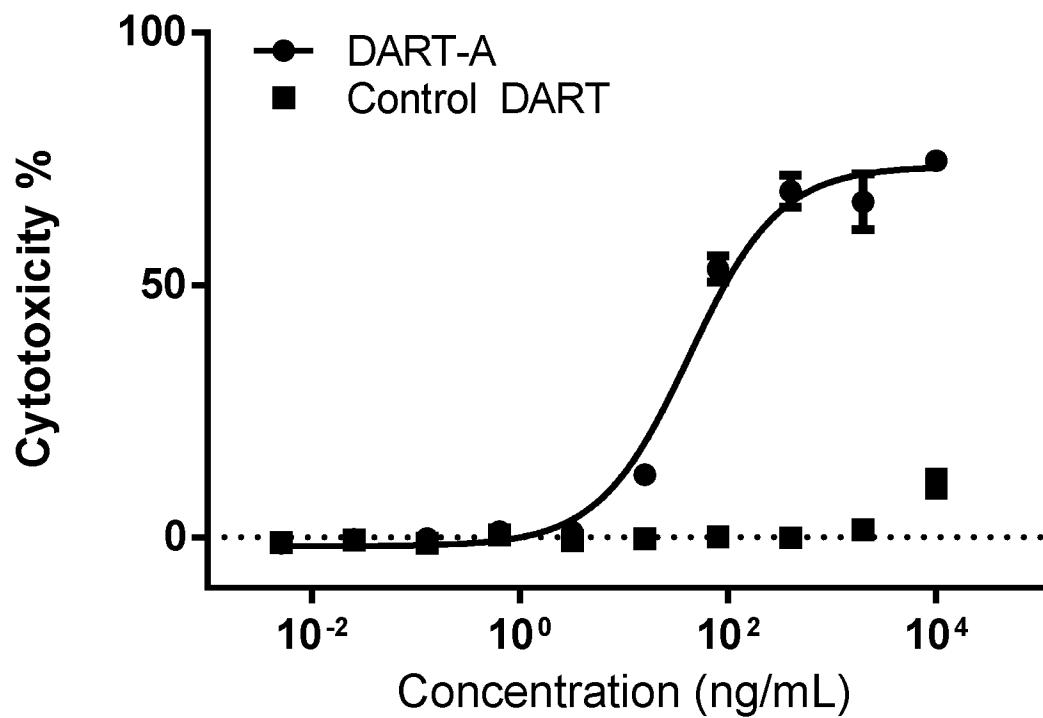
**A375 + T Cells (D59346) E:T=10:1  
24h LDH**



**Figure 6B**

26/50

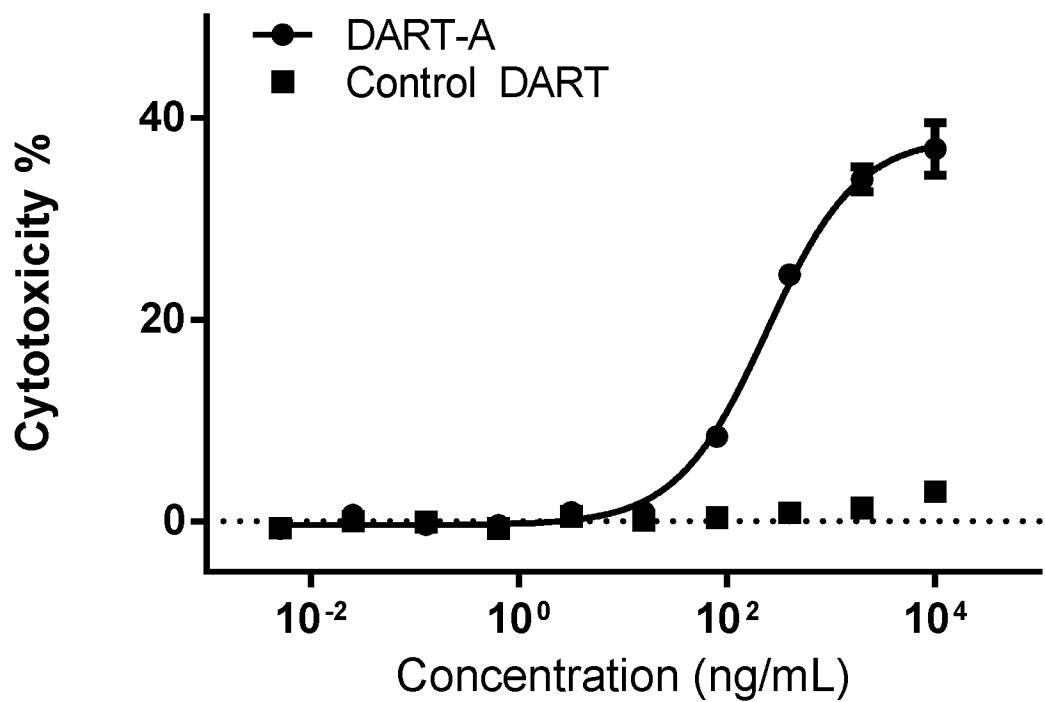
**A498 + T Cells (D59346) E:T=5:1  
24h LDH**



**Figure 6C**

27/50

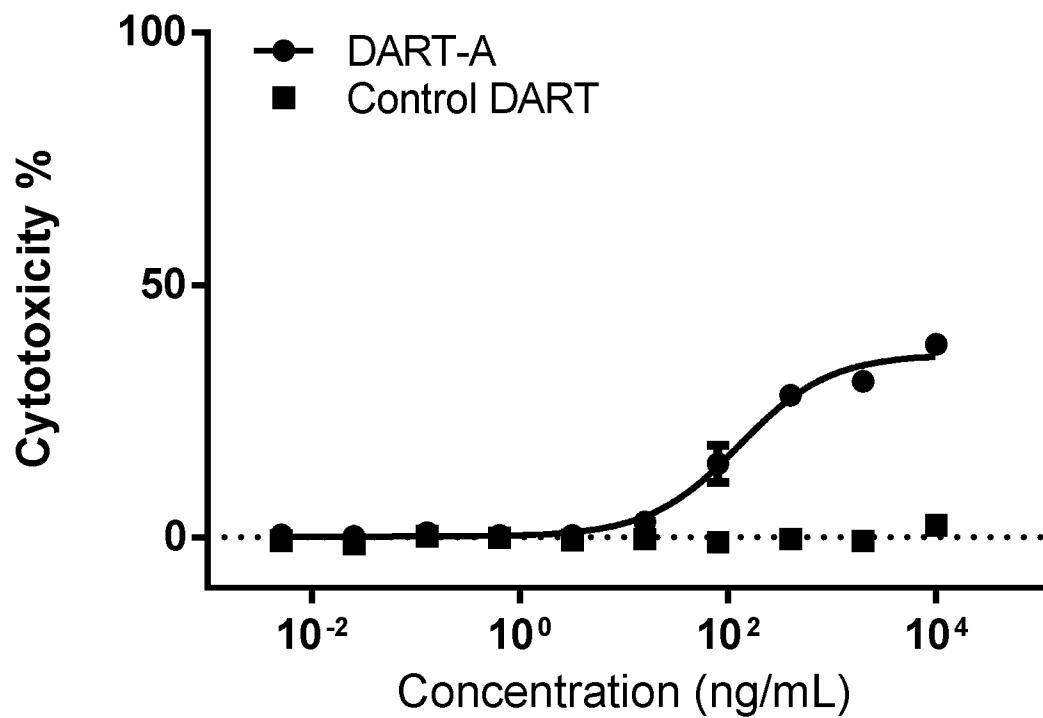
**A375 + T Cells (D59346) E:T=5:1  
24h LDH**



**Figure 6D**

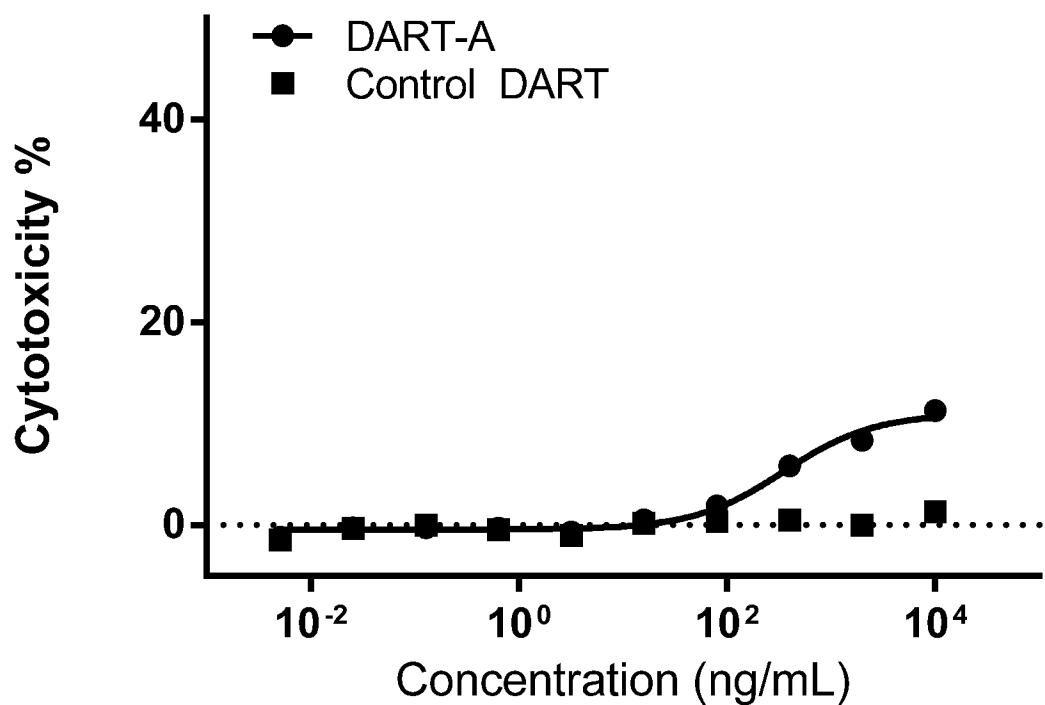
28/50

**A498 + T Cells (D59346) E:T=1:1  
24h LDH**



**Figure 6E**

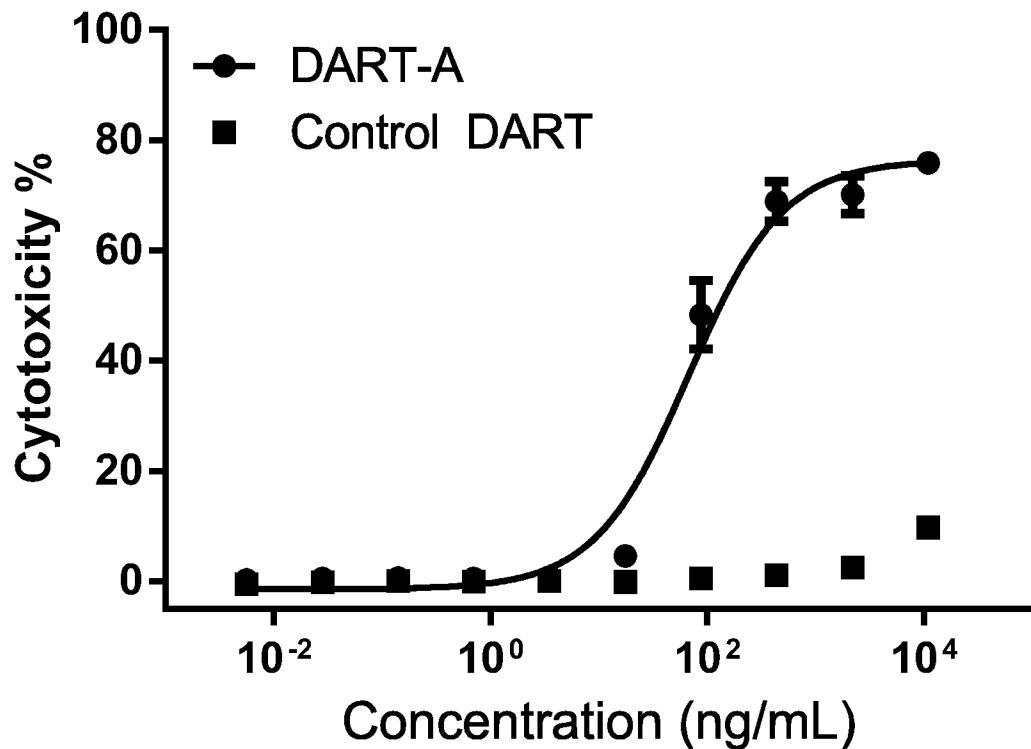
**A375 + T Cells (D59346) E:T=1:1  
24h LDH**



**Figure 6F**

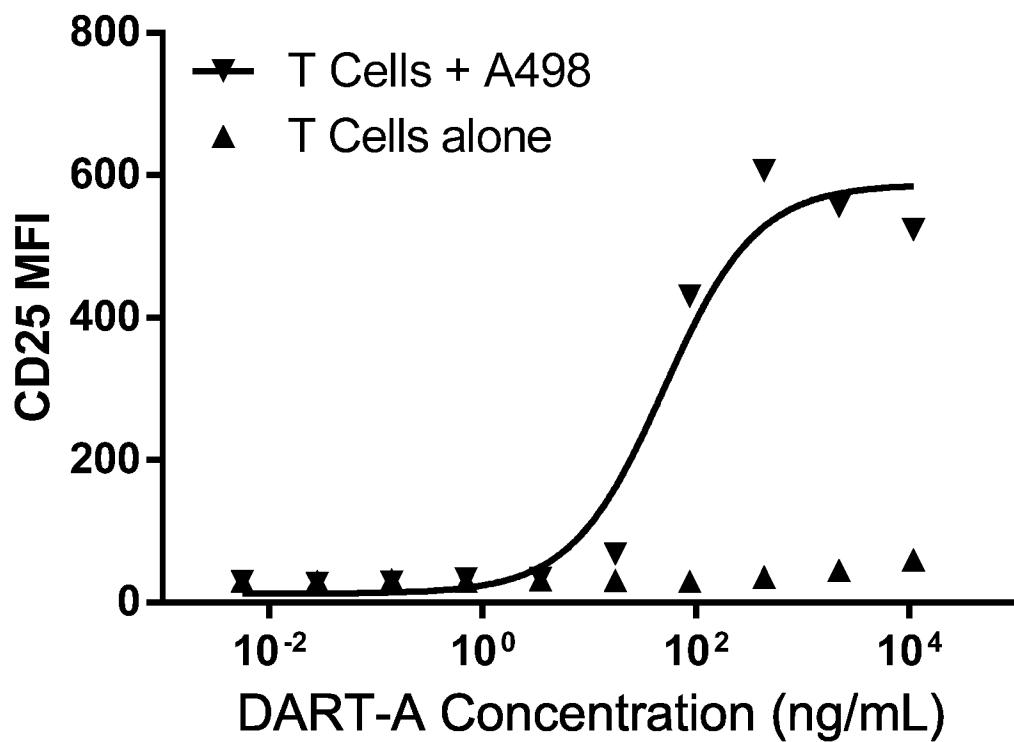
30/50

**A498 + T Cells (D47766) E:T=10:1  
LDH 24h**

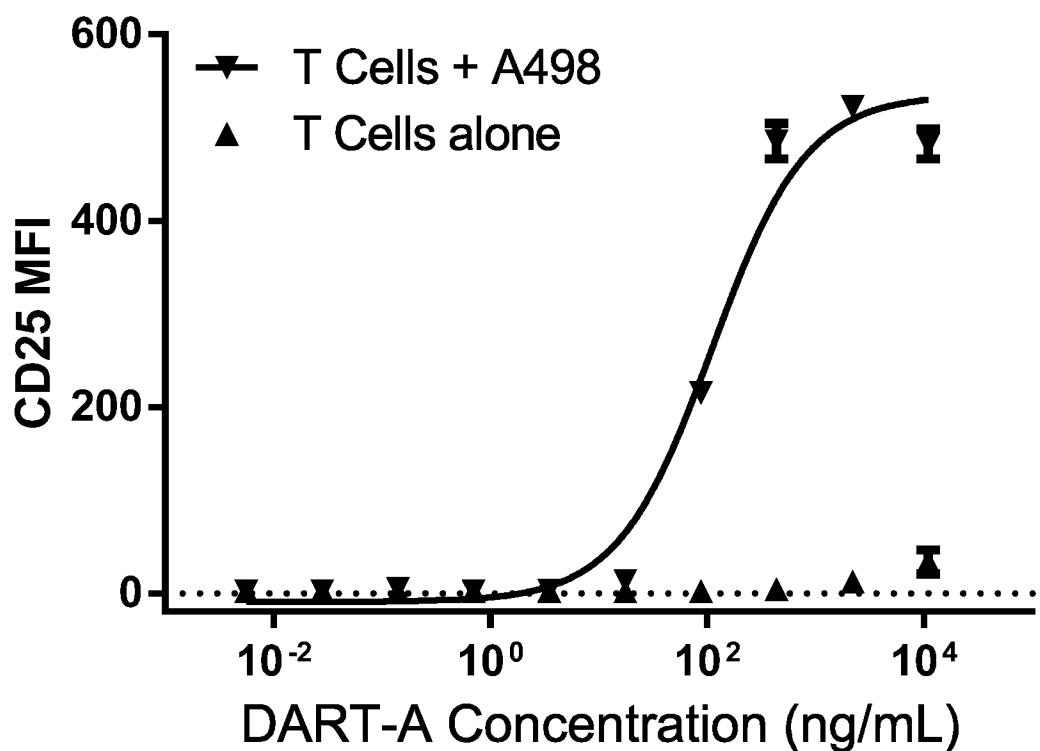


**Figure 7A**

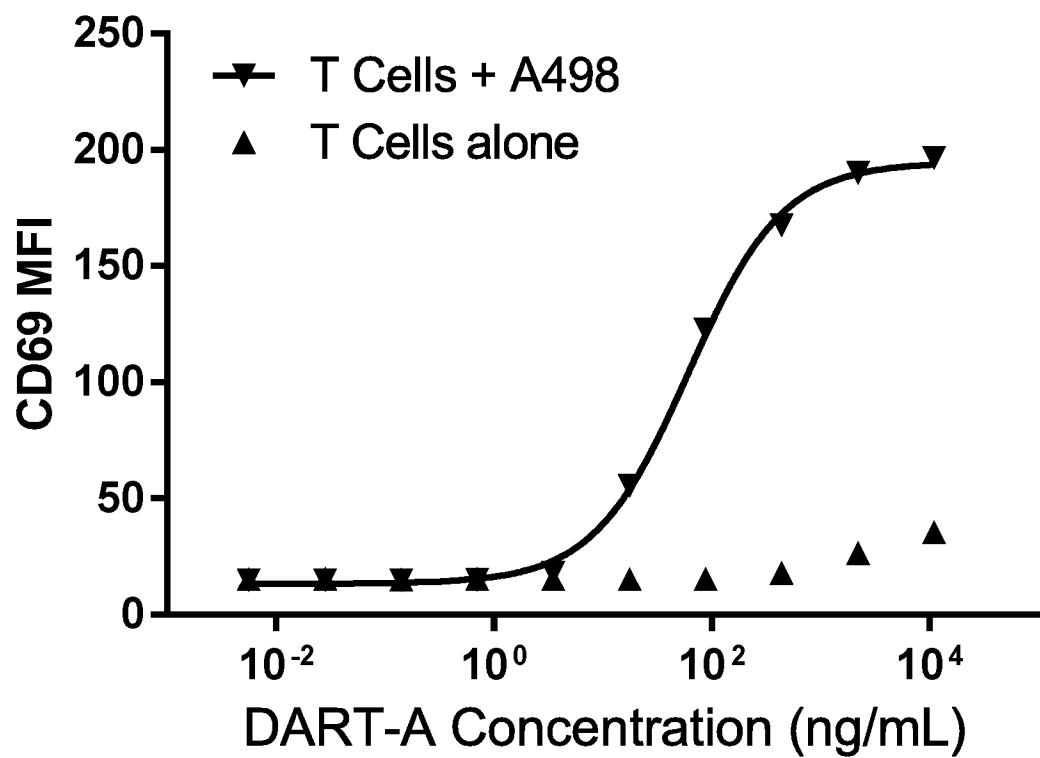
31/50

**CD4/CD25 (D47766)****Figure 7B**

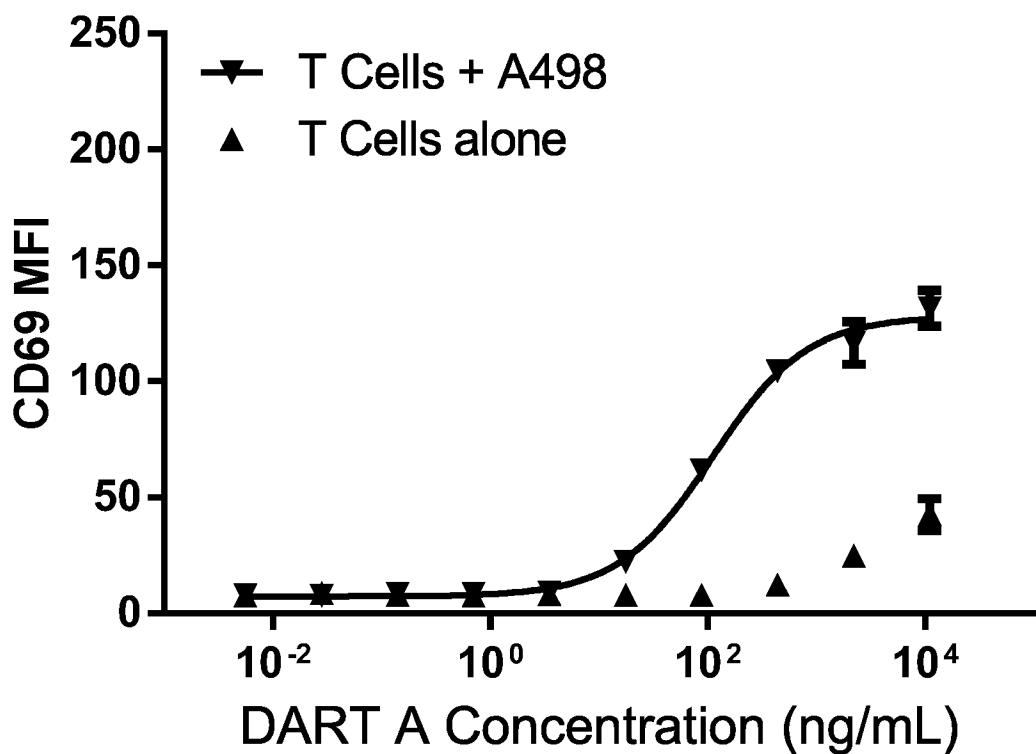
32/50

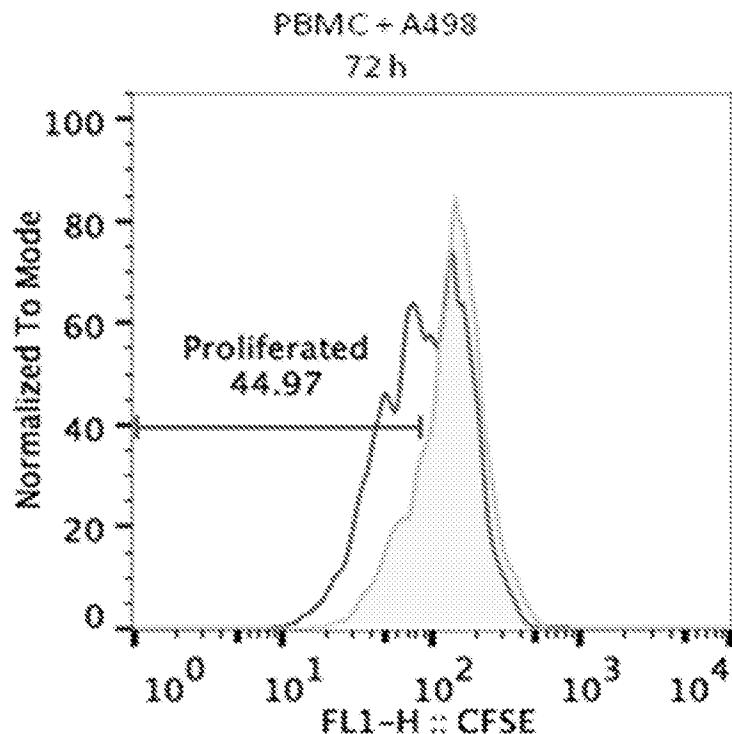
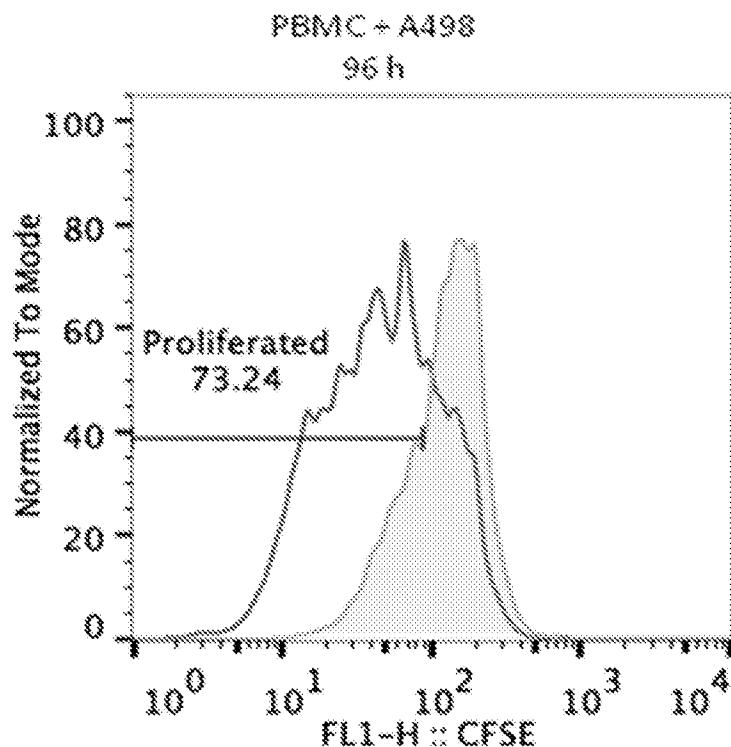
**CD8/CD25 (D47766)****Figure 7C**

33/50

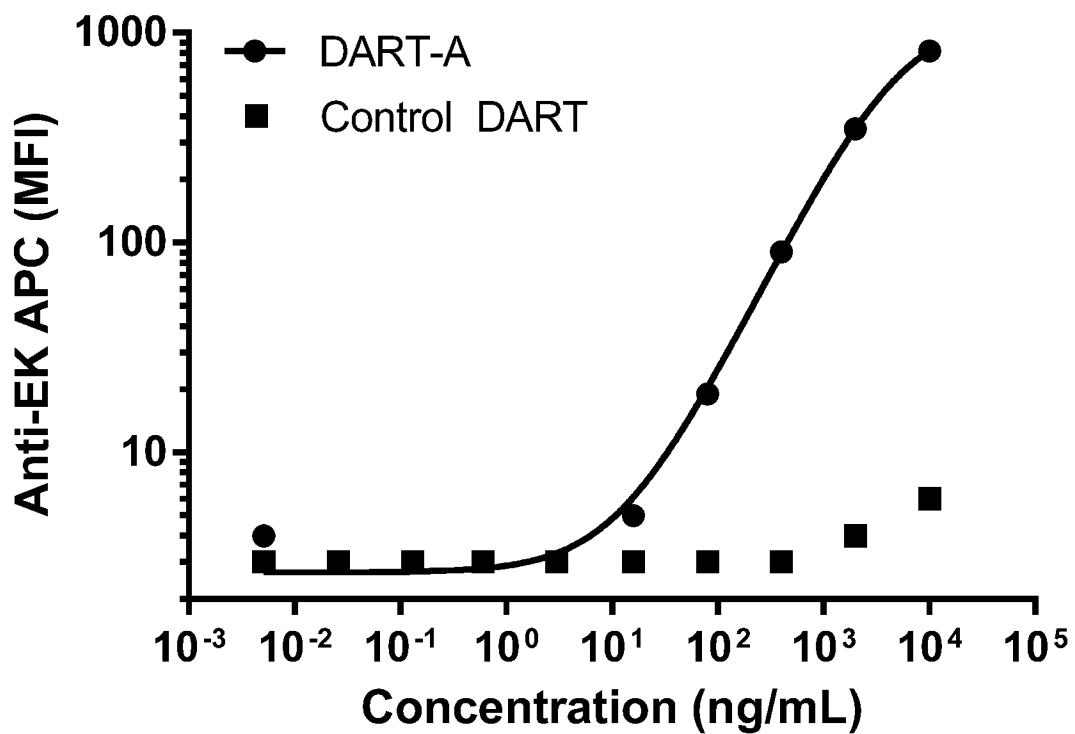
**CD4/CD69 (D47766)****Figure 7D**

34/50

**CD8/CD69 (D47766)****Figure 7E**

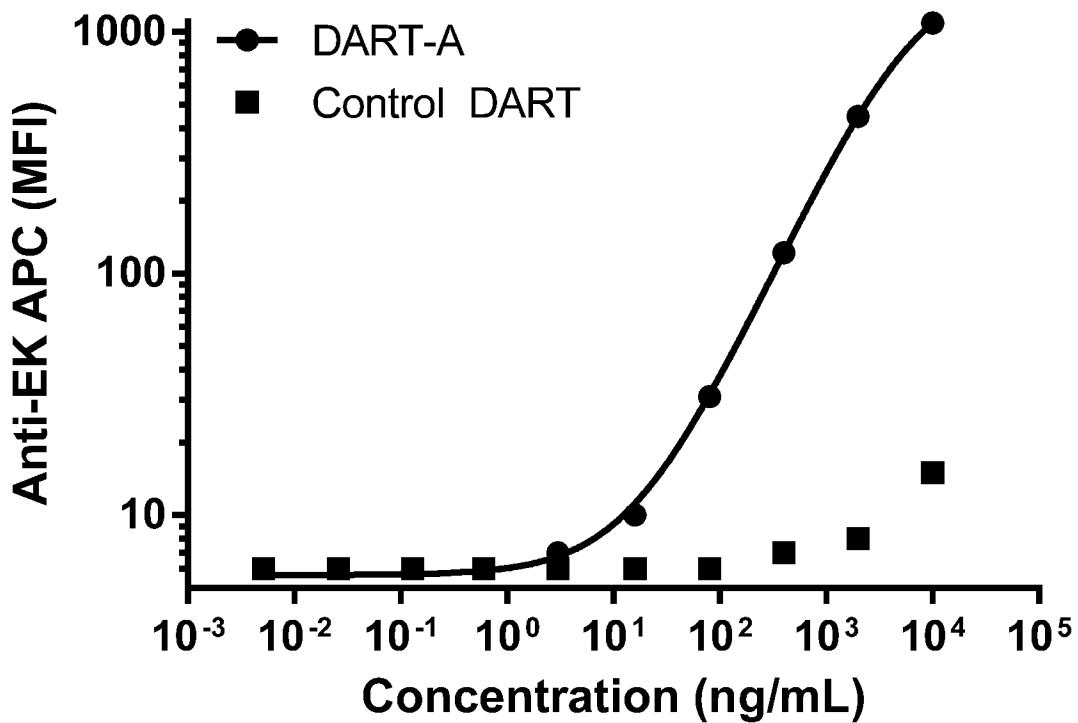
**35/50****Figure 8A****Figure 8B**

### Binding to Human B7-H3-CHO Cells (FACS)



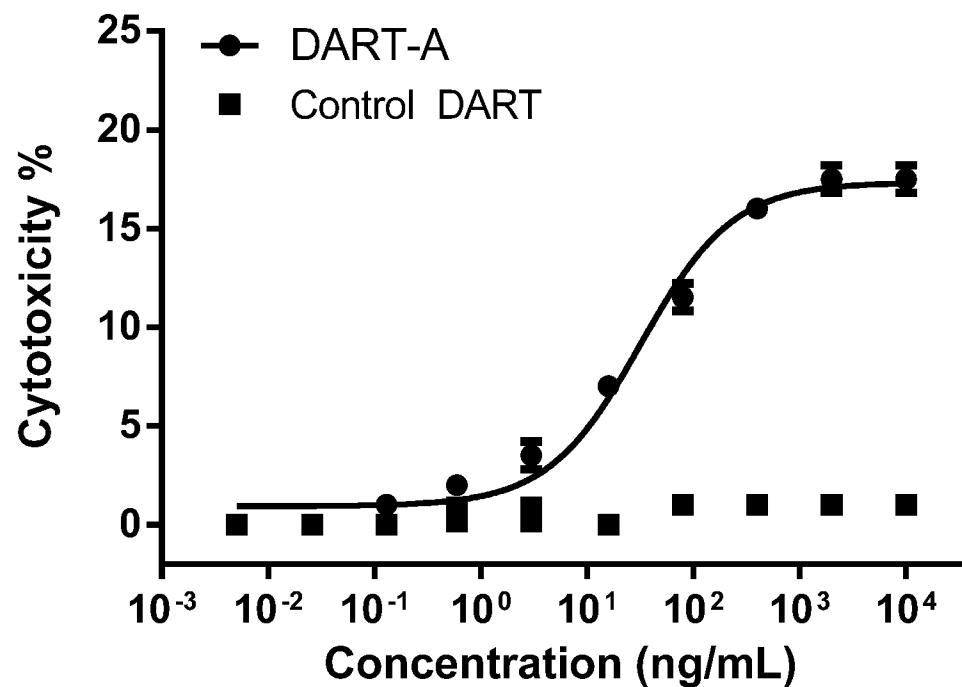
**Figure 9A**

### Binding to Cynomolgus B7-H3-CHO Cells (FACS)



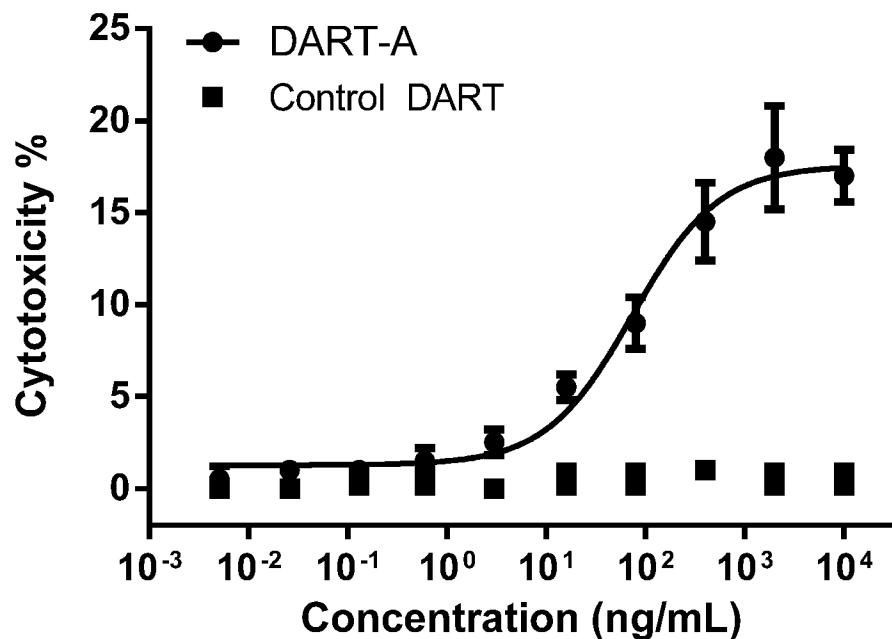
**Figure 9B**

**Human B7-H3-CHO (C27 H) + Human T Cells (D55778)**  
**24h LDH E:T=5:1**

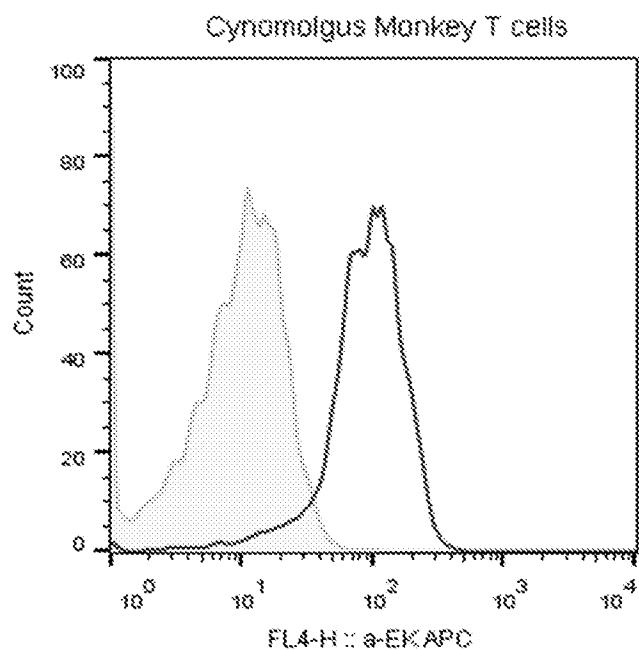
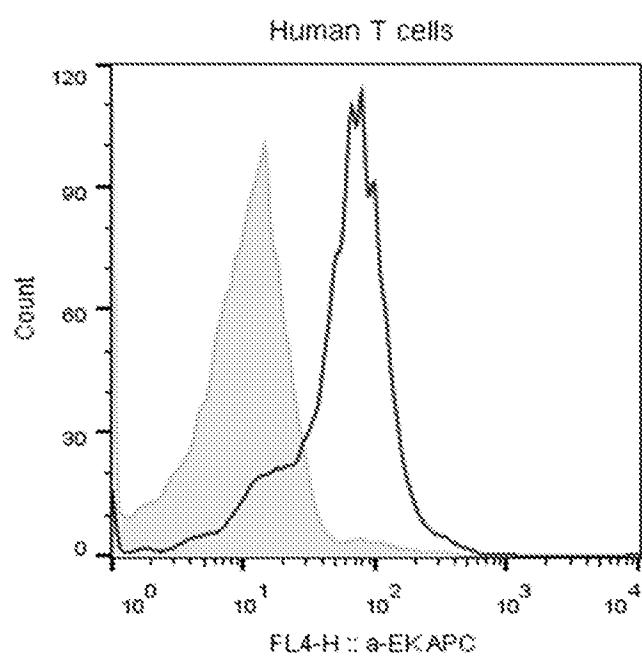


**Figure 9C**

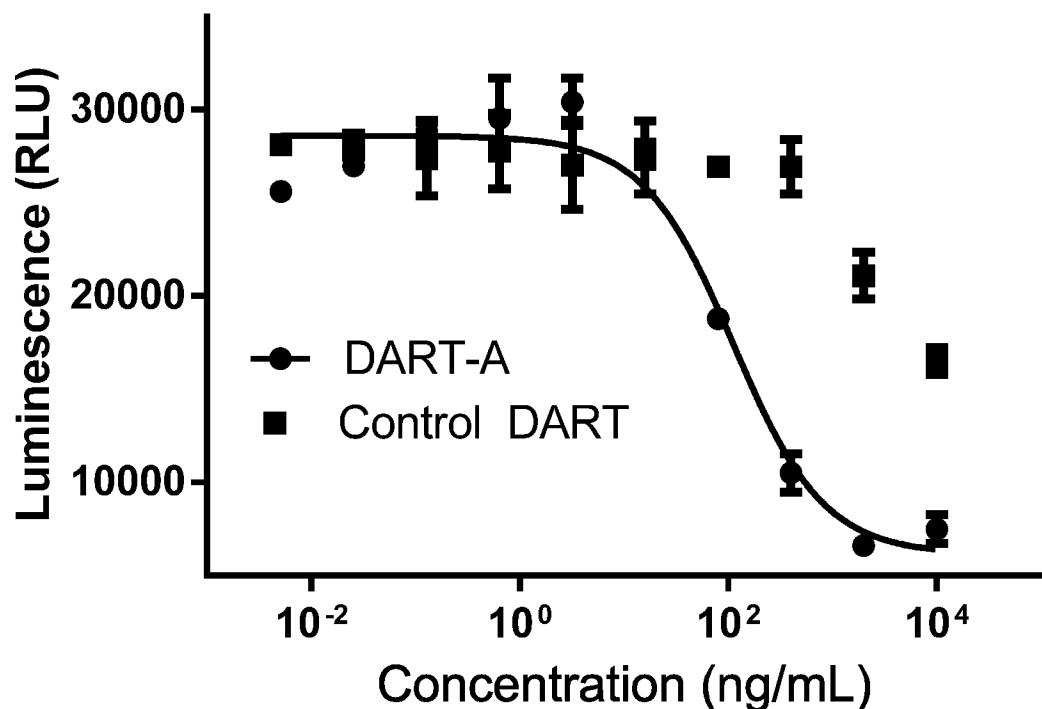
**Cynomolgus B7-H3-CHO (7229) + Human T Cells (D55778)**  
**24h LDH E:T=5:1**



**Figure 9D**

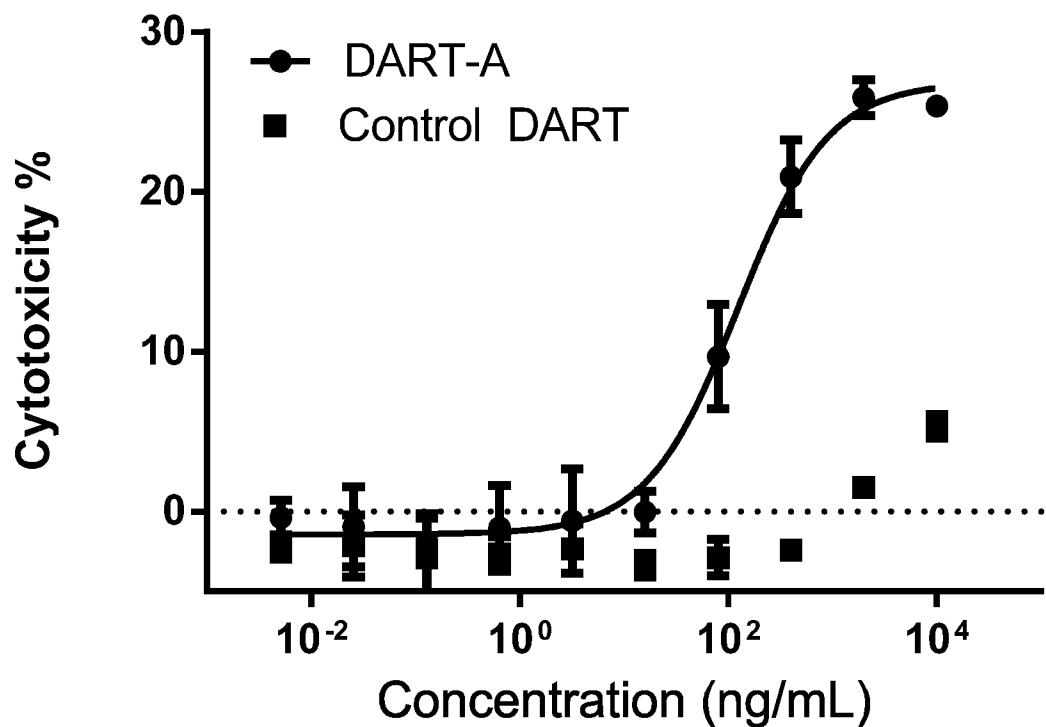
**40/50****Figure 10A****Figure 10B**

**JIMT-1/Luc + Cynomolgus PBMCs (5371)**  
**24h LUM E:T=30:1**



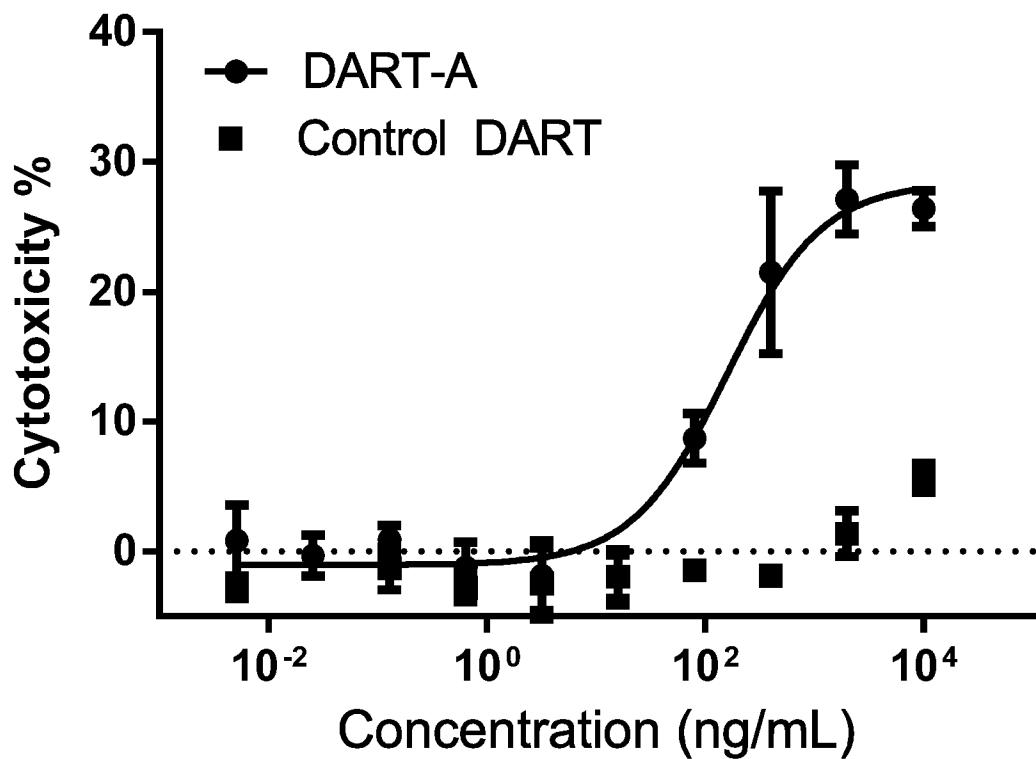
**Figure 11A**

**JIMT-1/Luc + Cynomolgus PBMCs (5371)**  
**24h LDH E:T=30:1**



**Figure 11B**

**A498 + Cynomolgus PBMCs (5371)**  
**24h LDH E:T=30:1**



**Figure 11C**

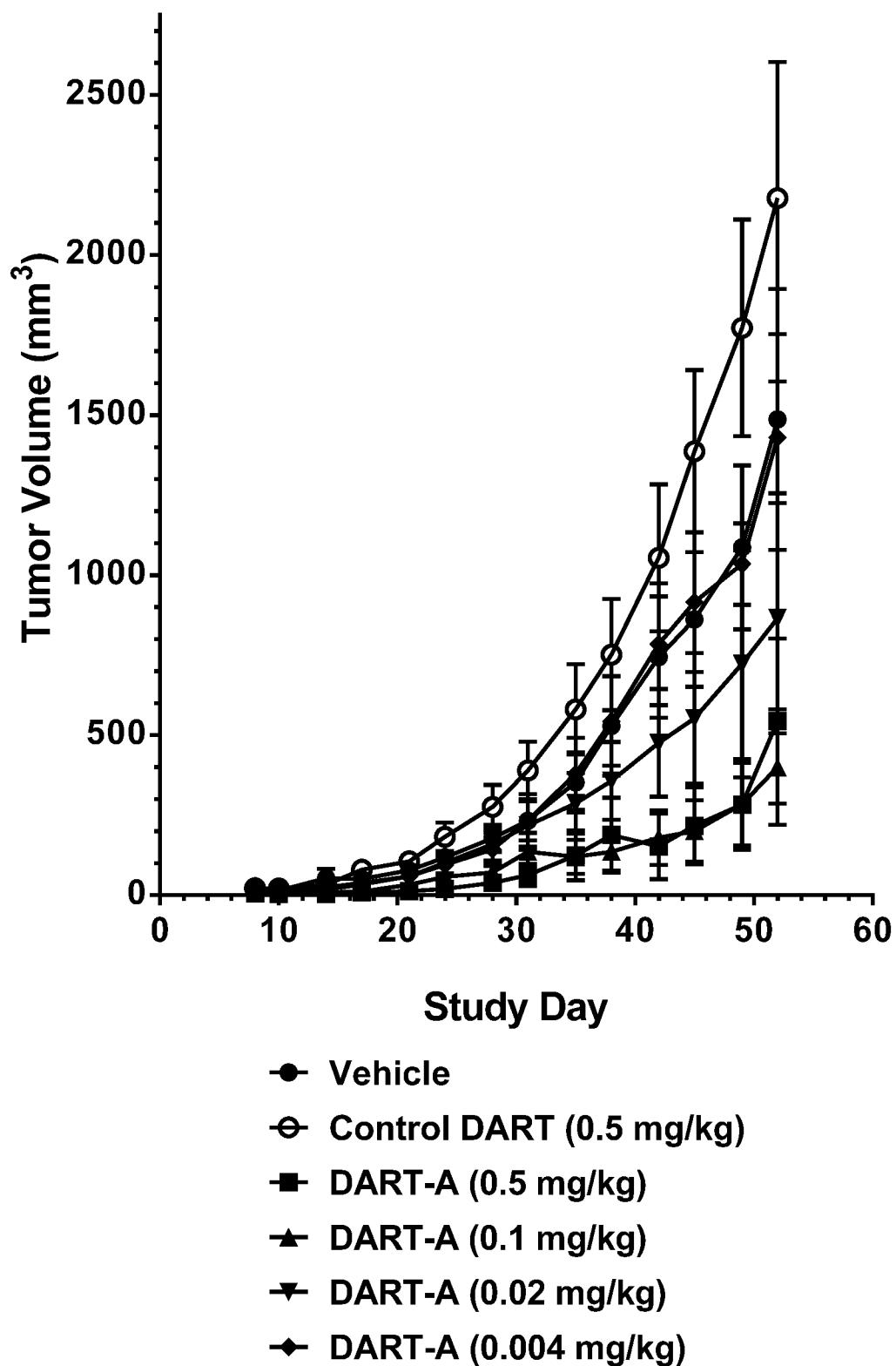


Figure 12

45/50

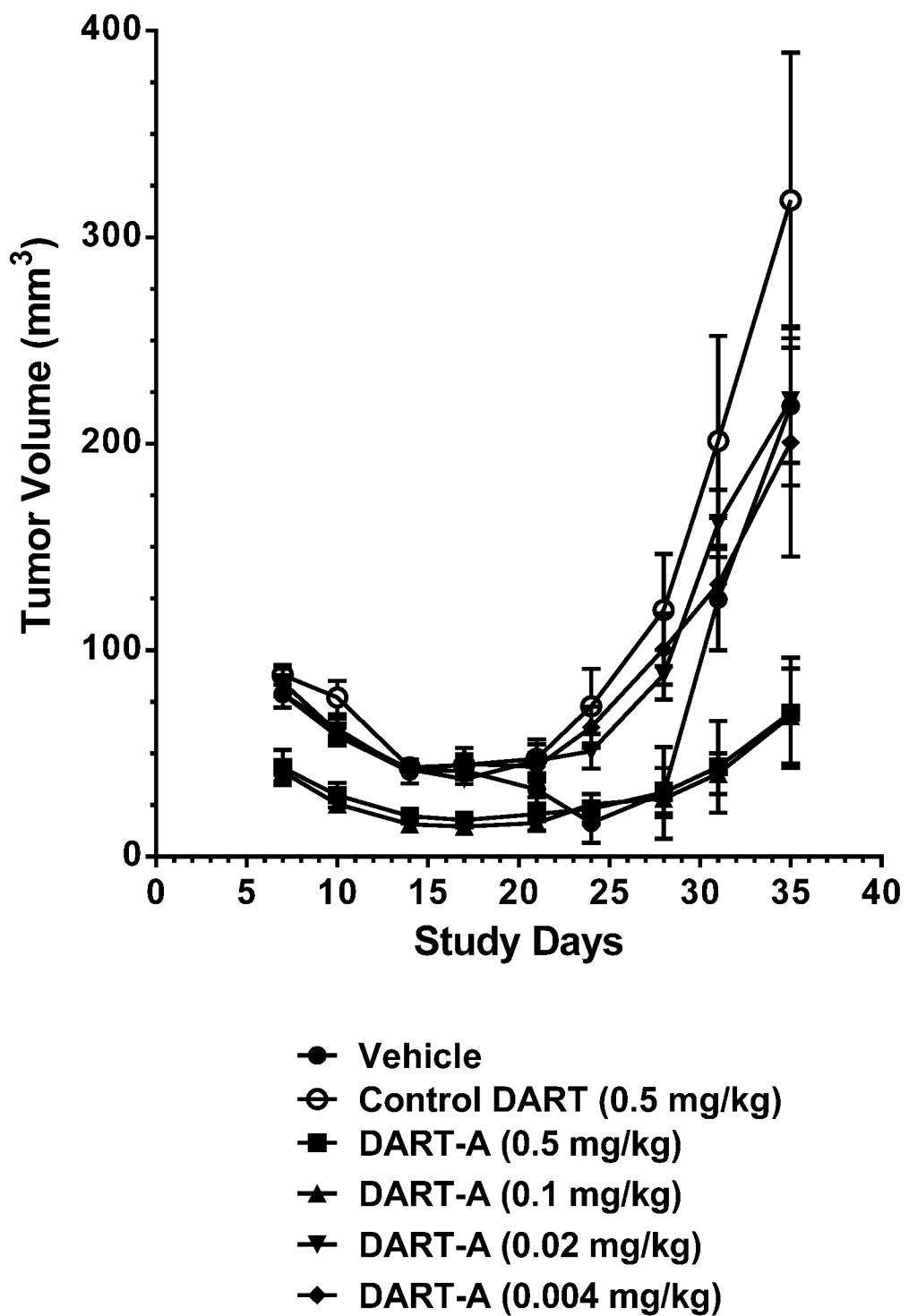
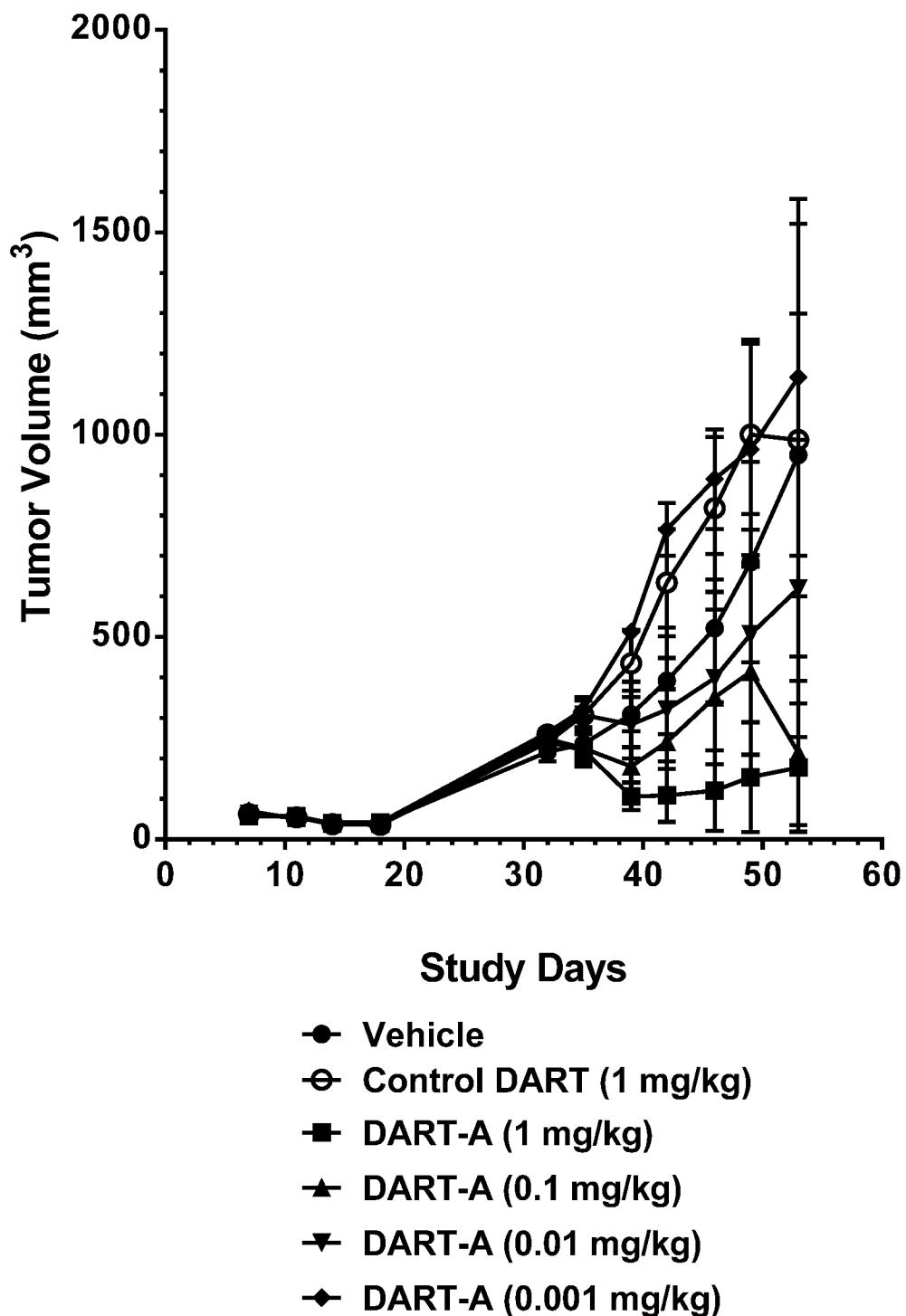
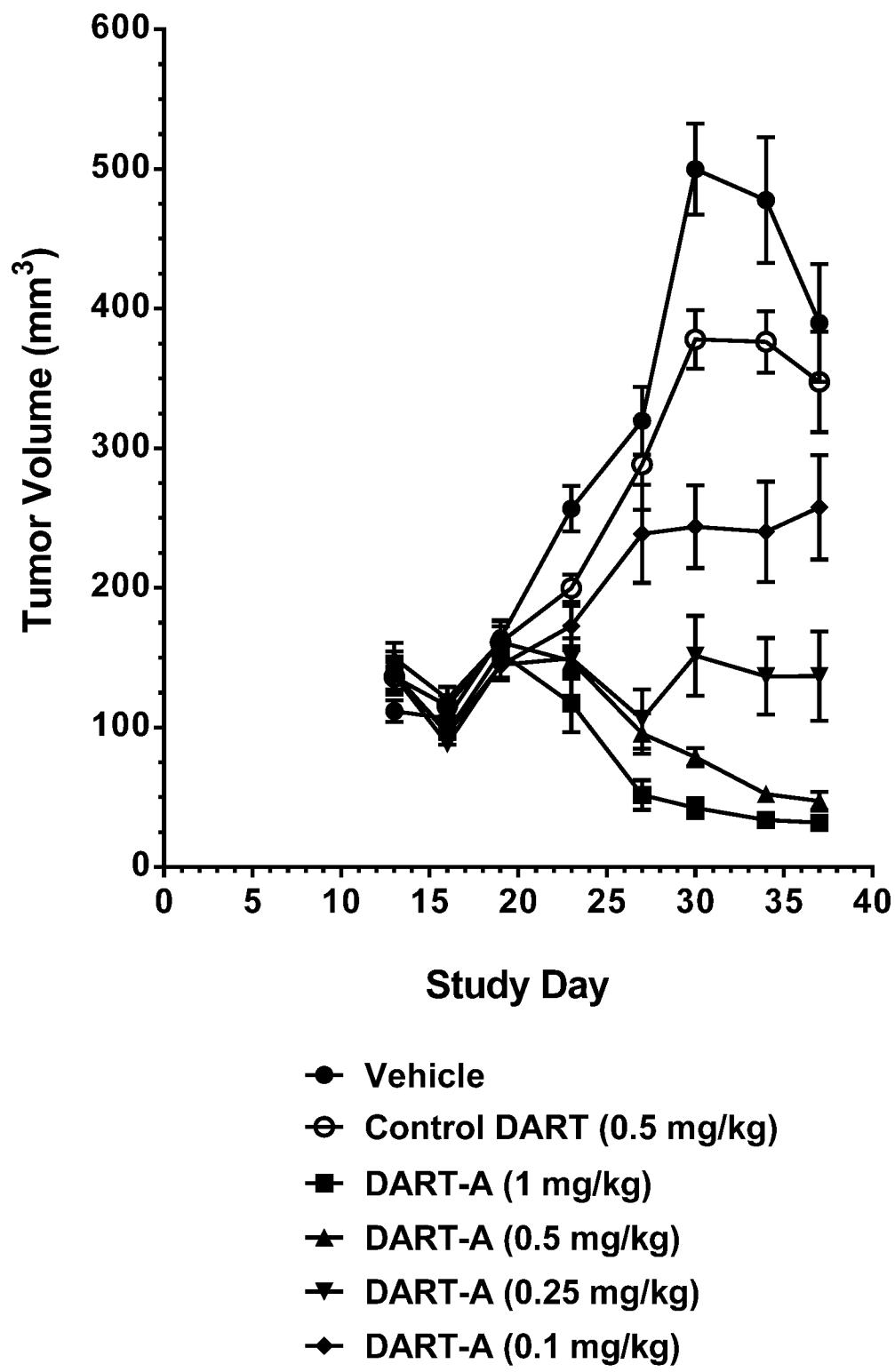
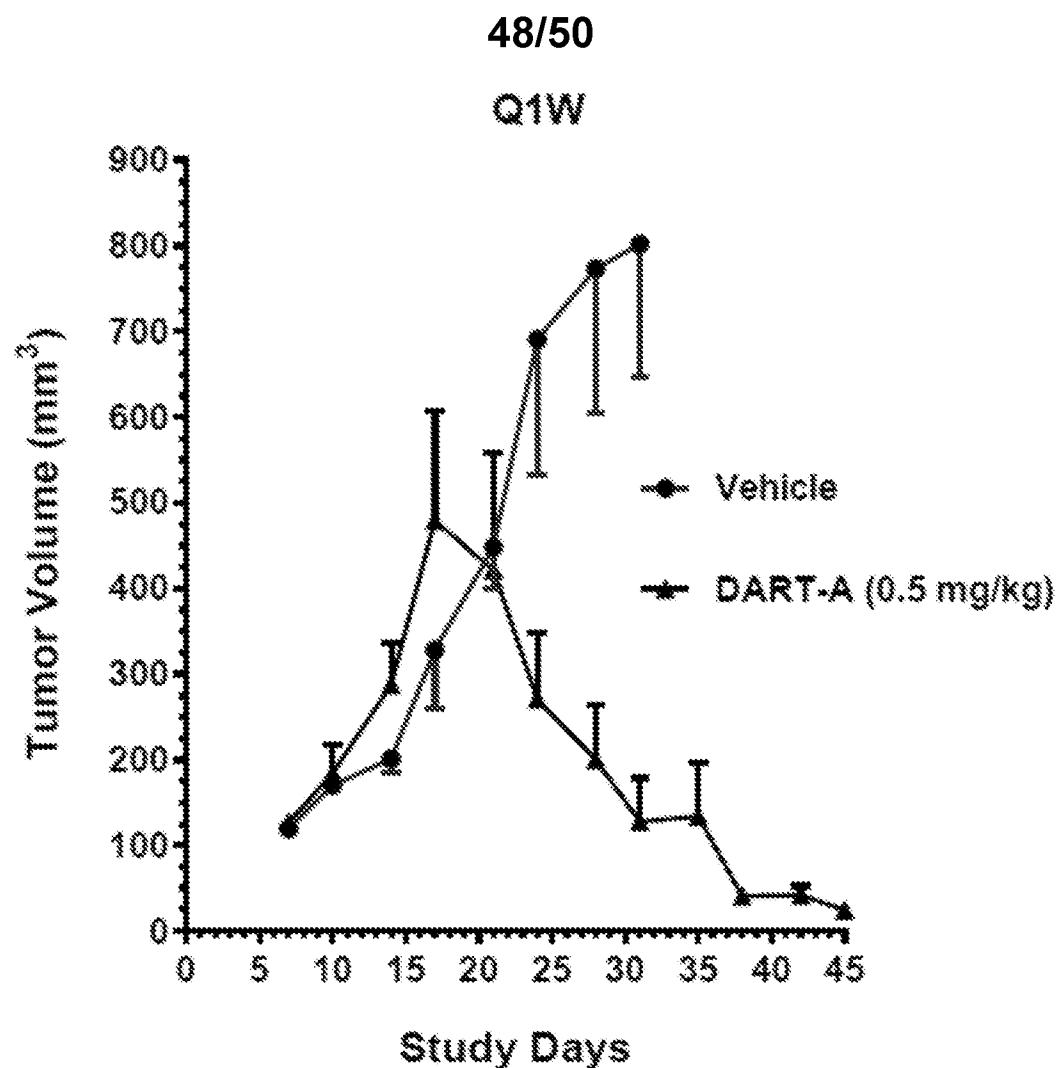


Figure 13

**Figure 14**

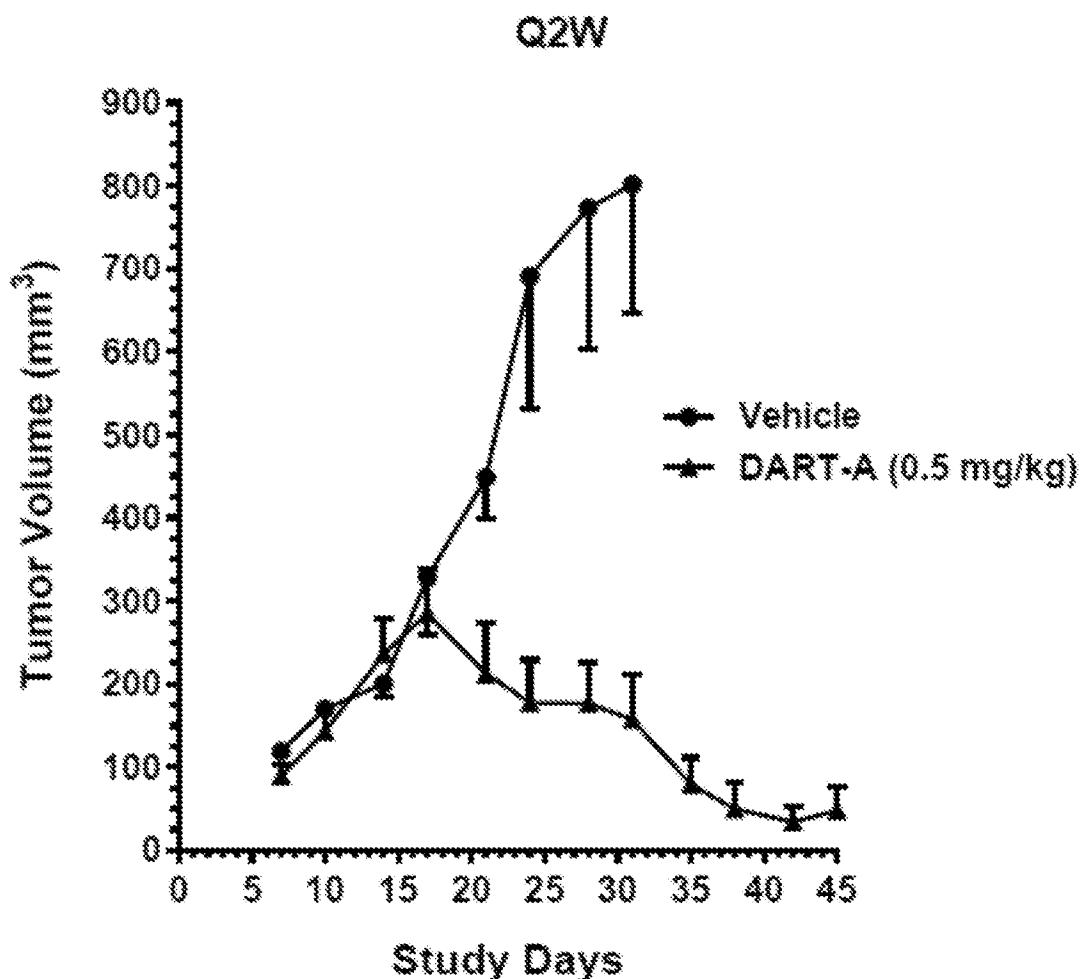
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**Figure 15**



**Figure 16A**

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**Figure 16B**

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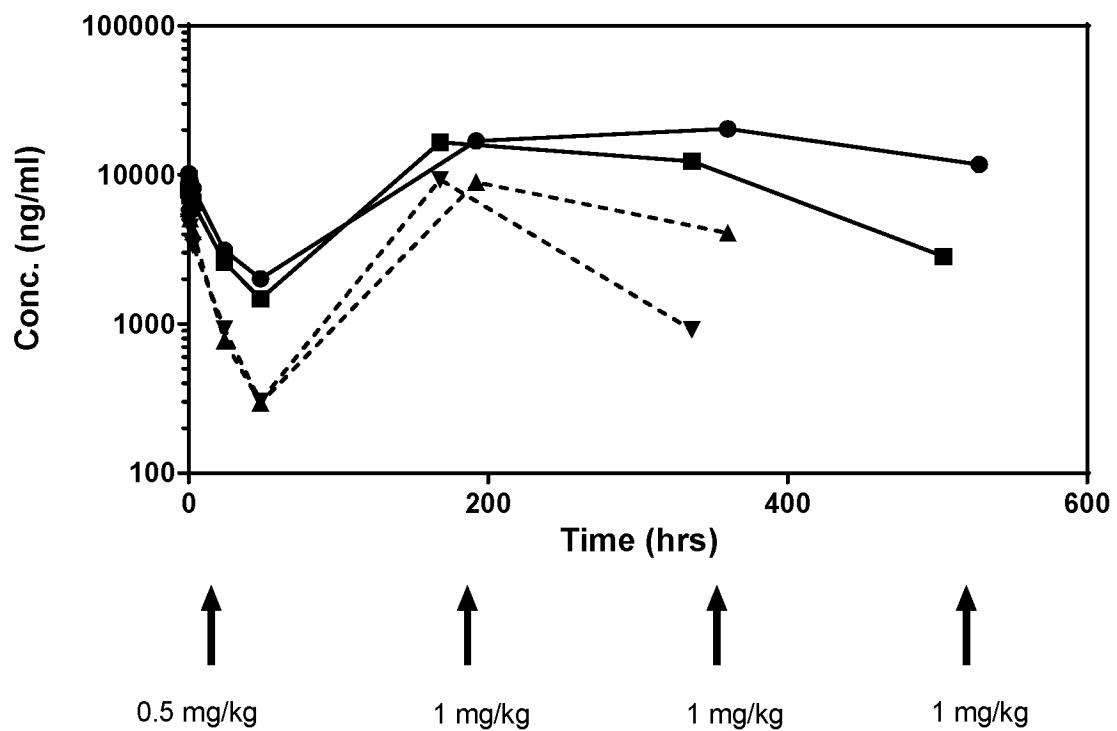


Figure 17

1301-0123PCT\_ST25  
SEQUENCE LISTING

<110> Macrogenics, Inc.  
Johnson, Leslie  
Moore, Paul  
Bonvini, Ezio  
Huang, Liang  
Shah, Kalpana  
Chichili, Gurunadh

<120> Bi specific Monovalent Diabodies That Are Capable of Binding B7-H3 and CD3, and Uses Thereof

<130> 1301.0123PCT

<150> US 62/206,051  
<151> 2015-08-17

<160> 68

<170> PatentIn version 3.5

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1 5 10 15

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Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln  
100 105 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met  
115 120 125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Glu Phe Tyr Pro  
130 135 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
145 150 155 160

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Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
165 170 175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Gly Asn Val  
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Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
50 55 60

Glu Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
65 70 75 80

Glu Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Glu  
100 105 110

Pro Arg Glu Pro Glu Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met  
115 120 125

Thr Lys Asn Glu Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro  
130 135 140

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Glu Pro Glu Asn Asn  
145 150 155 160

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
Page 5

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170

175

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Gly Asn Val  
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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Glu  
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Lys Ser Leu Ser Leu Ser Pro Gly Lys  
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Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val  
 20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr  
 35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 50 55 60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
 65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln  
 100 105 110

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met  
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Thr Lys Asn Gln Val Ser Leu Ser Cys Ala Val Lys Gly Phe Tyr Pro  
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Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
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Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu  
 165 170 175

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Val Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Gly Asn Val  
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Lys Ser Leu Ser Leu Ser Pro Gly Lys  
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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Asn Val Asp Thr Asn  
20 25 30

Val Ala Trp Tyr Glu Glu Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile  
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Glu Val Pro Ser Arg Phe Ser Glu  
50 55 60

Ser Glu Ser Glu Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro  
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Glu Asp Phe Ala Thr Tyr Tyr Cys Glu Glu Tyr Asn Asn Tyr Pro Phe  
85 90 95

Thr Phe Glu Glu Glu Thr Lys Leu Glu Ile Lys  
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Page 7

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10

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Ser Ala Ser Tyr Arg Tyr Ser  
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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe  
 20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr  
 65 70 75 80

Leu Glu Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp  
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Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val Lys  
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Gly

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr  
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
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Thr Phe Gly Gly Thr Lys Leu Gl u Ile Lys  
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Arg Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn  
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Tyr Thr Ser Arg Leu His Ser  
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Gln Gln Gly Asn Thr Leu Pro Pro Thr  
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1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
20 25 30

Trp Met Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
35 40 45

Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr Arg Tyr Thr Gln Lys Phe  
50 55 60

Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

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Thr Ile Tyr Pro Gly Asp Gly Asp Thr Arg Tyr Thr Gln Lys Phe Lys  
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Gly

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 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr  
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gln Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Tyr Thr Ser Arg Leu Gln Ser Gln Val Pro Ser Arg Phe Ser Gln  
 50 55 60

Ser Gln Ser Gln Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gln Asn Thr Leu Pro Pro  
 85 90 95

Thr Phe Gln Gln Gln Thr Lys Leu Glu Ile Lys  
 100 105

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&lt;223&gt; Light Chain CDR1 of B7-H3 mAb C

&lt;400&gt; 34

Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn  
 1 5 10

&lt;210&gt; 35

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; MI SC\_FEATURE

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&lt;223&gt; Light Chain CDR2 of B7-H3 mAb C

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Tyr Thr Ser Arg Leu Gln Ser  
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&lt;210&gt; 36

&lt;211&gt; 9

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&lt;213&gt; Mus musculus

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala  
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr  
 20 25 30

Trp Met Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met  
 35 40 45

Gly Thr Ile Tyr Pro Gly Gly Asp Thr Arg Tyr Thr Gln Lys Phe  
 50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

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 100 105 110

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1 5 10 15

Gly

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Glu Ala Val Val Thr Glu Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30Asn Tyr Ala Asn Trp Val Glu Glu Lys Pro Gly Glu Ala Pro Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe  
 50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala  
 65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
 85 90 95

Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Gly  
 100 105 110

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Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn  
 1 5 10

<210> 43  
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Gly Thr Asn Lys Arg Ala Pro  
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Ala Leu Trp Tyr Ser Asn Leu Trp Val  
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 1 5 10 15

Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl y Phe Thr Phe Ser Thr Tyr  
 20 25 30

Al a Met Asn Trp Val Arg Gl n Al a Pro Gl y Lys Gl y Leu Gl u Trp Val  
 35 40 45

Gl y Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Al a Thr Tyr Tyr Al a Asp  
 50 55 60

Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser  
 65 70 75 80

Leu Tyr Leu Gl n Met Asn Ser Leu Lys Thr Gl u Asp Thr Al a Val Tyr  
 85 90 95

Tyr Cys Val Arg His Gl y Asn Phe Gl y Asn Ser Tyr Val Ser Trp Phe  
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Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser  
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Val Lys Asp

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His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr  
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 Substitution at Kabat Position 65

<400> 49

Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser  
 1 5 10 15

Val Lys Gly

<210> 50  
 <211> 125  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Heavy Chain Variable Domain of CD3 mAb A Containing Aspartate to  
 Glycine Substitution at Kabat Position 65

<400> 50

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Thr Tyr  
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25

30

Ala Met Asn Trp Val Arg Glu Ala Pro Gly Lys Glu Leu Glu Trp Val  
35 40 45

Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr Tyr Ala Asp  
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser  
65 70 75 80

Leu Tyr Leu Glu Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val Ser Trp Phe  
100 105 110

Ala Tyr Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser  
115 120 125

<210> 51

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker 4

<400> 51

Ala Pro Ser Ser Ser  
1 5

<210> 52

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Alternative Linker 4

<400> 52

Ala Pro Ser Ser Ser Pro Met Glu  
1 5

<210> 53

<211> 504

<212> PRT

<213> Artificial Sequence

<220>

<223> First Polypeptide Chain of B7-H3 x CD3 Bi-specific DART-A Diabody

<400> 53

Asp Ile Glu Leu Thr Glu Ser Pro Ser Phe Leu Ser Ala Ser Val Glu  
1 5 10 15

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Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asp Thr Asn  
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile  
35 40 45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Asn Tyr Pro Phe  
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly  
100 105 110

Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln  
115 120 125

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe  
130 135 140

Ser Thr Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
145 150 155 160

Gl u Trp Val G l y Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr  
165 170 175

Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser  
180 185 190

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Gl u Asp Thr  
195 200 205

Ala Val Tyr Tyr Cys Val Arg His G l y Asn Phe G l y Asn Ser Tyr Val  
210 215 220

Ser Trp Phe Ala Tyr Trp G l y Gln G l y Thr Leu Val Thr Val Ser Ser  
225 230 235 240

Gly G l y Cys G l y G l y Gl u Val Ala Ala Leu Gl u Lys Gl u Val Ala  
245 250 255

Ala Leu Gl u Lys Gl u Val Ala Ala Leu Gl u Lys Gl u Val Ala Ala Leu  
260 265 270

Gl u Lys G l y G l y Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
275 280 285

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Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
290 295 300

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
305 310 315 320

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
325 330 335

Asp Glu Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Glu  
340 345 350

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Glu  
355 360 365

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
370 375 380

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Glu Glu Pro  
385 390 395 400

Arg Glu Pro Glu Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr  
405 410 415

Lys Asn Glu Val Ser Leu Trp Cys Leu Val Lys Glu Phe Tyr Pro Ser  
420 425 430

Asp Ile Ala Val Glu Trp Glu Ser Asn Glu Glu Pro Glu Asn Asn Tyr  
435 440 445

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Glu Ser Phe Phe Leu Tyr  
450 455 460

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Glu Asn Val Phe  
465 470 475 480

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Glu Lys  
485 490 495

Ser Leu Ser Leu Ser Pro Glu Lys  
500

<210> 54

<211> 1512

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide Encoding the First Polypeptide Chain of B7-H3 x  
CD3 Bi-specific DART-A Diabody

<400> 54

gacatccagtc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga cagagtgacc

60

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atcacatgca	aggcctccca	gaacgtggac	accaacgtgg	cctggtatca	gcagaagcct	120
ggcaaggccc	ctaaggcgct	gatctactcc	gcctcctacc	ggtactccgg	cgtccctcc	180
aggttctccg	gctccggctc	tggcaccgac	ttcaccctga	ccatctccag	cctgcagcct	240
gaggacttcg	ccacctacta	ctgccagcag	tacaacaact	accctttcac	cttcggccag	300
ggcaccaagc	tggaaatcaa	gggaggcgg	tccggcggcg	gaggcgaggt	gcagctggtg	360
gagtctgggg	gaggcttgg	ccagcctgga	gggtccctga	gactctcctg	tgcagcctct	420
ggattcacct	tcagcacata	cgctatgaat	tgggtccgccc	aggctccagg	gaaggggctg	480
gagtgggttg	gaaggatcag	gtccaagtac	aacaattatg	caacctacta	tgccgactct	540
gtgaaggata	gattcaccat	ctcaagagat	gattcaaaga	actcactgta	tctgcaaatg	600
aacagcctga	aaaccgagga	cacggccgtg	tattactgtg	tgagacacgg	taacttcggc	660
aattcttacg	tgtcttgggt	tgcttattgg	ggacagggga	cactggtgac	tgtgtcttcc	720
ggaggatgtg	gcggtgagaa	agtggccgca	ctggagaaag	agttgctgc	tttggagaag	780
gaggtcgctg	cacttgaaaa	ggaggtcgca	gccctggaga	aaggcggcgg	ggacaaaact	840
cacacatgcc	caccgtgccc	agcacctgaa	gccgcggggg	gaccgtcagt	cttcctcttc	900
cccccaaaac	ccaaggacac	cctcatgatc	tcccgaccc	ctgaggtcac	atgcgtggtg	960
gtggacgtga	gccacgaaga	ccctgaggc	aagttcaact	ggtacgtgga	cggcgtggag	1020
gtgcataatg	ccaagacaaa	gccgcggag	gagcagtaca	acagcacgta	ccgtgtggtc	1080
agcgtcctca	ccgtcctgca	ccaggactgg	ctgaatggca	aggagtacaa	gtgcaaggc	1140
tccaacaaag	ccctcccagc	ccccatcgag	aaaaccatct	ccaaagccaa	agggcagccc	1200
cgagaaccac	aggtgtacac	cctgccccca	tcccggagg	agatgaccaa	gaaccaggc	1260
agcctgtgg	gcctggtcaa	aggctctat	cccagcgaca	tcgccgtgga	gtggagagc	1320
aatggcagc	cggagaacaa	ctacaagacc	acgcctcccg	tgctggactc	cgacggctcc	1380
ttcttcctct	acagcaagct	caccgtggac	aagagcaggt	ggcagcaggg	gaacgtcttc	1440
tcatgctccg	tgtatgcatga	ggctctgac	aaccactaca	cgcagaagag	cctctccctg	1500
tctccggta	aa					1512

<210> 55  
 <211> 274  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Second Polypeptide Chain of B7-H3 x CD3 Bifunctional DART-A Diabody  
 <400> 55

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
 1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser  
 20 25 30

Asn Tyr Al a Asn Trp Val Gl n Gl n Lys Pro Gl y Gl n Al a Pro Arg Gl y  
 35 40 45

Leu Ile Gl y Gl y Thr Asn Lys Arg Al a Pro Trp Thr Pro Al a Arg Phe  
 50 55 60

Ser Gl y Ser Leu Leu Gl y Gl y Lys Al a Al a Leu Thr Ile Thr Gl y Al a  
 65 70 75 80

Gl n Al a Gl u Asp Gl u Al a Asp Tyr Tyr Cys Al a Leu Trp Tyr Ser Asn  
 85 90 95

Leu Trp Val Phe Gl y Gl y Thr Lys Leu Thr Val Leu Gl y Gl y Gl y  
 100 105 110

Gl y Ser Gl y Gl y Gl y Gl u Val Gl n Leu Val Gl u Ser Gl y Gl y Gl y  
 115 120 125

Leu Val Gl n Pro Gl y Gl y Ser Leu Arg Leu Ser Cys Al a Al a Ser Gl y  
 130 135 140

Phe Thr Phe Ser Ser Phe Gl y Met His Trp Val Arg Gl n Al a Pro Gl y  
 145 150 155 160

Lys Gl y Leu Gl u Trp Val Al a Tyr Ile Ser Ser Asp Ser Ser Al a Ile  
 165 170 175

Tyr Tyr Al a Asp Thr Val Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asn  
 180 185 190

Al a Lys Asn Ser Leu Tyr Leu Gl n Met Asn Ser Leu Arg Asp Gl u Asp  
 195 200 205

Thr Al a Val Tyr Tyr Cys Gl y Arg Gl y Arg Gl u Asn Ile Tyr Tyr Gl y  
 210 215 220

Ser Arg Leu Asp Tyr Trp Gl y Gl n Gl y Thr Thr Val Thr Val Ser Ser  
 225 230 235 240

Gl y Gl y Cys Gl y Gl y Lys Val Al a Al a Leu Lys Gl u Lys Val Al a  
 245 250 255

Al a Leu Lys Gl u Lys Val Al a Al a Leu Lys Gl u Lys Val Al a Al a Leu  
 260 265 270

Lys Gl u

<210> 56  
 <211> 822

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<212> DNA  
<213> Artificial Sequence

<220>  
<223> Polynucleotide Encoding the Second Polypeptide Chain of B7-H3 x CD3 Bispecific DART-A Diabody

<400> 56  
caggctgtgg tgactcagga gccttcactg accgtgtccc caggcggAAC tgtgaccctg 60  
acatgcagat ccagcacagg cgcaGTgacc acatctaact acgccaattg ggtgcagcag 120  
aagccaggac aggcaccaag gggcctgatc ggggttacaa aaaaaaggc tccctggacc 180  
cctgcacggt tttcttggaaag tctgtggc ggaaaggccg ctctgactat taccggggca 240  
caggccgagg acgaagccga ttactattgt gctctgtggt atagcaatct gtgggtgttc 300  
gggggtggca caaaactgac tgtgtggaa ggggtggat ccggcggcgg aggccagggtg 360  
cagctggtcg agtctggcgg aggactggc cagcctggcg gctccctgag actgtcttgc 420  
gccgcctccg gcttcaccc ttccagcttc ggcatgcact gggccggcca ggctccaggc 480  
aaggactgg aatgggtggc ctacatctcc tccgactcct ccgcctatcta ctacggcagc 540  
accgtgaagg gcaggttcac catctccgg gacaacgcca agaactccct gtacctgcag 600  
atgaactccc tgcgggacga ggacaccgccc gtgtactact gccggcaggagg ccgggagaat 660  
atctactacg gctcccgct ggattattgg ggccaggcga ccaccgtgac cgtgtccctcc 720  
ggaggatgtg gcggtgaaa agtggccgca ctgaaggaga aagttgtgc tttgaaagag 780  
aaggctgccc cacttaagga aaaggctgca gccctgaaag ag 822

<210> 57  
<211> 227  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Third Polypeptide Chain of B7-H3 x CD3 Bispecific DART-A Diabody

<400> 57

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Glu  
1 5 10 15

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
35 40 45

Gl u Asp Pro Gl u Val Lys Phe Asn Trp Tyr Val Asp Gl y Val Gl u Val  
50 55 60

His Asn Ala Lys Thr Lys Pro Arg Gl u Gl u Gl n Tyr Asn Ser Thr Tyr  
65 70 75 80

Arg Val Val Ser Val Leu Thr Val Leu His Gl n Asp Trp Leu Asn Gl y  
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85

90

95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
100 105 110

Gl u Lys Thr Ile Ser Lys Ala Lys Gl y Gl n Pro Arg Gl u Pro Gl n Val  
115 120 125

Tyr Thr Leu Pro Pro Ser Arg Gl u Gl u Met Thr Lys Asn Gl n Val Ser  
130 135 140

Leu Ser Cys Ala Val Lys Gl y Phe Tyr Pro Ser Asp Ile Ala Val Gl u  
145 150 155 160

Trp Gl u Ser Asn Gl y Gl n Pro Gl u Asn Asn Tyr Lys Thr Thr Pro Pro  
165 170 175

Val Leu Asp Ser Asp Gl y Ser Phe Phe Leu Val Ser Lys Leu Thr Val  
180 185 190

Asp Lys Ser Arg Trp Gl n Gl n Gl y Asn Val Phe Ser Cys Ser Val Met  
195 200 205

Hi s Gl u Ala Leu Hi s Asn Arg Tyr Thr Gl n Lys Ser Leu Ser Leu Ser  
210 215 220

Pro Gl y Lys  
225

<210> 58

<211> 681

<212> DNA

<213> Artificial Sequence

<220>

<223> Polynucleotide Encoding the Third Polypeptide Chain of B7-H3 x CD3 Bi specific DART-A Diabody

<400> 58

gacaaaactc acacatgcc accgtgccca gcacctgaag ccgcgggggg accgtcagtc 60

ttccctttcc ccccaaaacc caaggacacc ctcatgatct cccggacccc tgaggtcaca 120

tgcgtggtag tggacgttag ccacgaagac cctgaggtagtca agttcaactg gtacgtggac 180

ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagtacaa cagcacgtac 240

cgtgtggta gcgtcctcac cgtcctgcac caggactggc tgaatggcaa ggagtacaag 300

tgcaaggtagt ccaacaaagc cctcccgacc cccatcgaga aaaccatctc caaagccaaa 360

ggcagcccc gagaaccaca ggtgtacacc ctgccccat cccgggagga gatgaccaag 420

aaccaggtagtca gcctgaggtagt cgcagtcaaa ggcttctatc ccagcgacat cgccgtggag 480

tgggagagca atggcagcc ggagaacaac tacaagacca cgcctcccggt gctggactcc 540

gacggctcct tcttcctcgt cagcaagctc accgtggaca agagcaggtagt gcagcagggg 600

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aacgtcttct catgctccgt gatgcatgag gctctgcaca accgctacac gcagaagagc 660  
ctctccctgt ctccggtaa a 681

<210> 59  
<211> 504  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> First Polypeptide Chain of B7-H3 x CD3 Bi specific DART-B Diabody

<400> 59

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Tyr Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro  
85 90 95

Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly  
100 105 110

Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Leu Val Gln  
115 120 125

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe  
130 135 140

Ser Thr Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
145 150 155 160

Glu Trp Val Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr  
165 170 175

Tyr Ala Asp Ser Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser  
180 185 190

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr  
195 200 205

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Ala Val Tyr Tyr Cys Val Arg His Glu Asn Phe Glu Asn Ser Tyr Val  
210 215 220

Ser Trp Phe Ala Tyr Trp Glu Glu Gly Thr Leu Val Thr Val Ser Ser  
225 230 235 240

Gly Gly Cys Gly Gly Glu Val Ala Ala Leu Glu Lys Glu Val Ala  
245 250 255

Ala Leu Glu Lys Glu Val Ala Ala Leu Glu Lys Glu Val Ala Ala Leu  
260 265 270

Glu Lys Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
275 280 285

Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
290 295 300

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
305 310 315 320

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
325 330 335

Asp Glu Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Glu  
340 345 350

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Glu  
355 360 365

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala  
370 375 380

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Glu Glu Pro  
385 390 395 400

Arg Glu Pro Glu Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr  
405 410 415

Lys Asn Glu Val Ser Leu Trp Cys Leu Val Lys Glu Phe Tyr Pro Ser  
420 425 430

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Glu Pro Glu Asn Asn Tyr  
435 440 445

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Glu Ser Phe Phe Leu Tyr  
450 455 460

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Glu Asn Val Phe  
465 470 475 480

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Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
485 490 495

Ser Leu Ser Leu Ser Pro Gly Lys  
500

<210> 60  
<211> 272  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Second Polypeptide Chain of B7-H3 x CD3 Bispecific DART-B Diabody

<400> 60

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala  
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly  
100 105 110

Gly Ser Gly Gly Gly Gln Val Gln Leu Val Gln Ser Gly Ala Glu  
115 120 125

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly  
130 135 140

Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Arg Gln Ala Pro Gly  
145 150 155 160

Gln Gly Leu Glu Trp Met Gly Thr Ile Tyr Pro Gly Asp Gly Asp Thr  
165 170 175

Arg Tyr Thr Gln Lys Phe Lys Gly Arg Val Thr Ile Thr Ala Asp Lys  
180 185 190

Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp  
195 200 205

Thr Al a Val Tyr Tyr Cys Al a Arg Arg Gly Ile Pro Arg Leu Trp Tyr  
 210 215 220

Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly  
 225 230 235 240

Cys Gly Gly Gly Lys Val Al a Al a Leu Lys Glu Lys Val Al a Al a Leu  
 245 250 255

Lys Glu Lys Val Al a Al a Leu Lys Glu Lys Val Al a Al a Leu Lys Glu  
 260 265 270

<210> 61

<211> 504

<212> PRT

<213> Artificial Sequence

<220>

<223> First Polypeptide Chain of B7-H3 x CD3 Bi specific DART-C Diabody

<400> 61

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Al a Ser Val Gly  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Al a Ser Gln Ser Ile Ser Ser Tyr  
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Al a Pro Lys Leu Leu Ile  
 35 40 45

Tyr Tyr Thr Ser Arg Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80

Glu Asp Ile Al a Thr Tyr Tyr Cys Gln Gln Gly Asn Thr Leu Pro Pro  
 85 90 95

Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Ser Gly  
 100 105 110

Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln  
 115 120 125

Pro Gly Gly Ser Leu Arg Leu Ser Cys Al a Al a Ser Gly Phe Thr Phe  
 130 135 140

Ser Thr Tyr Al a Met Asn Trp Val Arg Gln Al a Pro Gly Lys Gly Leu  
 145 150 155 160

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Gl u Trp Val Gl y Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr  
165 170 175

Tyr Ala Asp Ser Val Lys Gl y Arg Phe Thr Ile Ser Arg Asp Asp Ser  
180 185 190

Lys Asn Ser Leu Tyr Leu Gl n Met Asn Ser Leu Lys Thr Gl u Asp Thr  
195 200 205

Al a Val Tyr Tyr Cys Val Arg His Gl y Asn Phe Gl y Asn Ser Tyr Val  
210 215 220

Ser Trp Phe Al a Tyr Trp Gl y Gl n Gl y Thr Leu Val Thr Val Ser Ser  
225 230 235 240

Gl y Gl y Cys Gl y Gl y Gl u Val Al a Al a Leu Gl u Lys Gl u Val Al a  
245 250 255

Al a Leu Gl u Lys Gl u Val Al a Al a Leu Gl u Lys Gl u Val Al a Al a Leu  
260 265 270

Gl u Lys Gl y Gl y Asp Lys Thr His Thr Cys Pro Pro Cys Pro Al a  
275 280 285

Pro Gl u Al a Al a Gl y Gl y Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
290 295 300

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Gl u Val Thr Cys Val Val  
305 310 315 320

Val Asp Val Ser His Gl u Asp Pro Gl u Val Lys Phe Asn Trp Tyr Val  
325 330 335

Asp Gl y Val Gl u Val His Asn Al a Lys Thr Lys Pro Arg Gl u Gl u Gl n  
340 345 350

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gl n  
355 360 365

Asp Trp Leu Asn Gl y Lys Gl u Tyr Lys Cys Lys Val Ser Asn Lys Al a  
370 375 380

Leu Pro Al a Pro Ile Gl u Lys Thr Ile Ser Lys Al a Lys Gl y Gl n Pro  
385 390 395 400

Arg Gl u Pro Gl n Val Tyr Thr Leu Pro Pro Ser Arg Gl u Gl u Met Thr  
405 410 415

Lys Asn Gl n Val Ser Leu Trp Cys Leu Val Lys Gl y Phe Tyr Pro Ser  
420 425 430

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Glu Pro Glu Asn Asn Tyr  
435 440 445

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
450 455 460

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Gly Asn Val Phe  
465 470 475 480

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Glu Lys  
485 490 495

Ser Leu Ser Leu Ser Pro Gly Lys  
500

<210> 62

<211> 272

<212> PRT

<213> Artificial Sequence

<220>

<223> Second Polypeptide Chain of B7-H3 x CD3 Bimpecific DART-C Diabody

<400> 62

Gln Ala Val Val Thr Gln Glu Pro Ser Leu Thr Val Ser Pro Gly Gly  
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser  
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly  
35 40 45

Leu Ile Gly Gly Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe  
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Ile Thr Gly Ala  
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Gly Gly Thr Lys Leu Thr Val Leu Gly Gly Gly  
100 105 110

Gly Ser Gly Gly Gly Gln Val Gln Leu Val Gln Ser Gly Ala Glu  
115 120 125

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly  
130 135 140

Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Arg Gln Ala Pro Gly  
145 150 155 160

Gl n Gl y Leu Gl u Trp Met Gl n Thr Ile Tyr Pro Gl y Gl y Gl y Asp Thr  
 165 170 175

Arg Tyr Thr Gl n Lys Phe Gl n Gl y Arg Val Thr Ile Thr Ala Asp Lys  
 180 185 190

Ser Thr Ser Thr Ala Tyr Met Gl u Leu Ser Ser Leu Arg Ser Gl u Asp  
 195 200 205

Thr Ala Val Tyr Tyr Cys Ala Arg Arg Gl y Ile Pro Arg Leu Trp Tyr  
 210 215 220

Phe Asp Val Trp Gl y Gl n Gl y Thr Thr Val Thr Val Ser Ser Gl y Gl y  
 225 230 235 240

Cys Gl y Gl y Gl y Lys Val Ala Ala Leu Lys Gl u Lys Val Ala Ala Leu  
 245 250 255

Lys Gl u Lys Val Ala Ala Leu Lys Gl u Lys Val Ala Ala Leu Lys Gl u  
 260 265 270

<210> 63

<211> 503

<212> PRT

<213> Artificial Sequence

<220>

<223> First Polypeptide Chain of B7-H3 x CD3 Bi specific DART-D Diabody

<400> 63

Asp Ile Gl n Met Thr Gl n Ser Pro Ser Ser Leu Ser Ala Ser Val Gl y  
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gl n Ser Ile Ser Ser Tyr  
 20 25 30

Leu Asn Trp Tyr Gl n Gl n Lys Pro Gl y Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Tyr Thr Ser Arg Leu Gl n Ser Gl y Val Pro Ser Arg Phe Ser Gl y  
 50 55 60

Ser Gl y Ser Gl y Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gl n Pro  
 65 70 75 80

Gl u Asp Ile Ala Thr Tyr Tyr Cys Gl n Gl n Gl y Asn Thr Leu Pro Pro  
 85 90 95

Thr Phe Gl y Gl y Gl y Thr Lys Leu Gl u Ile Lys Gl y Gl y Gl y Ser Gl y  
 100 105 110

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Gly Gly Gly Glu Val Gln Leu Val 115 120 Glu Ser Gly Gly Leu Val Gln 125

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe 130 135 140

Ser Thr Tyr Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu 145 150 155 160

Glu Trp Val Gly Arg Ile Arg Ser Lys Tyr Asn Asn Tyr Ala Thr Tyr 165 170 175

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser 180 185 190

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr 195 200 205

Ala Val Tyr Tyr Cys Val Arg His Gly Asn Phe Gly Asn Ser Tyr Val 210 215 220

Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 225 230 235 240

Ala Ser Thr Lys Gly Glu Val Ala Ala Cys Glu Lys Glu Val Ala Ala 245 250 255

Leu Glu Lys Glu Val Ala Ala Leu Glu Lys Glu Val Ala Ala Leu Glu 260 265 270

Lys Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro 275 280 285

Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys 290 295 300

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val 305 310 315 320

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp 325 330 335

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr 340 345 350

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp 355 360 365

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu 370 375 380

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Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Glu Pro Arg  
385 390 395 400

Glu Pro Glu Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys  
405 410 415

Asn Glu Val Ser Leu Trp Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp  
420 425 430

Ile Ala Val Glu Trp Glu Ser Asn Glu Glu Pro Glu Asn Asn Tyr Lys  
435 440 445

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser  
450 455 460

Lys Leu Thr Val Asp Lys Ser Arg Trp Glu Glu Gly Asn Val Phe Ser  
465 470 475 480

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Glu Lys Ser  
485 490 495

Leu Ser Leu Ser Pro Glu Lys  
500

<210> 64

<211> 271

<212> PRT

<213> Artificial Sequence

<220>

<223> Second Polypeptide Chain of B7-H3 x CD3 Bispecific DART-D Diabody

<400> 64

Glu Ala Val Val Thr Glu Glu Pro Ser Leu Thr Val Ser Pro Glu Glu  
1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Glu Ala Val Thr Thr Ser  
20 25 30

Asn Tyr Ala Asn Trp Val Glu Glu Lys Pro Glu Glu Ala Pro Arg Glu  
35 40 45

Leu Ile Glu Glu Thr Asn Lys Arg Ala Pro Trp Thr Pro Ala Arg Phe  
50 55 60

Ser Glu Ser Leu Leu Glu Glu Lys Ala Ala Leu Thr Ile Thr Glu Ala  
65 70 75 80

Glu Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn  
85 90 95

Leu Trp Val Phe Glu Glu Glu Thr Lys Leu Thr Val Leu Glu Glu Glu  
100 105 110

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Gly Ser Gly Gly Gly Glu Val Glu Leu Val Glu Ser Gly Ala Glu  
115 120 125

Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly  
130 135 140

Tyr Thr Phe Thr Ser Tyr Trp Met Glu Trp Val Arg Glu Ala Pro Gly  
145 150 155 160

Gln Gly Leu Glu Trp Met Gly Thr Ile Tyr Pro Gly Gly Asp Thr  
165 170 175

Arg Tyr Thr Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys  
180 185 190

Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp  
195 200 205

Thr Ala Val Tyr Tyr Cys Ala Arg Arg Gly Ile Pro Arg Leu Trp Tyr  
210 215 220

Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser  
225 230 235 240

Thr Lys Gly Lys Val Ala Ala Cys Lys Glu Lys Val Ala Ala Leu Lys  
245 250 255

Gl u Lys Val Ala Ala Leu Lys Glu Lys Val Ala Ala Leu Lys Gl u  
260 265 270

<210> 65  
<211> 112  
<212> PRT  
<213> Mus musculus

<220>  
<221> MI SC\_FEATURE  
<222> (1)..(112)  
<223> Light Chain Variable Domain of Anti -Fluorescein Antibody 4-4-20

<400> 65

Asp Val Val Met Thr Glu Thr Pro Phe Ser Leu Pro Val Ser Leu Gly  
1 5 10 15

Asp Glu Ala Ser Ile Ser Cys Arg Ser Ser Glu Ser Leu Val His Ser  
20 25 30

Asn Glu Asn Thr Tyr Leu Arg Trp Tyr Leu Glu Lys Pro Gly Glu Ser  
35 40 45

Pro Lys Val Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
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50

55

60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser  
 85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

<210> 66  
 <211> 118  
 <212> PRT  
 <213> Mus musculus

<220>  
 <221> MI SC\_FEATURE  
 <222> (1)..(118)  
 <223> Heavy Chain Variable Domain of Anti -Fluorescein Antibody 4-4-20

<400> 66

Gl u Val Lys Leu Asp Gl u Thr Gly Gly Leu Val Gln Pro Gly Arg  
 1 5 10 15

Pro Met Lys Leu Ser Cys Val Ala Ser Gly Phe Thr Phe Ser Asp Tyr  
 20 25 30

Trp Met Asn Trp Val Arg Gln Ser Pro Gl u Lys Gly Leu Gl u Trp Val  
 35 40 45

Al a Gln Ile Arg Asn Lys Pro Tyr Asn Tyr Gl u Thr Tyr Ser Asp  
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ser  
 65 70 75 80

Val Tyr Leu Gln Met Asn Asn Leu Arg Val Gl u Asp Met Gly Ile Tyr  
 85 90 95

Tyr Cys Thr Gly Ser Tyr Tyr Gly Met Asp Tyr Trp Gl y Gln Gl y Thr  
 100 105 110

Ser Val Thr Val Ser Ser  
 115

<210> 67  
 <211> 509  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> First Polypeptide of Anti -Fluorescein Diabody (Control DART)

&lt;400&gt; 67

Asp Val Val Met Thr Gln Thr Pro Phe Ser Leu Pro Val Ser Leu Gly  
 1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser  
 20 25 30

Asn Gly Asn Thr Tyr Leu Arg Trp Tyr Leu Gln Lys Pro Gly Gln Ser  
 35 40 45

Pro Lys Val Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro  
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile  
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser  
 85 90 95

Thr His Val Pro Trp Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105 110

Gly Gly Gly Ser Gly Gly Gly Glu Val Gln Leu Val Glu Ser Gly  
 115 120 125

Gly Gly Leu Val Glu Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala  
 130 135 140

Ser Gly Phe Thr Phe Ser Thr Tyr Ala Met Asn Trp Val Arg Gln Ala  
 145 150 155 160

Pro Gly Lys Gly Leu Glu Trp Val Gly Arg Ile Arg Ser Lys Tyr Asn  
 165 170 175

Asn Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile  
 180 185 190

Ser Arg Asp Asp Ser Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu  
 195 200 205

Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg His Gly Asn Phe  
 210 215 220

Gly Asn Ser Tyr Val Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu  
 225 230 235 240

Val Thr Val Ser Ser Gly Gly Cys Gly Gly Glu Val Ala Ala Leu  
 245 250 255

Glu Lys Glu Val Ala Ala Leu Glu Lys Glu Val Ala Ala Leu Glu Lys  
 260 265 270

Gl u Val Al a Al a Leu Gl u Lys Gl y Gl y Asp Lys Thr His Thr Cys  
 275 280 285

Pro Pro Cys Pro Al a Pro Gl u Al a Al a Gl y Gl y Pro Ser Val Phe Leu  
 290 295 300

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Gl u  
 305 310 315 320

Val Thr Cys Val Val Asp Val Ser His Gl u Asp Pro Gl u Val Lys  
 325 330 335

Phe Asn Trp Tyr Val Asp Gl y Val Gl u Val His Asn Al a Lys Thr Lys  
 340 345 350

Pro Arg Gl u Gl u Gl n Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
 355 360 365

Thr Val Leu His Gl n Asp Trp Leu Asn Gl y Lys Gl u Tyr Lys Cys Lys  
 370 375 380

Val Ser Asn Lys Al a Leu Pro Al a Pro Ile Gl u Lys Thr Ile Ser Lys  
 385 390 395 400

Al a Lys Gl y Gl n Pro Arg Gl u Pro Gl n Val Tyr Thr Leu Pro Pro Ser  
 405 410 415

Arg Gl u Gl u Met Thr Lys Asn Gl n Val Ser Leu Trp Cys Leu Val Lys  
 420 425 430

Gl y Phe Tyr Pro Ser Asp Ile Al a Val Gl u Trp Gl u Ser Asn Gl y Gl n  
 435 440 445

Pro Gl u Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gl y  
 450 455 460

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gl n  
 465 470 475 480

Gl n Gl y Asn Val Phe Ser Cys Ser Val Met His Gl u Al a Leu His Asn  
 485 490 495

His Tyr Thr Gl n Lys Ser Leu Ser Leu Ser Pro Gl y Lys  
 500 505

<210> 68  
 <211> 270

<212> PRT  
 <213> Artificial Sequence

<220>

&lt;223&gt; Second Polypeptide of Anti -Fluorescein Diabody (Control DART)

&lt;400&gt; 68

Gl n Al a Val Val Thr Gl n Gl u Pro Ser Leu Thr Val Ser Pro Gl y Gl y  
 1 5 10 15

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gl y Al a Val Thr Thr Ser  
 20 25 30

Asn Tyr Al a Asn Trp Val Gl n Gl n Lys Pro Gl y Gl n Al a Pro Arg Gl y  
 35 40 45

Leu Ile Gl y Gl y Thr Asn Lys Arg Al a Pro Trp Thr Pro Al a Arg Phe  
 50 55 60

Ser Gl y Ser Leu Leu Gl y Gl y Lys Al a Al a Leu Thr Ile Thr Gl y Al a  
 65 70 75 80

Gl n Al a Gl u Asp Gl u Al a Asp Tyr Tyr Cys Al a Leu Trp Tyr Ser Asn  
 85 90 95

Leu Trp Val Phe Gl y Gl y Thr Lys Leu Thr Val Leu Gl y Gl y Gl y  
 100 105 110

Gl y Ser Gl y Gl y Gl y Gl u Val Lys Leu Asp Gl u Thr Gl y Gl y Gl y  
 115 120 125

Leu Val Gl n Pro Gl y Arg Pro Met Lys Leu Ser Cys Val Al a Ser Gl y  
 130 135 140

Phe Thr Phe Ser Asp Tyr Trp Met Asn Trp Val Arg Gl n Ser Pro Gl u  
 145 150 155 160

Lys Gl y Leu Gl u Trp Val Al a Gl n Ile Arg Asn Lys Pro Tyr Asn Tyr  
 165 170 175

Gl u Thr Tyr Tyr Ser Asp Ser Val Lys Gl y Arg Phe Thr Ile Ser Arg  
 180 185 190

Asp Asp Ser Lys Ser Ser Val Tyr Leu Gl n Met Asn Asn Leu Arg Val  
 195 200 205

Gl u Asp Met Gl y Ile Tyr Tyr Cys Thr Gl y Ser Tyr Tyr Gl y Met Asp  
 210 215 220

Tyr Trp Gl y Gl n Gl y Thr Ser Val Thr Val Ser Ser Gl y Gl y Cys Gl y  
 225 230 235 240

Gl y Gl y Lys Val Al a Al a Leu Lys Gl u Lys Val Al a Al a Leu Lys Gl u  
 245 250 255

Lys Val Al a Al a Leu Lys Gl u Lys 1301-0123PCT\_ST25  
260 Val Al a Al a Leu Lys Gl u  
265 270