Title of the Invention:

Antibodies Reactive with B7-H3, Immunologically Active Fragments Thereof and Uses Thereof

Cross-Reference to Related Applications:

[0001] This application claims priority to United States Patent Applications Serial Nos. 61/310,692 (filed March 4, 2010; pending); 61/310,695 (filed March 4, 2010; pending) 61/311,057 (filed March 5, 2010; pending), each of which applications is herein incorporated by reference in its entirety.

Reference to Sequence Listing:

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entireties.

Background of the Invention:

Field of the Invention:

[0003] The present invention relates to antibodies and their fragments that are immunoreactive to the mammalian, and more particularly, the human B7-H3 receptor and to uses thereof, particularly in the treatment of cancer and inflammation. The invention thus particularly concerns humanized B7-H3-reactive antibodies and their immunoreactive fragments that are capable of mediating, and more preferably enhancing the activation of the immune system against cancer cells that are associated with a variety of human cancers.

Description of Related Art:

[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] CD4+ T-lymphocytes are the essential organizers of most mammalian immune and autoimmune responses (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48). The activation of CD4+ helper T-cells has been found to be mediated through co-stimulatory interactions between Antigen Presenting Cells and naive CD4+ T-lymphocytes. Two interactions are required (Viglietta, V. *et al.* (2007) "*Modulating Co-Stimulation*," Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in*

Cancer Immunotherapy," Adv. Immunol. 90:297-339). In the first interaction, an Antigen Presenting Cell must display the relevant target antigen bound to the cell's major histocompatibility complex so that it can bind to the T-cell Receptor ("TCR") of a naive CD4+ T-lymphocyte. In the second interaction, a ligand of the Antigen Presenting Cell must bind to a CD28 receptor of the CD4+ T-lymphocyte (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321). CD4+ helper T-cells experiencing both stimulatory signals are then capable of responding to cytokines (such as Interleukin-2 and Interleukin-12 to develop into Th1 cells. Such cells produce interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which mediate inflammatory responses to target cells expressing the target antigen. B-cell activation and proliferation also occurs, resulting in antibody production specific for the target antigen (Bernard, A. *et al.* (2005) "*T and B Cell Cooperation: A Dance of Life and Death*," Transplantation 79:S8-S11). In the absence of both co-stimulatory signals during TCR engagement, T cells enter a functionally unresponsive state, referred to as clonal anergy (Khawli, L.A. *et al.* (2008) "*Cytokine, Chemokine, and Co-Stimulatory Fusion Proteins for the Immunotherapy of Solid Tumors*," Exper. Pharmacol. 181:291-328). In pathologic states, Th1 cells are the key players of various organ-specific autoimmune diseases, such as type I diabetes, rheumatoid arthritis, and multiple sclerosis (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48).

I. The B7 Superfamily and B7-H3

[0006] Investigations into the ligands of the CD28 receptor have led to the characterization of a set of related molecules known as the B7 Superfamily (Coyle, A.J. *et al.* (2001) "*The Expanding B7 Superfamily: Increasing Complexity In Costimulatory Signals Regulating T Cell Function*," Nature Immunol. 2(3):203-209; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548; Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Loke, P. *et al.* (2004) "*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T Cells*." Arthritis Res. Ther. 6:208-214; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339; Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; Agarwal, A. *et al.* (2008) "*The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance*," Curr. Opin. Organ Transplant. 13:366-372; Lenschow, D.J. *et al.* (1996) "*CD28/B7 System of T Cell Costimulation*," Ann. Rev. Immunol. 14:233-258; Wang, S. *et al.* (2004) "*Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte Responses*," Microbes Infect. 6:759-766). There are currently seven known members of the family: B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L), the programmed death-1

ligand (PD-L1), the programmed death-2 ligand (PD-L2), B7-H3 and B7-H4 (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7).

[0007] B7 family members are immunoglobulin superfamily members with an immunoglobulin-V-like and an immunoglobulin-C-like domain (*e.g.*, IgV–IgC) (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). The IgV and IgC domains of B7-family 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). B7-H3 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). Members of the B7 family 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).

[0008] 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).

[0009] 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-γ Production*," Nature Immunol. 2:269–274; Sun, M. *et al.* (2002) "*Characterization of Mouse and Human B7-H3 Genes*," J. Immunol. 168:6294-6297) a four immunoglobulin extracellular domain variant ("4Ig-B7-H3") has been identified and found to be more common human form of the protein (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). No functional difference has been observed between these two forms, since the natural murine form (2Ig) 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.* "*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 human B7-H3 (2Ig form) has been found to promote T-cell activation and IFN-γ 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-γ 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). Both B7-H4 and B7-H1 are 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).

[0010] 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 receptor (TCR) regulates gene transcription (*e.g.*, NFTA, NF-κB, or AP-1 factors).

[0011] B7-H3 co-stimulates CD4+ and CD8+ T-cell proliferation. B7-H3 also stimulates IFN-γ production and CD8+ lytic activity (Chapoval, A. *et al.* (2001) "*B7-H3: A Costimulatory Molecule For T Cell Activation and IFN-γ Production*," Nature Immunol. 2:269–274; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). However, the protein also possibly acts through NFAT (nuclear factor for activated T cells), NF-κB (nuclear factor kappa B), and AP-1 (Activator Protein-1) factors to inhibit T-cell activation (Yi. K.H. *et al.* (2009) "Fine *Tuning The Immune Response Through B7-H3 And B7-H4*," Immunol. Rev. 229:145-151). B7-H3 is also believed to inhibit Th1, Th2, or Th17 *in vivo* (Prasad, D.V. *et al.* (2004) "*Murine B7–H3 Is A Negative Regulator Of T Cells*," J. Immunol. 173:2500-2506; Fukushima, A. *et al.* (2007) "*B7–H3 Regulates The Development Of Experimental Allergic Conjunctivitis In Mice*," Immunol. Lett. 113:52-57; Yi. K.H. *et al.* (2009) "Fine *Tuning The Immune Response Through B7-H3 And B7-H4*," Immunol. Rev. 229:145-151). 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).

[0012] 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*," Immunolog. 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 B7H3 (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).

[0013] B7-H3 is not expressed on resting B or T cells, monocytes, or dendritic cells, but it is induced on dendritic cells by IFN-γ 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/Granuloid 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).

[0014] 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-γ 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 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. Therapeutic Antibodies

[0015] In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. For example, immunotherapy, or the use of antibodies for therapeutic purposes has been used in recent years to treat cancer. Passive immunotherapy involves the use of monoclonal antibodies in cancer treatments (see for example, DEVITA, HELLMAN, AND ROSENBERG'S CANCER: PRINCIPLES & PRACTICE OF ONCOLOGY, EIGHTH EDITION (2008), DeVita, V. *et al.* Eds., Lippincott Williams & Wilkins, Philadelphia, PA, pp. 537-547, 2979-2990). These antibodies can have inherent therapeutic biological activity both by direct inhibition of tumor cell growth or survival and by their ability to recruit the natural cell killing activity of the body's immune system. These agents can be administered alone or in conjunction with radiation or chemotherapeutic agents. Rituximab and Trastuzumab, approved for treatment of non-Hodgkin's lymphoma and breast cancer, respectively, are examples of such therapeutics. Alternatively, antibodies can be used to make antibody conjugates in which the antibody is linked to a toxic agent and directs that agent to the tumor by specifically binding to the tumor. Gemtuzumab ozogamicin is an example of an approved antibody conjugate used for the treatment of leukemia.

[0016] Monoclonal antibodies that bind to cancer cells and have potential uses for diagnosis and therapy have been disclosed (see, for example, the following patent applications which disclose, *inter alia*, some molecular weights of target proteins: U.S. Patent No. 6,054,561 (200 kD c-erbB-2 (Her2), and other unknown antigens 40-200 KD in size) and U.S. Patent No. 5,656,444 (50 kD and 55 kD oncofetal protein)). Examples of antibodies in clinical trials and /or approved for treatment of solid tumors include: Trastuzumab (antigen: 180 kD, HER2/neu), Edrecolomab (antigen: 40-50 kD, Ep-CAM), Anti-human milk fat globules (HMFGl) (antigen >200 kD, HMW Mucin), Cetuximab (antigens: 150 kD and 170 kD, EGF receptor), Alemtuzumab (antigen: 21-28 kD, CD52), and Rituximab (antigen: 35 kD, CD20).

[0017] The antigen targets of trastuzumab (Her-2 receptor), which is used to treat breast cancer, and cetuximab (EGF receptor), which is in clinical trials for the treatment of several cancers, are present at some detectable level on a large number of normal human adult tissues including skin, colon, lung, ovary, liver, and pancreas. The margin of safety in using these therapeutics is possibly provided by the difference in the levels of antigen expression or in access of or activity of the antibody at these sites.

[0018] Another type of immunotherapy is active immunotherapy, or vaccination, with an antigen present on a specific cancer(s) or a DNA construct that directs the expression of the antigen, which then evokes the immune response in the individual, *i.e.*, to induce the individual to actively produce antibodies against their own cancer. Active immunization has not been used as often as passive immunotherapy or immunotoxins.

[0019] Several models of disease (including cancer) progression have been suggested. Theories range from causation by a single infective /transforming event to the evolution of an increasingly "disease-like" or "cancer-like" tissue type leading ultimately to one with fully pathogenic or malignant capability. Some argue that with cancer, for example, a single mutational event is sufficient to cause malignancy, while others argue that subsequent alterations are also necessary. Some others have suggested that increasing mutational load and tumor grade are necessary for both initiation as well as progression of neoplasia via a continuum of mutation-selection events at the cellular level. Some cancer targets are found only in tumor tissues, while others are present in normal tissues and are up regulated and /or over-expressed in tumor tissues. In such situations, some researchers have suggested that the over-expression is linked to the acquisition of malignancy, while others suggest that the over-expression is merely a marker of a trend along a path to an increasing disease state.

[0020] In some cases, cancer targets, such as oncoproteins expressed or over-expressed in tumors, have been shown to be present during embryonic and fetal development and serve as a regulator of growth and differentiation. Some researchers have found that the expression of these oncoproteins during embryonic and fetal development appear to be restricted to specific tissues and also restricted to specific stages of development. In contrast, the expression of these oncoproteins in the adult has been shown to be associated with over-expression in tumor growth and /or a malfunction of tumor suppressor proteins.

[0021] An ideal diagnostic and /or therapeutic antibody would be specific for an antigen present on a large number of cancers, but absent or present only at low levels on any normal tissue. The discovery, characterization, and isolation of a novel antibody capable of binding to an antigen that is specifically associated with cancer(s) would be useful in many ways. First, the antibody would have biological activity against such cancer cells and be able to recruit the immune system's response to thereby treat the disease. The antibody could be administered as a therapeutic alone or in combination with current treatments or used to prepare immunoconjugates linked to toxic agents. An antibody with the same specificity but with low or no biological activity when administered alone could also be useful in that an antibody could be used to prepare an immunoconjugate with a radioisotope, a toxin, or a chemotherapeutic agent or liposome containing a chemotherapeutic agent, with the conjugated

form being biologically active by virtue of the antibody directing the toxin to the antigen-containing cells.

[0022] As discussed above, antibodies and other molecules that that specifically bind to B7-H3 have been described (see, United States Patent No. 7,527,969; 7,368,554; 7,358,354; and 7,279,567; United States Patent Application Publications Nos. US 20090087416; US 20090022747; US 20090018315; US2008116219; US20080081346; US 20050202536; US20030103963; US20020168762; PCT Publications Nos. WO 2008/116219; WO 2006/016276; WO 2004/093894; WO 04/001381; WO 2002/32375; WO 2002/10187 and WO 2001/094413; EP 1292619B; Modak, S. *et al.* (March 1999) "*Disialoganglioside GD2 And Antigen 8H9: Potential Targets For Antibody-Based Immunotherapy Against Desmoplastic Small Round Cell Tumor (DSRCT) And Rhabdomyosarcoma (RMS)*," Proceedings Of The American Association For Cancer Research Annual Meeting, Vol. 40:474 (90th Annual Meeting Of The American Association For Cancer Research; Philadelphia, Pennsylvania, US; April 10-14, 1999; Modak, S. *et al.* (March 2000) "*Radioimmunotargeting To Human Rhabdomyosarcoma Using Monoclonal Antibody 8H9*," Proc. Am. Assoc. Cancer Res.41:724; Modak, S. *et al.* (2001) "*Monoclonal Antibody 8H9 Targets A Novel Cell Surface Antigen Expressed By A Wide Spectrum Of Human Solid Tumors*," Cancer Res. 61(10):4048-4054; 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; 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).

[0023] Nevertheless, one aspect desirable for an ideal diagnostic and /or therapeutic antibody would be the discovery and characterization of novel antibodies capable of mediating, and particularly of enhancing the activation of the immune system against cancer cells (especially human cancer cells) that are associated with a variety of cancers. Such compositions would also be useful for drug discovery (*e.g.*, small molecules) and for further characterization of cellular regulation, growth, and differentiation.

[0024] Thus, despite all prior advances, a need remains for improved compositions capable of binding to cancer cells and of facilitating or mediating an immune response against cancer cells. Such compositions may be used to diagnose and treat such cancers. There exists a further need, based on the discoveries disclosed herein, for novel compositions that specifically recognize dual targets on the surface of cells, and which can thereby modulate, either by reducing or enhancing, the capabilities of B7-H3 to mediate T-cell activation or by recognizing and killing cancer cells that express B7-H3. It is an object of this invention to identify such compositions. It is another object to provide novel compounds for use in the assay of B7-H3 expression.

[0025] As described in detail below, the present invention relates to novel antibodies, including in particular dual affinity retargeting reagents ("DART™s") that comprise modulators of B7-H3 T-cell activation, that are capable of influencing T-cell activation as well as novel antibodies that bind to B7-H3 receptors of cancer cells and facilitate or mediate the death of such cells. The present invention is directed to such compositions and to their uses in diagnostics and in the treatment of diseases such as cancer.

Summary of the Invention:

[0026] The present invention relates to antibodies and their fragments that are immunoreactive to the mammalian, and more particularly, the human B7-H3 receptor and to uses thereof, particularly in the treatment of cancer and inflammation. The invention thus particularly concerns humanized B7-H3-reactive antibodies and their immunoreactive fragments that are capable of mediating, and more preferably enhancing the activation of the immune system against cancer cells that are associated with a variety of human cancers.

[0027] In detail, the invention concerns an isolated antibody or an immunoreactive fragment thereof, wherein the isolated antibody or the fragment comprises a variable domain that specifically binds an extracellular domain of B7-H3, wherein the antibody competes for binding to the B7-H3 with any of antibodies: BRCA69D, BRCA84D, or PRCA157.

[0028] The invention further concerns the above-described isolated antibody or immunoreactive fragment thereof, wherein the antibody or the fragment comprises a variable domain that comprises:

(A) CDR_1 (**SEQ ID NO: 21**), CDR_2 (**SEQ ID NO: 23**) and CDR_3 (**SEQ ID NO: 25**) of the light chain of BRCA69D and CDR_1 (**SEQ ID NO: 29**), CDR_2 (**SEQ ID NO: 31**) and CDR_3 (**SEQ ID NO: 33**) of the heavy chain of BRCA69D;

(B) CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; or

(C) CDR_1 (**SEQ ID NO: 37**), CDR_2 (**SEQ ID NO: 39**) and CDR_3 (**SEQ ID NO: 41**) of the light chain of PRCA157 and CDR_1 (**SEQ ID NO: 45**), CDR_2 (**SEQ ID NO: 47**) and CDR_3 (**SEQ ID NO: 49**) of the heavy chain of PRCA157.

[0029] The invention further concerns any of the above-described isolated antibodies or immunoreactive fragments thereof, wherein the antibody binds to B7-H3 that is endogenously expressed on the surface of a cancer cell.

[0030] The invention further concerns any of the above-described isolated antibodies or immunoreactive fragments thereof, wherein the antibody binds to B7-H3 that is internalized upon binding to B7-H3 expressed on the surface of a cancer cell.

[0031] The invention further concerns any of the above-described isolated antibodies or immunoreactive fragments thereof, which is a humanized monoclonal antibody.

[0032] The invention further concerns any of the above-described isolated antibodies or immunoreactive fragments thereof, wherein the antibody is a modified antibody that comprises a variant human IgG1 Fc region, wherein the variant human IgG1 Fc region comprises at least one amino acid modification relative to the Fc region of the parent of the antibody, the amino acid modification(s) comprising amino acid modification(s) that alters the affinity or avidity of the variant Fc region for binding to an FcγR such that the modified antibody exhibits enhanced effector function relative to the parent antibody.

[0033] The invention further concerns any of the above-described isolated antibodies or immunoreactive fragments thereof, wherein the Fc region modification comprises:

(A) at least one substitution selected from the group consisting of:

(1)	F243L;	(5)	Y300L;
(2)	D270E;	(6)	V305I;
(3)	R292P;	(7)	A330V; and
(4)	S298N;	(8)	P396L;

(B) at least one substitution of two amino acid residues, the substitutions being selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) at least one substitution of three amino acid residues, the substitutions being selected from the group consisting of:

(1) F243L, R292P and Y300L;

(2) F243L, R292P and V305I;

(3) F243L, R292P and P396L; and

(4) R292P, V305I and P396L;

(D) at least one substitution of four amino acid residues, the substitutions being selected from the group consisting of:

(1) F243L, R292P, Y300L and P396L; and

(2) F243L, R292P, V305I and P396L;

or

(E) a substitution of at least the five amino acid residues: F243L, R292P, Y300L, V305I and P396.

[0034] The invention further concerns the above-described antibody, wherein the antibody comprises substitutions of:

(A) F243L, R292P, and Y300L;

(B) L235V, F243L, R292P, Y300L, and P396L; or

(C) F243L, R292P, Y300L, V305I, and P396L.

[0035] The invention further concerns the above-described antibody, wherein the antibody comprises comprises:

(A) a variable domain that comprises CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; and

(B) an Fc region modification that comprises the substitutions: L235V, F243L, R292P, Y300L, and P396L.

[0036] The invention further concerns the above-described antibody, wherein the antibody is a chimeric antibody or a humanized antibody.

[0037] The invention further concerns the above-described isolated antibodies or immunoreactive fragments thereof, wherein the antibody comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO: 89**);

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO: 99**); and

(C) an Fc-region having the substitutions: L235V, F243L, R292P, Y300L, and P396L.

[0038] The invention further concerns a hybridoma that secretes a monoclonal antibody that specifically binds an extracellular domain of B7-H3, wherein the antibody competes for binding to the B7-H3 with any of antibodies: BRCA69D, BRCA84D, or PRCA157.

[0039] The invention further concerns a nucleic acid molecule that encodes any of the above-described isolated antibodies or immunoreactive fragments.

[0040] The invention further concerns a dual affinity retargeting reagent (DART™), the reagent comprising:

(A) a polypeptide chain I that comprises an immunoglobulin VL epitope binding domain specific for binding B7-H3 and a VH epitope binding domain specific for binding a molecule other than B7-H3; and

(B) a polypeptide chain II that comprises an immunoglobulin VH epitope binding domain specific for binding B7-H3 and a VL epitope binding domain specific for binding the molecule other than B7-H3;

wherein the polypeptide chains I and II are associated together so as to form functional epitope binding domains capable of binding to B7-H3 and the molecule other than B7-H3.

[0041] The invention further concerns the above-described dual affinity retargeting reagent (DART™), wherein the molecule other than B7-H3 that may be bound by the DART™ is a hapten, and particularly wherein the hapten is fluorescein isothiocyanate.

[0042] The invention further concerns the above-described dual affinity retargeting reagent (DART™), wherein the molecule other than B7-H3 that may be bound by the DART™ is a T-Cell Receptor or the NKG2D receptor.

[0043] The invention further concerns the above-described dual affinity retargeting reagent (DART™), wherein the molecule other than B7-H3 that may be bound by the DART™ is a tumor-associated antigen, and particularly, wherein the tumor-associated antigen is selected from the group consisting of A33; ADAM-9; ALCAM; BAGE; beta-catenin; CA125; Carboxypeptidase M; CD103; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD36; CD40/CD154; CD45; CD46; CD5; CD56; CD79a/CD79b; CDK4; CEA; CTLA4; Cytokeratin 8; EGF-R; EphA2; ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; gp100; HER-2/neu; human papillomavirus-E6; human papillomavirus-E7; Integrin Alpha-V-Beta-6; JAM-3; KID3; KID31; KSA (17-1A); LUCA-2; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; N-acetylglucosaminyltransferase; Oncostatin M; p15; PIPA; PSA; PSMA; ROR1; sTn; TNF-ß receptor; TNF-α receptor; TNF-γ receptor; Transferrin Receptor; and VEGF receptor.

[0044] The invention further concerns a nucleic acid molecule that encodes a polypeptide chain of any of the above-described dual affinity retargeting reagents (DART™s).

[0045] The invention further concerns a pharmaceutical composition comprising (i) a therapeutically effective amount of any of the above-described isolated antibodies or immunoreactive fragments or dual affinity retargeting reagents (DART™s) and (ii) a pharmaceutically acceptable carrier.

[0046] The invention further concerns the above-described pharmaceutical composition, wherein the antibody is a humanized antibody that comprises:

(A) a variable domain that comprises CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; and

(B) an Fc region modification that comprises the substitutions: L235V, F243L, R292P, Y300L, and P396L.

[0047] The invention further concerns the above-described pharmaceutical composition, wherein the antibody is a humanized antibody that comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO: 89**);

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO: 99**); and

(C) an Fc-region having the substitutions: L235V, F243L, R292P, Y300L, and P396L.

[0048] The invention further concerns any of the above-described pharmaceutical compositions, which further comprises one or more additional anti-cancer agents, and particularly wherein the additional anti-cancer agent is a chemotherapeutic agent, a radiation therapeutic agent, a hormonal therapeutic agent, or an immunotherapeutic agent.

[0049] The invention further concerns the use of any of the above-described antibodies or immunoreactive fragments or dual affinity retargeting reagents (DART™s) in the diagnosis of cancer, wherein the isolated antibody, immunoreactive fragment, or DART™ is detectably labeled.

[0050] The invention further concerns the above-described use characterised in that the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of 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 dhordoma, 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, 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

multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 unveal melanoma, a rare hematologic disorder, 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.

[0051] The invention further concerns the use of any of the above-described antibodies or immunoreactive fragments or dual affinity retargeting reagents (DART™s) in the preparation of a medicament for the treatment or the prevention of cancer in a patient. The invention further concerns such uses characterised in that the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of 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 dhordoma, 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, 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 multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 unveal melanoma, a rare hematologic disorder, 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.

[0052] The invention further concerns the above described uses characterised in that the use further comprises administration of one or more additional cancer therapies selected from the group consisting of chemotherapy, immunotherapy, radiation therapy, hormonal therapy, and surgery.

Brief Description of the Drawings:

[0053] **Figures 1A-1B** show the results of IHC investigations conducted using normal pancreas, liver, lung and colon tissue specimens with BRCA84D at 0.625 μg/ml and 0.078 μg/ml (**Figure 1A**) and normal heart, kidney and adrenal tissue with BRCA84D at 0.625 μg/ml (**Figure 1B**).

[0054] **Figure 2** shows the results of IHC investigations conducted using cancerous pancreas, breast, colon and lung tissue specimens with BRCA84D at 0.625 μg/ml and 0.078 μg/ml.

[0055] **Figures 3A-3D** show dose-dependent redirected killing mediated by the antibodies of the present invention. **Figures 3A-3B** show dose-dependent redirected killing of A498 renal carcinoma cells (with resting PBMC at 18 hours (LDH)) by monoclonal antibodies reactive against B7-H3 (effector:target ratio of 20:1) (**Figure 3A**: BRCA68D, BRCA69D, PRCA157, GB8, TCR-4420; **Figure 3B**: OVCA22, BRCA84D, TDH6, TES7, TCR-4420). **Figures 3C-3D** show dose-dependent redirected killing of A549 lung cancer cells (with resting PBMC at 18 hours (LDH)) by monoclonal antibodies reactive against B7-H3 (effector:target ratio of 30:1) (**Figure 3C**: BRCA84D, OVCA22, PRCA157, TES7; **Figure 3D**: TDH6, BRCA68D, BRCA69D).

[0056] **Figures 4A-4B** show the abilities of anti-B7-H3 antibodies to bind to soluble B7H3-2Ig (**Figure 4A**) and soluble B7H3-4Ig B7-H3 (**Figure 4B**) (antibody concentration is 100 nM). Legend: **(A)** BLA8; **(B)** BRCA165; **(C)** BRCA68D; **(D)** BRCA69D; **(E)** BRCA84D; **(F)** GB8; **(G)** LUCA1; **(H)** LUCA50; **(I)** OVCA21; **(J)** OVCA22; **(K)** PA20; **(L)** PRCA123; **(M)** SG24; **(N)** SG27; **(O)** STO9; **(P)** TDH4 (184-192); **(Q)** TDH4; **(R)** TDH5; **(S)** TES7. The vertical position of legend correlates with the position of the corresponding curve.

[0057] **Figures 5A-5S** demonstrate the binding affinity between antigens in solution and captured monoclonal antibodies (solid lines; B7-H3(4Ig) 100 nM; dashed lines; B7-H3, 100 nM).

[0058] **Figures 6A-6I** show the results of BIACORE™ analyses of B7-H3 antibodies immobilized to B7-H3-2Ig (dashed gray lines) or B7-H3-4Ig (solid black lines). Antibodies were titrated from 0.063 μM to 1 μM. Time is in seconds.

[0059] **Figure 7** provides a comparison BIACORE™ analysis of antibodies PRCA157, BRCA69D, BLA8, PA20, BRCA84D, GB8 and SG27.

[0060] **Figure 8** provides a BIACORE™ analysis demonstrating that antibodies BRCA68D, BRCA69D, and PRCA157 do not compete with BRCA84D for binding to human B7-H3.

[0061] **Figures 9A-9B** show the results of studies on the ability of the anti-B7-H3 antibodies of the present invention to become internalized upon binding to cancer cells (**Figure 9A**, prostate CSC cells; **Figure 9B**, Hs700t pancreatic cells).

[0062] **Figures 10A-10F** show the ability of the anti-B7-H3 antibodies of the present invention to cross-block one another thereby revealing overlapping or distinct epitopes. A ten-fold excess of competitor antibody was employed.

[0063] **Figures 11A-11B** show the alignment of the amino acid residues of the variable light chains (**Figure 11A**) or variable heavy chains (**Figure 11B**) of BRCA84D and its humanized derivative, hBRCA84D.

[0064] **Figure 12** shows the relative binding affinities of the hBRCA84D light chain derivatives BRCA84D-3VL, BRCA84D-4VL and BRCA84D-5VL for human B7-H3.

[0065] **Figure 13** shows the relative binding affinities of the hBRCA84D heavy chain derivatives BRCA84D-2VH, BRCA84D-3VH and BRCA84D-4VH for human B7-H3.

[0066] **Figure 14** shows the relative binding affinities of (1) antibodies containing hBRCA84D-2VL and hBRCA84D-2VH (trials 1 and 2), (2) chimeric BRCA84D, (3) antibody containing hBRCA84D-5VL and chimeric BRCA84D-HC and (4) antibody containing hBRCA84D-5VL and hBRCA84D-2VH.

[0067] **Figure 15** shows the ability of Fc-modified humanized anti-B7-H3 antibodies to inhibit tumor growth of HT-1197 urinary bladder carcinoma cells *in vivo* in a murine xenograft model system. The Fc-modified hBRCA84D-2 antibody (comprising Fc modifications L235V, F243L, R292P, Y300L, and P396L) was administered to the mice (at a dose of 1 μg/kg, 10 μg/kg, or 20 μg/kg) 7 days, 14 days, 21 days and 28 days post implantation of the cancer cells.

[0068] **Figure 16** shows the ability of Fc-modified humanized anti-B7-H3 antibodies to inhibit tumor growth of A498 renal carcinoma cells *in vivo* in a murine xenograft model system. The Fc-modified hBRCA84D-2 antibody (comprising Fc modifications L235V, F243L, R292P, Y300L, and P396L) was administered to the mice (at a dose of 1 μg/kg, 10 μg/kg, or 20 μg/kg) 7 days, 14 days, 21 days and 28 days post implantation of the cancer cells.

[0069] **Figures 17A-17D** demonstrate the ability of the hBRCA84D-2 / anti-TCR DART™ ("T-DART™") to mediate redirected killing of SK-MES-1 lung cancer cells, A498 renal carcinoma cells, LNCaP prostate cancer cells, and UACC-62 melanoma cells.

[0070] **Figures 18A-18C** show the pharmacokinetic decay ofanti-B7-H3 Mab1 in the sera of male tumor-free male mCD16-/-, hCD16A_FOXN1 mice (**Figures 18A-18B**). **Figure 18C** shows the predicted pharmacokinetic profiles generated using a 2-compartment model with parameters from the 5mg/kg dose at 0.1, 0.5, 1, 5, and 10 mg/kg.

[0071] **Figure 19** shows the relative expression of HER2 and PRCA135 by the HT-1197 bladder cancer line.

[0072] **Figure 20** shows the binding affinity of anti-B7-H3 antibody hBRCA84D variants to HT-1197 cells.

[0073] **Figures 21A-21C** show the results of a murine xenograft analysis for HT-1197. Groups of 8 female mice received vehicle or 10 mg/kg IgG control, or centuximab at a dose of 1, 5, or 15 mg/kg or anti-B7-H3 antibody Mab1 at a dose of 0.1, 0.5, 1, 5, or 10 mg/kg (Q7D x5). Tumor measurements were made every 3-4 days. **Figure 21A** shows the ability of anti-B7-H3 antibody Mab1 to prevent or inhibit tumor development in the murine xenograft model. Comparisons vs IgG control: Mab1 (1 and 5 mg/kg) vs IgG control *** from day 51; Mab1 (10 mg/kg) vs IgG control ** from day 48. **Figure 21B** shows the ability of centuximab to prevent or inhibit tumor development in the murine xenograft model. Cetuximab (7 mg/kg) vs IgG control ** from day 51; Cetuximab (15 mg/kg) vs IgG control *** from day 58. **Figure 21C** compares the results obtained at the maximum doses tested.

[0074] **Figures 22A-22B** show the relative expression of HER2 and PMSA by the HT-1376 bladder cancer line.

[0075] **Figure 23** shows the results of a murine xenograft analysis for HT-1376. Groups of mice received vehicle or 1.0 mg/kg of anti-B7-H3 antibody Mab1 (Q7D x4).

[0076] **Figure 24** show the results of a murine xenograft analysis for AGS. Groups of mice received vehicle or 10 mg/kg anti-B7-H3 antibody Mab1 at a dose of 0.5, 1, 5, or 10 mg/kg (Q7D x5).

[0077] **Figure 25** show the results an *in vitro* cytotoxicity assay of A549 lung cancer cells upon incubation with hBRCA84D, chBRCA84D and hBRCA84 (Fc Var1) variant anti-B7-H3 antibodies (E:T Ratio = 25:1; Effector = Human PBMC; LDH Assay readout).

[0078] **Figure 26** shows the results of a murine xenograft analysis for A549. Groups of mice received vehicle or 1.0 mg/kg of anti-B7-H3 antibody Mab1 (Q7D x4).

[0079] **Figure 27** show the results of a murine xenograft analysis for CaLu3. Groups of mice received vehicle or 0.5, 1, or 5 mg/kg (Q7D x5) anti-B7-H3 antibody Mab1 or IgG control (10 mg/ml).

[0080] **Figures 28A-28C** show the results of a murine xenograft analysis for LOX-IMVI melanoma cancer cells. Groups of 8 female mice received vehicle or 5 mg/kg IgG control, or Docetaxel at a dose of 5, 10 or 20 mg/kg or anti-B7-H3 antibody Mab1 at a dose of 0.5, 1, 5, or 10 mg/kg. **Figure 28A** shows the ability of anti-B7-H3 antibody Mab1 to prevent or inhibit tumor development in the murine xenograft model. **Figure 28B** shows the ability of Docetaxel to prevent or inhibit tumor development in the murine xenograft model. **Figure 28C** compares the results obtained at the maximum doses tested.

[0081] **Figure 29** shows the result of a murine xenograft analysis for UACC-62 melanoma cancer cells. Groups of mice received vehicle or 5 mg/kg IgG control, or anti-B7-H3 antibody Mab1 at a dose of 0.5, 1, 5, or 10 mg/kg.

[0082] **Figures 30A-30C** show the results of a murine xenograft analysis for 2rv prostate cancer cells. Groups of 8 female mice received vehicle or 10 mg/kg IgG control, or trastuzumab at a dose of 1, 7 or 15 mg/kg or anti-B7-H3 antibody Mab1 at a dose of 0.5, 1, 5, or 10 mg/kg. **Figure 30A** shows the ability of anti-B7-H3 antibody Mab1 to prevent or inhibit tumor development in the murine xenograft model. **Figure 30B** shows the ability of trastuzumab to prevent or inhibit tumor development in the murine xenograft model. **Figure 30C** compares the results obtained at the maximum doses tested.

[0083] **Figure 31** show the results an *in vitro* cytotoxicity assay of A498 renal cancer cells upon incubation with hBRCA84D, chBRCA84D and hBRCA84 (Fc Var1) variant anti-B7-H3 antibodies (E:T Ratio = 25:1; Effector = Human PBMC; LDH Assay readout).

[0084] **Figure 32** shows the result of a murine xenograft analysis for A498 renal cancer cells. Groups of mice received vehicle or 10 mg/kg IgG control, or anti-B7-H3 antibody Mab1 at a dose of 0.1, 0.5, 1, 5, or 10 mg/kg. Centuximab (anti-EGRF antibody) was administered to a control group of mice at doses of 1, 7, or 15 mg/kg.

[0085] **Figures 33A-33B** show the result of a murine xenograft analysis for 786-0 renal cancer cells compared to centuximab. Groups of mice received vehicle or 10 mg/kg IgG control, or anti-B7-H3 antibody Mab1 at a dose of 0.1, 0.5, 1, 5, or 10 mg/kg. Centuximab (anti-EGRF antibody) was administered to a control group of mice at doses of 1, 7, or 15 mg/kg.

[0086] **Figure 34** shows the result of a murine xenograft analysis for 786-0 renal cancer cells compared to paclitaxel. Groups of mice received vehicle or 5 mg/kg IgG control, or anti-B7-H3 antibody Mab1 at a dose of 0.1, 0.5, 1, 5, or 10 mg/kg. Paclitaxel was administered to a control group of eight such mice at a dose of 2.5 mg/kg.

Detailed Description of the Invention:

[0087] The present invention relates to antibodies and their fragments that are immunoreactive to the mammalian, and more particularly, the human B7-H3 receptor and to uses thereof, particularly in the treatment of cancer and inflammation. The invention thus particularly concerns humanized B7-H3-reactive antibodies and their immunoreactive fragments that are capable of mediating, and more preferably enhancing the activation of the immune system against cancer cells that are associated with a variety of human cancers.

I. General Techniques

[0088] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, MOLECULAR CLONING: A LABORATORY MANUAL, Third Edition (Sambrook *et al.* Eds., 2001) Cold Spring Harbor Press, Cold Spring Harbor, NY; OLIGONUCLEOTIDE SYNTHESIS: METHODS AND APPLICATIONS (Methods in Molecular Biology), Herdewijn, P., Ed., Humana Press, Totowa, NJ; OLIGONUCLEOTIDE SYNTHESIS (Gait, M.J., Ed., 1984); METHODS IN MOLECULAR BIOLOGY, Humana Press, Totowa, NJ; CELL BIOLOGY: A LABORATORY NOTEBOOK (Cellis, J.E., Ed., 1998) Academic Press, New York, NY; ANIMAL CELL CULTURE (Freshney, R.I., Ed., 1987); INTRODUCTION TO CELL AND TISSUE CULTURE (Mather, J.P. and Roberts, P.E., Eds., 1998) Plenum Press, New York, NY; CELL AND TISSUE CULTURE: LABORATORY PROCEDURES (Doyle, A. *et al.*, Eds., 1993-8) John Wiley and Sons, Hoboken, NJ; METHODS IN ENZYMOLOGY (Academic Press, Inc.) New York, NY; WEIR'S HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (Herzenberg, L.A. *et al.* Eds. 1997) Wiley-Blackwell Publishers, New York, NY; GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (Miller, J.M. *et al.* Eds., 1987) Cold Spring Harbor Press, Cold Spring Harbor, NY; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (Ausubel, F.M. *et al.*, Eds., 1987) Greene Pub. Associates, New York, NY; PCR: THE POLYMERASE CHAIN REACTION, (Mullis, K. *et al.*, Eds., 1994) Birkhäuser, Boston MA; CURRENT PROTOCOLS IN IMMUNOLOGY (Coligan, J.E. *et al.*, eds., 1991) John Wiley and Sons, Hoboken, NJ; SHORT PROTOCOLS IN MOLECULAR BIOLOGY (John Wiley and Sons, 1999) Hoboken, NJ; IMMUNOBIOLOGY 7 (Janeway, C.A. *et al.* 2007) Garland Science, London, UK; Antibodies (P. Finch, 1997) Stride Publications, Devoran, UK; ANTIBODIES: A PRACTICAL APPROACH (D. Catty., ed., 1989) Oxford University Press, USA, New York NY); MONOCLONAL ANTIBODIES: A PRACTICAL APPROACH (Shepherd, P. *et al.* Eds., 2000) Oxford University Press, USA, New York NY; USING ANTIBODIES: A LABORATORY MANUAL (Harlow, E. *et al.* Eds., 1998) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; THE ANTIBODIES (Zanetti, M. *et al.* Eds. 1995) Harwood Academic Publishers, London, UK); and DEVITA, HELLMAN,

AND ROSENBERG'S CANCER: PRINCIPLES & PRACTICE OF ONCOLOGY, EIGHTH EDITION, DeVita, V. *et al.* Eds. 2008, Lippincott Williams & Wilkins, Philadelphia, PA.

II. Definitions

[0089] As used herein, the term "B7-H3" refers to a member of the human B7 family of proteins, a type I membrane protein with Ig-like domains also known as CD276. The term "2Ig-B7-H3" denotes the B7-H3 form that comprises only two Ig-like domains; the term "4Ig-B7-H3" denotes the B7-H3 form that comprises four Ig-like domains (see, Sun, M. *et al.* (2002) "*Characterization of Mouse and Human B7-H3 Genes*," J. Immunol. 168:6294-6297; Steinberger *et al.* (2004), "*Molecular Characterization Of Human 4Ig-B7-H3, A Member Of The B7 Family With Four Ig-Like Domains*," J. Immunol. 2004, 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). The antigen "TES7" (WO 2008/066691) is an antigen sharing characteristics of the 4Ig-B7-H3. Accordingly, antibodies that specifically bind to TES7 bind to 4Ig-B7-H3. The TES7 antigen may have more than one different epitope, and epitopes may be non-linear. Several anti-B7-H3 antibodies are known to bind to non-linear epitopes, including some only present on the 4Ig-B7-H3 isoform. It is currently believed that TES7 may be over-expressed in certain cancer cells in comparison to their normal tissue counterparts.

[0090] Agonists, antagonists, and other modulators of B7-H3 function are expressly included within the scope of this invention. These agonists, antagonists and modulators are polypeptides that comprise one or more of the antigenic determinant sites of B7-H3, or comprise one or more fragments of such sites, variants of such sites, or peptidomimetics of such sites. These agonistic, antagonistic, and B7-H37 modulatory compounds are provided in linear or cyclized form, and optionally comprise at least one amino acid residue that is not commonly found in nature or at least one amide isostere. These compounds may be glycosylated.

[0091] More specifically, the terms "B7-H3 modulator" as used herein are defined as any compound that (1) is capable of disrupting or blocking the interaction between human B7-H3 and its native ligands or an anti-B7-H3 antibody; (2) is capable of binding to human B7-H3 and its native ligands or an anti-B7-H3 antibody; (3) contains an antigenic site that can be used in the raising of antibodies capable of binding to human B7-H3 and its native ligands or an anti-B7-H3 antibody; (4) contains an antigenic site that can be used in the screening of antibodies capable of binding to human B7-H3 and its native ligands or an anti-B7-H3 antibody; (5) contains an antigenic site that can be used in the raising of antibodies capable of disrupting or blocking the interaction between human B7-H3 and its native ligands or an anti-B7-H3 antibody; (6) contains an antigenic site that can be used in the screening of antibodies capable of disrupting or blocking the interaction between human B7-H3 and

its native ligands or an anti-B7-H3 antibody. B7-H3 modulators may be "B7-H3 agonists" or "B7-H3 antagonists" depending on whether their activity enhances T cell activation or inhibits Tcell activation, respectively.

[0092] B7-H3 agonists, antagonists and modulators include B7-H3 variants, B7-H3 peptide antagonists, peptidomimetics, and small molecules, anti-B7-H3 antibodies and immunoglobulin variants, amino acid variants of human B7-H3 including amino acid substitution, deletion, and addition variants, or any combination thereof, and chimeric immunoglobulins. The B7-H3 agonists, antagonists and modulators of this invention are based on the identification of the B7-H3 domains involved in the binding of human B7-H3 to its native ligands or anti-B7-H3 antibodies. Thus, the invention provides B7-H3 agonists, antagonists and modulators with molecular structures that duplicate or mimic one or more of the anti-B7-H3 binding domains of human B7-H3.

[0093] As used herein, the term "B7-H3 variant" denotes any amino acid variant of human B7-H3, including amino acid substitution, deletion, and addition variants, or any combination thereof. The definition encompasses chimeric molecules such as human B7-H3/ non-human chimeras and other hybrid molecules. Also included in the definition is any fragment of a B7-H3 variant molecule that comprises the variant or hybrid region(s) of the molecule.

[0094] As used herein, an "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition 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 fragments thereof (such as Fab, Fab', F(ab')2 Fv), single chain (ScFv), mutants thereof, naturally occurring variants, fusion proteins comprising an antibody portion with an antigen recognition site of the required specificity, humanized antibodies, chimeric antibodies, "BiTEs®," "DART™" molecules and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity.

[0095] The term "BiTEs" (bi-specific T-cell engagers) refers to a single polypeptide chain molecule that having two antigen binding domains, one of which binds to a T-cell antigen and the second of which binds to an antigen present on the surface of a target (WO 05/061547; Baeuerle, P *et al.* (2008) "*BiTE®: A New Class Of Antibodies That Recruit T Cells*," Drugs of the Future 33: 137-147; Bargou, *et al.* 2008) "*Tumor Regression in Cancer Patients by Very Low Doses of a T Cell-Engaging Antibody*," Science 321: 974-977).

[0096] The term "DART™" (dual affinity retargeting reagent) refers to an immunoglobulin molecule that comprises at least two polypeptide chains that associate (especially through a covalent

interaction) to form at least two epitope binding sites, which may recognize the same or different epitopes. Each of the polypeptide chains of a DART™ comprise an immunoglobulin light chain variable region and an immunoglobulin heavy chain variable region, but these regions do not interact to form an epitope binding site. Rather, the immunoglobulin heavy chain variable region of one (*e.g.*, the first) of the DART™ polypeptide chains interacts with the immunoglobulin light chain variable region of a different (*e.g.*, the second) DART™ polypeptide chain to form an epitope binding site. Similarly, the immunoglobulin light chain variable region of one (*e.g.*, the first) of the DART™ polypeptide chains interacts with the immunoglobulin heavy chain variable region of a different (*e.g.*, the second) DART™ polypeptide chain to form an epitope binding site. DART™s may be monospecific, bispecific, trispecific, etc., thus being able to simultaneously bind one, two, three or more different epitopes (which may be of the same or of different antigens). DART™s may additionally be monovalent, bivalent, trivalent, tetravalent, pentavalent, hexavelent, *etc.*, thus being able to simultaneously bind one, two, three, four, five, six or more molecules. These two attributes of DART™s (*i.e.*, degree of specificity and valency may be combined, for example to produce bispecific antibodies (*i.e.*, capable of binding two epitopes) that are tetravalent (*i.e.*, capable of binding four sets of epitopes), etc. DART™ molecules are disclosed in PCT Publications WO 2006/113665, WO 2008/157379, and WO 2010/080538.

[0097] The term "monoclonal antibody" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non- naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full- length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', F(ab')2 Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (e.g., by hybridoma, phage selection, recombinant expression, transgenic animals, etc.). The term includes whole immunoglobulins as well as the fragments etc. described above under the definition of "antibody."

[0098] The term "humanized antibody" refer to a chimeric molecule, generally prepared using recombinant techniques, having an antigen binding site derived from an immunoglobulin from a non-human species and the remaining immunoglobulin structure of the molecule based upon the structure and /or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete variable domains fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen binding

sites may be wild type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A.F. *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity-determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular antigen, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[0099] As used herein, an antibody or a polypeptide is said to "specifically" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that specifically binds to a B7-H3 epitope is an antibody that binds this B7-H3 epitope with greater affinity, avidity, more readily, and /or with greater duration than it binds to other B7-H3

epitopes or non-B7-H3 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "specific" binding.

[00100] As used herein, the term "immunologically active" in reference to an epitope being or "remaining immunologically active" refers to the ability of an antibody (*e.g.*, an anti-B7-H3 antibody) to bind to the epitope under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

[00101] Different biological functions are associated with anti-B7-H3 antibodies, including, but not limited to one or more of: an ability to specifically bind to B7-H3 (and in particular B7-H3 molecules that are expressed on the surfaces of cancer cells, including but not limited to kidney, prostate, or lung, cancer cells); an ability to competitively inhibits preferential binding of a known anti-B7-H3 antibody to B7-H3, including the ability to preferentially bind to the same B7-H3 epitope to which the original antibody preferentially binds; an ability to bind to a portion of B7-H3 that is exposed on the surface of a living cell *in vitro* or *in vivo*; an ability to bind to a portion of B7-H3 that is exposed on the surface of living cancer cells, such as but not limited to prostate, lung or kidney cancer cells; an ability to deliver a chemotherapeutic agent to cancerous cells (such as kidney, prostate, or lung cancer cells) expressing B7-H3 on their surface; and/oran ability to deliver a therapeutic agent or detectable marker into cancer cells expressing B7-H3 on their surface. As discussed herein, polypeptides (including antibodies) of the invention may have any one or more of these characteristics.

[00102] An "anti-B7-H3 equivalent antibody" or "anti-B7-H3 equivalent polypeptide" refers to an antibody or a polypeptide having one or more biological functions associated with an anti-B7-H3 antibody, such as, for example binding specificity.

[00103] As used herein, the term "agent" refers to a biological, pharmaceutical, or chemical compound. Non-limiting examples include simple or complex organic or inorganic molecule, a peptide, a protein, an oligonucleotide, an antibody, an antibody derivative, antibody fragment, a vitamin derivative, a carbohydrate, a toxin, or a chemotherapeutic compound. Various compounds can be synthesized, for example, small molecules and oligomers (e.g., oligopeptides and oligonucleotides), and synthetic organic compounds based on various core structures. In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like.

[00104] Agents that are employed in the methods of this invention can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen without prior consideration or knowledge of the specific amino acid or other chemical moieties involved in the association of the molecule with its native binding partner(s) or known antibodies. An example of a randomly selected agent is an agent that is identified through the use and screening of a chemical library or a peptide combinatorial library.

[00105] As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a non-random basis that takes into account the sequence of the target site and /or its conformation in connection with the agent's action. With respect to anti-B7-H3 agents, it is currently believed that there are at least three epitopes on B7-H3 against which antibodies can be raised and therefore at least three sites of action for agents that block B7-H3/anti-B7-H3 interaction. This invention also encompasses agents that act at the sites of interaction between B7-H3 and its native binding partner, although other ligands and their active B7-H3-interactive sites are also encompassed within the scope of this invention, whether currently known or later identified. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the contact sites of the receptor /ligand and /or B7-H3/anti-B7-H3 antibody complex. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to an epitope appearing on B7-H3 as it is exposed on the surface of a living cell in its native environment. Such an agent will reduce or block the association of the anti-B7-H3 antibody with B7-H3, or the association of B7-H3 with its native ligand, as desired, by binding to the anti-B7-H3 antibody or to the native ligand.

[00106] As used herein, the term “labeled,” with regard to an antibody, is intended to encompass direct labeling of the antibody by coupling (i.e., physically linking) a detectable substance, such as a radioactive agent or a fluorophore (e.g. phycoerythrin (PE) or fluorescein isothiocyanate (also known as fluoroisothiocyanate or FITC)) to the antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance.

[00107] As used herein, the term “association”, with regard to an antibody, includes covalent and non-covalent attachment or binding of an agent (e.g., chemotherapeutic agent) to the antibody. The antibody can be associated with an agent (e.g., chemotherapeutic agent) by direct binding or indirect binding via attachment to a common platform, such that the antibody directs the localization of the agent to the cancerous cell to which the antibody binds and wherein the antibody and agent do not substantially dissociate under physiological conditions such that the agent is not targeted to the same cancerous cell to which the antibody binds or such that the agent's potency is not decreased.

[00108] The term "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses saliva, blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom, and the progeny thereof, for example, cells obtained from a tissue sample collected from an individual suspected of having cancer, in preferred embodiments from ovary, lung, prostate, pancreas, colon, and breast tissue. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[00109] The term "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

[00110] As used herein, the term "delaying development of metastasis" means to defer, hinder, slow, retard, stabilize, and /or postpone development of metastasis. This delay can be of varying lengths of time, depending on the history of the cancer and /or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the metastasis.

[00111] 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 shrinking the size of the tumor (in the cancer context, for example, breast or prostate cancer), retardation of cancerous cell growth, delaying the development of metastasis, decreasing symptoms resulting from the disease, 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. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to reduce the proliferation of (or destroy) cancerous cells and to reduce and /or delay the development, or growth, of metastases of cancerous cells, 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. Typical dosages comprise 0.1-to 100 mg/kg/body weight. The preferred dosages comprise 1 to 100 mg/kg/body weight. The most preferred dosages comprise 10 to 100 mg/kg/body weight.

[00112] As used herein, a nucleic acid molecule or agent, antibody, composition or cell, etc., is said to be "isolated" when that nucleic acid molecule, agent, antibody, composition, or cell, etc. is substantially separated from contaminant nucleic acid molecules, antibodies, agents, compositions, or cells, etc. naturally present in its original source.

[00113] The term "individual" refers to a vertebrate animal, preferably a mammal. Mammals include, but are not limited to, humans, farm animals, sport animals, pets, primates, mice and rats. In the most preferred embodiment, the term individual denotes a human.

[00114] The terms "polypeptide," "oligopeptide," "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon an antibody, the polypeptides can occur as single chains or as associated chains.

[00115] Also encompassed within the scope of the invention are peptidomimetics of the B7-H3 peptide agonists, antagonists and modulators (including anti-B7-H3 antibodies) described herein. Such peptidomimetics include peptides wherein at least one amino acid residue is substituted with an amino acid residue that is not commonly found in nature, such as the D isomer of the amino acid or an N-alkylated species of the amino acid. In other embodiments, peptidomimetics are constructed by replacing at least one amide bond (−C(=O)−NH−) in a B7-H3 peptide agonist, antagonist or modulators with an amide isostere. Suitable amide isosteres include $-CH_2-NH-$, $-CH_2-S-$, $-CH_2-S(O)-$, $-CH_2-S(O)_2-$, $-CH_2-CH_2-$, –CH=CH− (E or Z form), −C(=O)−CH_2−, –CH(CN)−NH−, –C(OH)−CH_2−, and −O−C(=O)−NH−. The amide bonds in a B7-H3 peptide agonist, antagonist or modulator that are suitable candidates for replacement with amide isosteres include bonds that are

hydrolyzable by the endogenous esterases or proteases of the intended subject of B7-H3 peptide agonist, antagonist or modulator treatment.

[00116] As used herein, the term "substantially pure" refers to material that is at least 50% pure (*i.e.*, free from contaminants), more preferably at least 90 % pure, more preferably at least 95% pure, more preferably at least 98% pure, more preferably at least 99% pure, and most preferably greater than 99% pure.

[00117] As used herein, the term "toxin" refers to any substance which effects an adverse response within a cell. For example, a toxin directed to a cancerous cell would have an adverse, sometimes deleterious effect, on the cancerous cell. Examples of toxins include, but are not limited to, a taxane, a maytansinoid, an auristatin (e.g., monomethyl auristatin (MMAE), monomethyl auristatin F (MMAF), auristatin E (AE), *etc.*) (such as those disclosed in United States Patent No. 5,208,020; 5,416,064; 6,333,410; 6,340,701; 6,372,738; 6,436,931; 6,441,163; 6,596,757; 7,276,497; 7,585,857; or 7,851,432), a calicheamicin, an anthracycline (*e.g.*, doxorubicin), a CC-1065 analog, docetaxel,; cathepsin B or E; ricin, gelonin, *Pseudomonas* exotoxin, diphtheria toxin, and RNase; radiolabeled antibodies (*e.g.*, tiuxetan-conjugated or labeled with a toxic radioisotope (for example, ^{90}Y; ^{131}I, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi, ^{225}Ac, etc.).

[00118] As used herein, the terms "treatment" or "treating" denote an approach for obtaining a beneficial or desired result including and preferably a beneficial or desired clinical result. Such beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) cancerous cells or other diseased, reducing metastasis of cancerous cells found in cancers, shrinking the size of the tumor, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and /or prolonging survival of individuals.

[00119] As used herein, the term cancer is intended to encompass cancers characterized by the presence of a cancer cell selected from the group consisting of a cell of an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a dhordoma, 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 canrcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer (nephroblastoma, papillary renal cell carcinoma), a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer (hepatoblastoma, hepatocellular carcinoma), a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 unveal melanoma, a rare hematologic disorder, 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 (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

III. Methods Of Making Antibodies And Polypeptides

[00120] 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 non-human species, such as mice. In general, a mouse or rat is used for immunization but other animals may also be used. The antibodies are produced by immunizing mice with an immunogenic amount of cells, cell extracts, or protein preparations that contain human B7-H3. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, nucleic acids, or tissue. In one embodiment, human lung carcinoma cells are used. Cells used for immunization, for example, human testis or pancreatic adenocarcinoma or stomach cells, may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells (e.g., human testis, stomach, or pancreatic adenocarcinoma 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., Freud's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, b i weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (e.g., in a tissue recombinant).

[00121] In one embodiment, monoclonal antibodies that bind to B7-H3 are obtained by using host cells that over-express B7-H3 as an immunogen. Such cells include, by way of example and not by limitation, human lung carcinoma cells and human colon cancer cells.

[00122] To monitor the antibody response, a small biological sample (*e.g.*, blood) may be obtained from the animal and tested for antibody titer against the immunogen. The spleen and /or several large lymph nodes can be removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of non-specifically adherent cells) by applying a cell suspension to a plate or to a well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, can then be fused with myeloma cells (e.g., X63- Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, CA). Polyethylene glycol (PEG) may be used to fuse spleen or lymphocytes with myeloma cells to form a hybridoma. The hybridoma is then cultured in a selective medium (e.g., hypoxanthine, aminopterin, thymidine medium, otherwise known as "HAT medium"). The resulting hybridomas are then plated by limiting dilution, and are assayed for the production of antibodies that bind specifically to the immunogen, using, for example, FACS (fluorescence activated cell sorting) or immunohistochemistry (IHC) screening. The selected monoclonal antibody-secreting hybridomas are then cultured either in vitro (e.g., in tissue culture bottles or hollow fiber reactors), or in vivo (e.g., as ascites in mice).

[00123] As another alternative to the cell fusion technique, Epstein-Barr Virus (EBV)-immortalized B cells may be used to produce monoclonal antibodies of the subject invention. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional assay procedures (e.g., FACS, IHC, radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, etc.).

[00124] In another alternative, anti-B7-H3 monoclonal antibody and any other equivalent antibodies can be sequenced and produced recombinantly by any means known in the art (e.g., humanization, use of transgenic mice to produce fully human antibodies, phage display technology, etc.). In one embodiment, anti-B7-H3 monoclonal 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.

[00125] The polynucleotide sequence of anti-B7-H3 monoclonal antibody and any other equivalent antibodies may be used for genetic manipulation to generate a "humanized" antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing

process (3) the actual humanizing methodologies /techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[00126] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Winter *et al.* (1991) "*Man-made Anditbodies*," Nature 349:293-299; Lobuglio *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989), Shaw *et al.* (1987) "*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*," J. Immunol. 138:4534-4538, and Brown *et al.* (1987) "*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*," Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain (see, for example, Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; and Jones *et al.* (1986) "*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*," Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) "*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*," Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

[00127] The invention also encompasses single chain variable region fragments ("scFv") of antibodies of this invention, such as mu-anti-B7-H3. Single chain variable region fragments are made by linking light and/ or heavy chain variable regions by using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For

synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[00128] The invention includes modifications to antibodies and polypeptides that bind to B7-H3 and its agonists, antagonists, and modulators, including functionally equivalent antibodies and polypeptides that do not significantly affect their properties and variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for one another include but are not limited to: glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine; lysine/arginine; and phenylalanine/tryosine. These polypeptides also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, i.e., the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00129] The invention also encompasses fusion proteins comprising one or more fragments or regions from the polypeptides and antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of variable light chain region and at least 10 amino acids of variable heavy chain region. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a light chain variable region and a heavy chain variable region of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody

fusion protein contains one or more polypeptide domains that specifically bind to B7-H3 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

[00130] An anti-B7-H3 polypeptide, and other B7-H3 agonists, antagonists and modulators can be created by methods known in the art, for example, synthetically or recombinantly. One method of producing B7-H3 peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well known to those skilled in the art (see, *e.g.*, Kelley, R. F. *et al.* (1990) In: GENETIC ENGINEERING PRINCIPLES AND METHODS, Setlow, J.K. Ed., Plenum Press, N.Y., vol. 12, pp 1-19; Stewart, J.M *et al.* (1984) SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL; see also United States Patents Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[00131] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "Solid Phase Synthesis," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) "*Solid-Phase Synthesis In The Twenty-First Century*," Mini Rev. Med. Chem. 6(1):3-10).

[00132] In yet another alternative, fully human antibodies may be obtained through the use of commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XENOMOUSE™ (Abgenix, Inc., Fremont, CA) and HUMAB-MOUSE® and TC MOUSE™ (both from Medarex, Inc., Princeton, NJ).

[00133] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method that may be employed is to express the antibody sequence in plants {e.g., tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) "*Production Of Antibodies And Antibody Fragments In Plants*," Vaccine 19:2756; Lonberg, N. *et al.* (1995) "*Human Antibodies From Transgenic Mice*," Int. Rev. Immunol 13:65-93; and Pollock *et al.*(1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*,

humanized, single chain, etc. are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) "*Making Antibodies By Phage Display Technology*," Annu. Rev. Immunol. 12.433-455).

[00134] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[00135] An alternative method of cloning the protein of interest is by "panning" using purified B7-H3 or portions thereof for cells expressing the antibody or protein of interest. B7-H3 exists in a "2Ig" form and as a "4Ig" form. The amino acid sequence of the "2Ig" form of human B7-H3 is (**SEQ ID NO:1**):

MLRRRGSPGM GVHVGAALGA LWFCLTGALE VQVPEDPVVA LVGTDATLCC
SFSPEPGFSL AQLNLIWQLT DTKQLVHSFA EGQDQGSAYA NRTALFPDLL
AQGNASLRLQ RVRVADEGSF TCFVSIRDFG SAAVSLQVAA PYSKPSMTLE
PNKDLRPGDT VTITCSSYRG YPEAEVFWQD GQGVPLTGNV TTSQMANEQG
LFDVHSVLRV VLGANGTYSC LVRNPVLQQD AHGSVTITGQ PMTFPPEALW
VTVGLSVCLI ALLVALAFVC WRKIKQSCEE ENAGAEDQDG EGEGSKTALQ
PLKHSDSKED DGQEIA

[00136] The cDNA sequence encoding the "2Ig" form of human B7-H3 is (**SEQ ID NO:2**):

atgctgcgtc ggcggggcag ccctggcatg ggtgtgcatg tgggtgcagc cctgggagca
ctgtggttct gcctcacagg agccctggag gtccaggtcc ctgaagaccc agtggtggca
ctggtgggca ccgatgccac cctgtgctgc tccttctccc ctgagcctgg cttcagcctg
gcacagctca acctcatctg gcagctgaca gataccaaac agctggtgca cagctttgct
gagggccagg accagggcag cgcctatgcc aaccgcacgg ccctcttccc ggacctgctg
gcacagggca acgcatccct gaggctgcag cgcgtgcgtg tggcggacga gggcagcttc
acctgcttcg tgagcatccg ggatttcggc agcgctgccg tcagcctgca ggtggccgct
ccctactcga agcccagcat gaccctggag cccaacaagg acctgcggcc aggggacacg
gtgaccatca cgtgctccag ctaccggggc taccctgagg ctgaggtgtt ctggcaggat
gggcagggtg tgcccctgac tggcaacgtg accacgtcgc agatggccaa cgagcagggc
ttgtttgatg tgcacagcgt cctgcgggtg gtgctgggtg cgaatggcac ctacagctgc
ctggtgcgca accccgtgct gcagcaggat gcgcacggct ctgtcaccat cacagggcag
cctatgacat tccccccaga ggccctgtgg gtgaccgtgg ggctgtctgt ctgtctcatt
gcactgctgg tggccctggc tttcgtgtgc tggagaaaga tcaaacagag ctgtgaggag
gagaatgcag gagctgagga ccaggatggg gagggagaag gctccaagac agccctgcag
cctctgaaac actctgacag caaagaagat gatggacaag aaatagcc

[00137] The amino acid sequence of the "2Ig" form of human B7-H3 (**SEQ ID NO:1**) (shown in bold and underline below) is completely embraced within the "4Ig" form of human B7-H3(**SEQ ID NO:76**):

MLRRRGSPGM GVHVGAALGA LWFCLTGALE VQVPEDPVVA LVGTDATLCC
SFSPEPGFSL AQLNLIWQLT DTKQLVHSFA EGQDQGSAYA NRTALFPDLL
AQGNASLRLQ RVRVADEGSF TCFVSIRDFG SAAVSLQVAA PYSKPSMTLE
PNKDLRPGDT VTITCSSYQG YPEAEVFWQD GQGVPLTGNV TTSQMANEQG

LFDVHSILRV VLGANGTYSC LVRNPVLQQD AHSSVTITPQ RSPTGAV**EVQ**
VPEDPVVALV GTDATLRCSF SPEPGFSLAQ LNLIWQLTDT KQLVHSFTEG
RDQGSAYANR TALFPDLLAQ GNASLRLQRV RVADEGSFTC FVSIRDFGSA
AVSLQVAAPY SKPSMTLEPN KDLRPGDTVT ITCSSYRGYP EAEVFWQDGQ
GVPLTGNVTT SQMANEQGLF DVHSVLRVVL GANGTYSCLV RNPVLQQDAH
GSVTITGQPM TFPPEALWVT VGLSVCLIAL LVALAFVCWR KIKQSCEEEN
AGAEDQDGEG EGSKTALQPL KHSDSKEDDG QEIA

[00138] The cDNA sequence encoding the "4Ig" form of human B7-H3 is (**SEQ ID NO:77**); residues encoding the "2Ig" form of B&-H3 are shown in bold and underlined:

atgctgcgtc ggcggggcag ccctggcatg ggtgtgcatg tgggtgcagc cctgggagca
ctgtggttct gcctcacagg agccctggag gtccaggtcc ctgaagaccc agtggtggca
ctggtgggca ccgatgccac cctgtgctgc tccttctccc ctgagcctgg cttcagcctg
gcacagctca acctcatctg gcagctgaca gataccaaac agctggtgca cagctttgct
gagggccagg accagggcag cgcctatgcc aaccgcacgg ccctcttccc ggacctgctg
gcacagggca acgcatccct gaggctgcag cgcgtgcgtg tggcggacga gggcagcttc
acctgcttcg tgagcatccg ggatttcggc agcgctgccg tcagcctgca ggtggccgct
ccctactcga agcccagcat gaccctggag cccaacaagg acctgcggcc aggggacacg
gtgaccatca cgtgctccag ctaccagggc taccctgagg ctgaggtgtt ctggcaggat
gggcagggtg tgcccctgac tggcaacgtg accacgtcgc agatggccaa cgagcagggc
ttgtttgatg tgcacagcat cctgcgggtg gtgctgggtg caaatggcac ctacagctgc
ctggtgcgca accccgtgct gcagcaggat gcgcacagct ctgtcaccat cacaccccag
agaagcccca caggagccgt g**gaggtccag gtccctgagg acccggtggt ggccctagtg**
ggcaccgatg ccaccctgcg ctgctccttc tcccccgagc ctggcttcag cctggcacag
ctcaacctca tctggcagct gacagacacc aaacagctgg tgcacagttt caccgaaggc
cgggaccagg gcagcgccta tgccaaccgc acggccctct tcccggacct gctggcacaa
ggcaatgcat ccctgaggct gcagcgcgtg cgtgtggcgg acgagggcag cttcacctgc
ttcgtgagca tccgggattt cggcagcgct gccgtcagcc tgcaggtggc cgctccctac
tcgaagccca gcatgaccct ggagcccaac aaggacctgc ggccagggga cacggtgacc
atcacgtgct ccagctaccg gggctaccct gaggctgagg tgttctggca ggatgggcag
ggtgtgcccc tgactggcaa cgtgaccacg tcgcagatgg ccaacgagca gggcttgttt
gatgtgcaca gcgtcctgcg ggtggtgctg ggtgcgaatg gcacctacag ctgcctggtg
cgcaaccccg tgctgcagca ggatgcgcac ggctctgtca ccatcacagg gcagcctatg
acattccccc cagaggccct gtgggtgacc gtggggctgt ctgtctgtct cattgcactg
ctggtggccc tggctttcgt gtgctggaga aagatcaaac agagctgtga ggaggagaat
gcaggagctg aggaccagga tggggaggga gaaggctcca agacagccct gcagcctctg
aaacactctg acagcaaaga agatgatgga caagaaatag cc

[00139] The "panning" procedure may be conducted by obtaining a cDNA library from tissues or cells that express B7-H3, over-expressing the cDNAs in a second cell type, and screening the transfected cells of the second cell type for a specific binding to B7-H3. Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by "panning" can be found in the art (see, for example, Aruffo, A. *et al.* (1987) "*Molecular Cloning Of A CD28 cDNA By A High-Efficiency COS Cell Expression System*," Proc. Natl. Acad. Sci. (U.S.A.) 84:8573-8577 and Stephan, J. *et al.* (1999) "*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation*," Endocrinol. 140:5841-5854).

[00140] cDNAs encoding anti-B7-H3 antibodies, and other B7-H3 peptide agonists, antagonists and modulators can be obtained by reverse transcribing the mRNAs from a particular cell type according

to standard methods in the art. Specifically, mRNA can be isolated using various lytic enzymes or chemical solutions according to the procedures set forth in Sambrook *et al.* supra or extracted by commercially available nucleic-acid- binding resins following the accompanying instructions provided by manufacturers (*e.g.*, Qiagen, Invitrogen, Promega). The synthesized cDNAs are then introduced into an expression vector to produce the antibody or protein of interest in cells of a second type. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, and cosmids.

[00141] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00142] Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5-fold higher, more preferably 10-fold higher, even more preferably 20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to B7-H3 is effected by an immunoassay or FACS. A cell over-expressing the antibody or protein of interest can be identified.

[00143] Various techniques are also available which may now be employed to produce mutant B7-H3 peptide agonists, antagonists, and modulators which encodes for additions, deletions, or changes in amino acid sequence of the resultant protein relative to the parent B7-H3 peptide agonist, antagonist or modulator molecule.

[00144] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are

commercially available. For example, an anti-B7-H3 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

IV. Methods For Screening Polypeptides And Monoclonal Antibodies

[00145] Several methods may be used to screen polypeptides and monoclonal antibodies that bind to B7-H3. It is understood that "binding" refers to biologically or immunologically relevant specific binding, and does not refer to non-specific binding that may occur, for example, when an immunoglobulin is used at a very high concentration against a non-specific target. In one embodiment, monoclonal antibodies are screened for binding to B7-H3 using standard screening techniques. In this manner, anti-B7-H3 monoclonal antibody was obtained. The preferred hybridomas of the present invention are those that produce antibodies BRCA69D, BRCA84D or PRCA157.

[00146] Additional monoclonal antibodies that bind to B7-H3 may be identified. For this purpose, monoclonal antibodies are screened for their differential ability to bind to cancerous tissues but not to non-cancerous cells. In one embodiment, monoclonal antibodies which bind to B7-H3 and that are also cross-reactive to human cancerous cells or tissues, but not to normal cells or tissues to the same degree, are selected. One method that may be employed for screening is immunohistochemistry (IHC). Standard immunohistochemical techniques are known to those of average skill in the art. See, for example, ANIMAL CELL CULTURE METHODS (J.P. Mather and D. Barnes, eds., Academic Press, NY, Vol. 57, Ch. 18 and 19, pp. 314-350, 1998). Biological samples (e.g., tissues) may be obtained from biopsies, autopsies, or necropsies. To ascertain if B7-H3 is present only on cancerous cells, anti-B7-H3 antibodies may be used to detect the presence of B7-H3 on tissues from individuals with cancer while other non-cancerous tissues from the individual suffering from cancer or tissues from individuals without cancer are used as a control. The tissue can be embedded in a solid or semi-solid substance that prevents damage during freezing (e.g., agarose gel or OCT) and then sectioned for staining. Cancers from different organs and at different grades can be used to screen monoclonal antibodies. Examples of tissues that may be used for screening purposes include but are not limited to ovary, breast, lung, prostate, colon, kidney, skin, thyroid, brain, heart, liver, stomach, nerve, blood vessels, bone, upper digestive tract, and pancreas. Examples of different cancer types that may be used for screening purposes include, but are not limited to, carcinomas, adenocarcinomas, sarcomas, adenosarcomas, lymphomas, and leukemias.

[00147] In yet another alternative, cancerous cells lines such as HMEC (BioWhittaker CC- 2251), HUVEC (Primary endothelial cells), BT-474 (ATCC# HTB-20), MCF7 (ATCC# HTB22), MDA-MB-175-VII (ATCC# HB-25), MDA-MB-361 (ATCC# HB-27), SKBR3 (ATCC# HTB-30), A549 (ATCC# CCL- 185), Calu-3 (ATCC# HTB-55), SKMES-I (ATCC# HTB-58), ES-2 (ATCC# CRL-1978), SKOV3 (ATCC# HTB- 77), Panc-1 (ATCC# CRL-1469), AsPC-I (ATCC# CRL-1682),

HPAF-II (ATCC# CRL-1997), Hs700T (ATCC# HTB-174), Colo205 (ATCC# CCL-222), HT-29 (ATCC# HTB-38), SW480 (ATCC# CCL-228), SW948 (ATCC# CCL- 237), 293 (ATCC # CRL-1573), 786-O (ATCC# CRL-1932), A498 (ATCC# HTB-44), Caki-2 (ATCC# HTB-47), COS-7 (ATCC# CRL-1651), RL-65 (ATCC # CRL-10345), SV-T2 (ATCC# CCL-163.1), 22RV1 (ATCC# CRL-2505), DU145 (ATCC# HTB-81), LNCaP (ATCC# CRL-1740), PC-3 (ATCC# CRL-1435), HT29 (ATCC# HTB-38), Hs746T (ATCC# HTB-135), NCI-N87 (ATCC# CRL-5822) and normal cells from their respective tissues may be used to screen for monoclonal antibodies which are specific for cancerous tissue. Primary, or low passage, cell cultures derived from normal tissues from different organs, including but not limited to, kidney, ovary, breast, lung, prostate, colon, kidney, skin, thyroid, aortic smooth muscle, and endothelial cells can be used as negative controls. The cancerous or non-cancerous cells can be grown on glass slides or coverslips, or on plastic surfaces, or prepared in a CellArray™ device, as described in WO 01/43869, and screened for the binding of antibody using IHC as described above for tissues. Alternatively, cells may be removed from the growth surface using non-proteolytic means and spun into a pellet, which is then embedded and treated as tissues for IHC analysis as described above. Cells may be inoculated into immunodeficient animals, a tumor allowed to grow, and then this tumor may be harvested, embedded, and used as a tissue source for IHC analysis. In another alternative, single cells may be screened by incubating with the primary antibody, a secondary "reporter" antibody linked to a fluorescent molecule and then analyzed using a fluorescent activated cell- sorting (FACS) machine.

[00148] Any of several different detection systems may be utilized to detect binding of antibodies to tissue section. Typically, immunohistochemistry involves the binding of a primary antibody to the tissue and then a secondary antibody reactive against the species from the primary antibody was generated and conjugated to a detectable marker (e.g., horseradish peroxidase, HRP, or diaminobenzedine, DAB). One alternative method that may be used is polyclonal mirror image complementary antibodies or polyMICA™ (polyclonal Mirror Image Complementary Antibodies; The Binding Site Limited, Birmingham, UK; Mangham, D.C. *et al.* (1999) "*A Novel Immunohistochemical Detection System Using Mirror Image Complementary Antibodies (MICA)*," Histopathology 35(2):129-33). The PolyMICA™ technique can be used to test binding of primary antibodies (e.g., anti-B7-H3 antibodies) to normal and cancerous tissue. Several kinds of polyMICA™ Detection kits are commercially available: Product No. HK004.D is a polyMICA™ Detection kit which uses DAB chromagen; Product No. HK004.A is a polyMICA™ Detection kit which uses AEC chromagen. Alternatively, the primary antibody may be directly labeled with the detectable marker.

[00149] The first step in IHC screening to select for an appropriate antibody is the binding of primary antibodies raised in mice (e.g., anti-B7-H3 antibodies) to one or more immunogens (e.g.,

cells or tissue samples). In one embodiment, the tissue sample is sections of frozen tissue from different organs. The cells or tissue samples can be either cancerous or non-cancerous.

[00150] Frozen tissues can be prepared, sectioned, with or without fixation, and IHC performed by any of a number of methods known to one familiar with the art (see, for example, Stephan *et al.* (1999) "*Distribution And Function Of The Adhesion Molecule BEN During Rat Development*," Dev. Biol. 212:264-277 and Stephan *et al.* (1999) "*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation*," Endocrinology 140:5841-5854).

V. Methods Of Characterizing Anti-B7-H3 Antibodies

[00151] Any of several methods can be used to characterize anti-B7-H3 antibodies. One method is to identify the epitope to which it binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Lelystad, The Netherlands). Epitope mapping can be used to determine the sequence to which an anti-B7-H3 antibody binds. The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch.

[00152] Peptides of varying lengths (e.g., preferably at least 4-6 amino acids long) can be isolated or synthesized {e.g., recombinantly) and used for binding assays with anti-B7-H3 antibody. The epitope to which anti-B7-H3 antibody binds can be determined in a systematic screening by using overlapping peptides derived from the extracellular sequence and determining binding by anti-B7-H3 antibody.

[00153] Yet another method that can be used to characterize an anti-B7-H3 antibody is to use competition assays with other antibodies known to bind to the same antigen, i.e., B7-H3 to determine if anti-B7-H3 antibodies binds to the same epitope as other antibodies. Examples of commercially available antibodies to B7-H3 may be available and may be identified using the binding assays taught herein. Competition assays are well known to those of skill in the art, and such procedures and illustrative data are detailed further in the Examples. Anti-B7-H3 antibodies can be further characterized by the tissues, type of cancer or type of tumor to which they bind.

[00154] Another method of characterizing anti-B7-H3 antibodies is by the antigen to which it binds. Anti-B7-H3 antibodies were used in Western blots with cell lysates from various human cancers. As is known to one of skill in the art, Western blotting can involve running cell lysates and/ or cell fractions on a denaturing or non-denaturing gel, transferring the proteins to nitrocellulose paper, and then probing the blot with an antibody (e.g., anti-B7-H3 antibody) to see which proteins are bound by the antibody. B7-H3 is associated with various human cancers of different tissues including, but not limited to colon, breast, ovary, pancreas and lung.

VI. Methods Of Diagnosing Cancer Using Anti-B7-H3 Antibodies And B7-H3 Modulators

[00155] Monoclonal antibodies to B7-H3 made by the methods disclosed herein may be used to identify the presence or absence of cancerous cells in a variety of tissues, including but not limited to, ovary, breast, lung, prostate, colon, kidney, pancreas, skin, thyroid, brain, heart, liver, stomach, nerve, blood vessels, bone, and upper digestive tract, for purposes of diagnosis. Monoclonal antibodies to B7-H3 made by the methods disclosed herein may also be used to identify the presence or absence of cancerous cells, or the level thereof, which are circulating in blood after their release from a solid tumor. Such circulating antigen may be an intact B7-H3 antigen, or a fragment thereof that retains the ability to be detected according to the methods taught herein. Such detection may be effected by FACS analysis using standard methods commonly used in the art.

[00156] These uses can involve the formation of a complex between B7-H3 and an antibody that binds specifically to B7-H3. Examples of such antibodies include but are not limited to those anti-B7-H3 monoclonal antibodies produced by the hybridomas BRCA84D, BRCA69D, and PRCA157. The formation of such a complex can be *in vitro* or *in vivo*. Without being bound by theory, monoclonal antibody anti-B7-H3 can bind to B7-H3 through the extracellular domain of B7-H3 and may then be internalized.

[00157] In a preferred embodiment of the diagnostic methods of this invention, the antibody bears a detectable label. Examples of labels that may be used include a radioactive agent or a fluorophore, such as phycoerythrin or fluorescein isothiocyanate (also known as fluoroisothiocyanate or FITC).

[00158] As with other known antibodies used commercially for diagnostic and therapeutic purposes, the target antigen of this invention is broadly expressed in normal tissue. It is also up regulated in some tumors. Therefore, the particular dosages and routes of delivery of the antibodies of this invention as used for diagnostic or therapeutic agents will be tailored to the particular tumor or disease state at hand, as well as to the particular individual being treated.

[00159] One method of using the antibodies for diagnosis is in vivo tumor imaging by linking the antibody to a radioactive or radio-opaque agent, administering the antibody to the individual and using an x-ray or other imaging machine to visualize the localization of the labeled antibody at the surface of cancer cells expressing the antigen. The antibody is administered at a concentration that promotes binding at physiological conditions.

[00160] In vitro techniques for detection of B7-H3 are routine in the art and include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis.

[00161] In aspects of this invention, methods of radioimaging of tumors or neoplasms, or of measuring the effectiveness of a method of treatment with a radiolabeled antibody, comprising the step of administering a radiolabeled, tumor-specific antibody to an individual following the practice of this invention. The radiolabeled antibody may be a monoclonal or polyclonal antibody comprising a radiolabel, preferably selected from the group consisting of Technetium-99m, Indium-111, Iodine-131, Rhenium-186, Rhenium-188, Samarium-153, Lutetium-177, Copper-64, Scandium-47, Yttrium-90. Monoclonal antibodies labeled with therapeutic radionuclides such as Iodine-131, Rhenium-188, Holmium-166, Samarium-153 and Scandium-47, which do not compromise the immunoreactivity of antibodies and are not broken down in vivo, are especially preferred. The person skilled in the art will appreciate that other radioactive isotopes are known, and may be suitable for specific applications. The radioimaging may be conducted using Single Photon Emission Computer Tomography (SPECT), Position Emission Tomography (PET), Computer Tomography (CT) or Magnetic Resonance Imaging (MRI). Correlative imaging, which permits greater anatomical definition of location of metastases located by radioimmunoimaging, is also contemplated.

[00162] In other methods, the cancerous cells are removed and the tissue prepared for immunohistochemistry by methods well known in the art (*e.g.*, embedding in a freezing compound, freezing and sectioning, with or without fixation; fixation and paraffin embedding with or without various methods of antigen retrieval and counterstaining). The monoclonal antibodies may also be used to identify cancerous cells at different stages of development. The antibodies may also be used to determine which individuals' tumors express the antigen on their surface at a pre-determined level and are thus candidates for immunotherapy using antibodies directed against said antigen. The antibodies may recognize both primary and metastasizing cancers that express B7-H3. As used herein, detection may include qualitative and /or quantitative detection and may include comparing the level measured to a normal cell for an increased level of expression of B7-H3 in cancerous cells.

[00163] The invention also provides methods of aiding diagnosis of cancer characterized by cancer cells that express B7-H3 in an individual using any antibody that binds to B7-H3 and any other methods that can be used determine the level of B7-H3 expression. As used herein, methods for "aiding diagnosis" means that these methods assist in making a clinical determination regarding the classification, or nature, of cancer, and may or may not be conclusive with respect to the definitive diagnosis. Accordingly, a method of aiding diagnosis of cancer can comprise the step of detecting the level of B7-H3 in a biological sample from the individual and /or determining the level of B7-H3 expression in the sample. Antibodies recognizing the antigen or a portion thereof may also be used to create diagnostic immunoassays for detecting antigen released or secreted from living or dying cancer cells in bodily fluids, including but not limited to, blood, saliva, urine, pulmonary fluid, or ascites fluid.

[00164] Not all cells in a particular tumor of interest will express B7-H3, and cancerous cells in other tissues may express B7-H3, thus an individual should be screened for the presence or absence of B7-H3 on cancerous cells to determine the usefulness of immunotherapy in the individual. The anti-B7-H3 antibodies made by the methods disclosed herein may be used to determine whether an individual diagnosed with cancer may be deemed a candidate for immunotherapy using antibodies directed against B7-H3. In one embodiment, a cancerous tumor or a biopsy sample may be tested for expression of B7-H3, using antibodies directed against B7-H3. Individuals with cancer cells that express B7-H3 are suitable candidates for immunotherapy using antibodies directed against B7-H3. Staining with anti-B7-H3 antibody may also be used to distinguish cancerous tissues from normal tissues.

[00165] Methods of using anti-B7-H3 antibodies for diagnostic purposes are useful both before and after any form of anti-cancer treatment, e.g., chemotherapy or radiation therapy, to determine which tumors are most likely to respond to a given treatment, prognosis for individual with cancer, tumor subtype or origin of metastatic disease, and progression of the disease or response to treatment.

[00166] The compositions of this invention are also suitable for diagnosis of disease states other than cancer, using the methods generally described above in application with other diseased (non-cancerous) cells. Disease states suitable for use in the methods of this invention include, but are not limited to, diseases or disorders associated with inflammatory or autoimmune responses in individuals. The methods described above may be used for modulating inflammatory or autoimmune responses in individuals. Diseases and conditions resulting from inflammation and autoimmune disorders that may be subject to diagnosis and /or treatment using the compositions and methods of the invention include, by way of illustration and not of limitation, multiple sclerosis, meningitis, encephalitis, stroke, other cerebral traumas, inflammatory bowel disease including ulcerative colitis and Crohn's disease, myasthenia gravis, lupus, rheumatoid arthritis, asthma, acute juvenile onset diabetes, AIDS dementia, atherosclerosis, nephritis, retinitis, atopic dermatitis, psoriasis, myocardial ischemia and acute leukocyte-mediated lung injury.

[00167] Still other indications for diagnostic and/or therapeutic use of antibodies and other therapeutic agents of the invention include administration to individuals at risk of organ or graft rejection. Over recent years there has been a considerable improvement in the efficiency of surgical techniques for transplanting tissues and organs such as skin, kidney, liver, heart, lung, pancreas and bone marrow. Perhaps the principal outstanding problem is the lack of satisfactory agents for inducing immunotolerance in the recipient to the transplanted allograft or organ. When allogeneic cells or organs are transplanted into a host (i.e., the donor and donee are different individuals from the same species), the host immune system is likely to mount an immune response to foreign antigens in the transplant (host-versus-graft disease) leading to destruction of the transplanted tissue.

[00168] Uses described anywhere in this application for anti-B7-H3 antibodies also encompass the use of other B7-H3 agonists, antagonists and modulators as described herein. In such embodiments, the B7-H3 agonist, antagonist or other non-antibody modulator is substituted for the B7-H3 antibody in the steps described, and alterations within the scope of the ordinarily skilled practitioner are made to tailor the method to the substituted B7-H3 modulatory composition.

[00169] Monoclonal antibodies to B7-H3 made by the methods disclosed herein may be used to identify the presence or absence of human cancer stem cells in a variety of tissues. Cancer stem cells (CSCs) have been hypothesized to play a role in tumor growth and metastasis (Ghotra, V.P. *et al.* (2009) "*The Cancer Stem Cell Microenvironment And Anti-Cancer Therapy*," Int. J. Radiat. Biol. 85(11):955-962; Gupta, P.B. *et al.* (2009) "*Cancer Stem Cells: Mirage Or Reality?*" Nat. Med. 15(9):1010-1012; Lawson, J.C. *et al.* (2009) "*Cancer Stem Cells In Breast Cancer And Metastasis*," Breast Cancer Res. Treat. 118(2):241-254; Hermann, P.C. *et al.* (2009) "*Pancreatic Cancer Stem Cells--Insights And Perspectives*," Expert Opin. Biol. Ther. 9(10):1271-1278; Schatton, T. *et al.* (2009) "*Identification And Targeting Of Cancer Stem Cells*," Bioessays 31(10):1038-1049; Mittal, S. *et al.* (2009) "*Cancer Stem Cells: The Other Face Of Janus*," Amer. J. Med. Sci. 338(2):107-112; Alison, M.R. *et al.* (2009) "*Stem Cells And Lung Cancer: Future Therapeutic Targets?*" Expert Opin. Biol. Ther. 9(9):1127-1141; Charafe-Jauffret, E. *et al.* (2009) "*Breast Cancer Stem Cells: Tools And Models To Rely On*," BMC Cancer 9:202; Scopelliti, A. *et al.* (2009) "*Therapeutic Implications Of Cancer Initiating Cells*," Expert Opin. Biol. Ther. 9(8):1005-1016; PCT Publication WO 2008/091908). Under this hypothesis, the CSCs provide a small, distinct subset of cells within each tumor that are capable of indefinite self-renewal and of developing into the more adult tumor cell(s) that are relatively limited in replication capacity. It has been hypothesized that these cancer stem cells might be more resistant to chemotherapeutic agents, radiation or other toxic conditions, and thus, persist after clinical therapies and later grow into secondary tumors, metastases or be responsible for relapse. It has been suggested that CSCs can arise either from 'normal' tissue stem cells or from more differentiated tissue progenitor cells.

[00170] Human cancer stem cells have several defining characteristics. Such characteristics are described in PCT Publication WO 2008/091908 and are hereby incorporated by reference. Monoclonal antibodies to cell surface targets on cancer stem cells can be used to identify the presence or absence of cancer stem cells in a variety of tissues. Monoclonal antibodies to B7-H3 made by the methods disclosed herein may also be used to identify the presence or absence of cancer stem cells, or the level of cancer stem cells in a sample or tissue or in circulation after their release from a solid tumor. Such circulating antigen may be an intact B7-H3 antigen, or a fragment thereof that retains the ability to be detected according to the methods taught herein. Such detection may be effected by FACS analysis using standard methods commonly used in the art. In another embodiment, such

detection may be effected by immunohistochemical analysis of tissue samples using standard methods commonly used in the art.

[00171] These uses can involve the formation of a complex between B7-H3 and an antibody that binds specifically to B7-H3 on cancer stem cells. Examples of such antibodies include but are not limited to those anti-B7-H3 monoclonal antibodies produced by the hybridomas BRCA84D, BRCA69D, and PRCA157. The formation of such a complex can be *in vitro* or *in vivo*.

[00172] Uses described in this application that recite their use for anti-B7-H3 antibodies also encompass the use of other B7-H3 agonists, antagonists and modulators as described herein for the use of identification and treatment of cancer stem cells. In such embodiments, anti-B7-H3 antibodies and other B7-H3 agonists, antagonists and modulators are used for identification, diagnosis or therapeutic treatment of cancer stem cells using similar methods described, and alterations within the scope of the ordinary skilled practitioner are made to tailor the method to the identification /diagnosis or treatment of cancer stem cells.

VII. Preferred Compositions Of The Present Invention

[00173] The present invention encompasses compositions, including pharmaceutical compositions, comprising anti-B7-H3 antibodies, polypeptides derived from anti-B7-H3 antibodies, polynucleotides comprising sequence encoding anti-B7-H3 antibodies, and other agents as described herein. As used herein, compositions further comprises one or more antibodies, polypeptides and /or proteins that bind to B7-H3, B7-H3 agonists, antagonists, modulators, and /or one or more polynucleotides comprising sequences encoding one or more antibodies, polypeptides and proteins that bind to B7-H3.

[00174] The invention further provides for conjugates of any B7-H3 peptide agonist, antagonist or modulator, and additional chemical structures that support the intended function or functions of the particular B7-H3 peptide agonist, antagonist or modulator.

[00175] These conjugates include B7-H3 peptide agonist, antagonist or modulator covalently bound to a macromolecule such as any insoluble, solid support matrix used in the diagnostic, screening or purification procedures discussed herein. Suitable matrix materials include any substance that is chemically inert, has high porosity and has large numbers of functional groups capable of forming covalent linkages with peptide ligands. Examples of matrix materials and procedures for preparation of matrix-ligand conjugates are described in Dean *et al.* (Eds) AFFINITY CHROMATOGRAPHY: A PRACTICAL APPROACH, IRL Press (1985); Lowe, "*An Introduction to Affinity Chromatography*", in Work *et al.* (eds) LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, Vol. 7, Part II, North-Holland (1979); Porath *et al.*, "*Biospecific Affinity Chromatography*", in Neurath, H. *et*

al. (eds), THE PROTEINS, 3rd ed., Vol. 1, pp. 95-178 (1975); and Schott, H. AFFINITY CHROMATOGRAPHY, Macel Dekker, Inc. NY (1984).

[00176] Also provided herein are conjugates of B7-H3 peptide agonist, antagonist or modulator and any reporter moiety used in the diagnostic procedures discussed herein. The B7-H3 peptide agonist, antagonist or modulator agents, polypeptides and proteins of this invention, including anti-B7-H3 antibodies, are further identified and characterized by any (one or more) of the following criteria:

(a) an ability to specifically bind to B7-H3 (and in particular B7-H3 molecules that are expressed on the surfaces of cancer cells, including but not limited to kidney, prostate, or lung, cancer cells);

(b) an ability to competitively inhibits preferential binding of a known anti-B7-H3 antibody to B7-H3, including the ability to preferentially bind to the same B7-H3 epitope to which the original antibody preferentially binds;

(c) an ability to bind to a portion of B7-H3 that is exposed on the surface of a living cell *in vitro* or *in vivo*;

(d) an ability to bind to a portion of B7-H3 that is exposed on the surface of living cancer cells that express B7-H3;

(e) an ability to deliver a chemotherapeutic agent to cancerous cells (such as kidney, prostate, or lung cancer cells) expressing B7-H3 on their surface; and/or

(f) an ability to deliver a therapeutic agent or detectable marker into cancer cells (such as but not limited to prostate cancer cells) expressing B7-H3 on their surface.

[00177] A preferred antibody of the invention will exhibit differential IHC staining of tumor tissue relative to normal, non-cancerous tissue, and will moreover be capable of testing in primate (and particularly cynomolgus monkey) models of antibody efficacy. Preferred antibodies of the present invention will additionally exhibit desirable levels of affinity and antigen specificity. Preferred antibodies of the present invention will additionally exhibit desirable levels of immunomodulatory activity and cellular internalization.

[00178] In some embodiments, the antibody of the invention is an antibody that is produced by hybridoma BRCA84D, BRCA69D, or PRCA157, or progeny thereof. The present invention also encompasses various formulations of antibodies produced by these deposited hybridomas and equivalent antibodies or polypeptide fragments (e.g., Fab, Fab', F(ab')2 Fv, Fc, etc.), chimeric antibodies, single chain (scFv), mutants thereof, fusion proteins comprising an antibody portion, humanized antibodies, and any other modified configuration of any of these or equivalent antibodies that comprises an antigen (B7-H3), recognition site of the required specificity. The invention also provides human antibodies displaying one or more of the biological characteristics of an anti-B7-H3 family member. The equivalent antibodies of the anti-B7-H3 family (including humanized antibodies

and human antibodies), polypeptide fragments, and polypeptides comprising any of these fragments are identified and characterized by any (one or more) of the five criteria described above. Murine and exemplary humanized variable domain sequences of an anti-B7-H3 antibody are provided in PCT Publication WO 2008/066691. Such sequences are provided by way of illustration not limitation, and different sequences as well as fragments and variants of the provided sequences, are encompassed within the scope of this invention.

[00179] BRCA84D, BRCA69D, and PRCA157 are the preferred B7-H3 antibodies of the present invention due to their cleaner normal tissue IHC profiles, stronger tumor/normal IHC differential, moderate to strong binding (BIACORE™)/IHC), cross-reactivity to B7-H3 of cynomolgus monkeys and potent activity toward universal DART™ molecules ("UDART™s") relative to the other antibodies. In particularly preferred embodiments, the invention encompasses chimeric and humanized variants of these preferred antibodies, as well as native and chimeric and humanized variants of these preferred antibodies that possess modified Fc regions as described below. The invention additionally encompasses DART™ molecules that possess the epitope binding regions of such antibodies, particularly in concert with epitope binding region(s) that bind to the T-cell receptor, NKG2D receptor, or to a tumor-associated antigen or to a hapten such as fluorescein (*e.g.*, fluorescein isothiocyanate (also known as fluoroisothiocyanate or FITC).

[00180] In some embodiments, the antibodies, polypeptides and proteins of the invention that bind to B7-H3 are antibodies, polypeptides and proteins that competitively inhibit preferential binding of a herein-specified anti-B7-H3 antibody to B7-H3. In some embodiments, the antibodies, the polypeptides and the proteins preferentially bind to the same epitope on B7-H3 as the antibody mu-anti-B7-H3 preferentially binds.

[00181] Accordingly, the invention provides any of the following (or compositions, including pharmaceutical compositions, comprising any of the following): (a) an antibody produced by the host cell with a deposit number identified above or its progeny; (b) a humanized form of such an antibody; (c) an antibody comprising one or more of the light chain and /or heavy chain variable regions of such an antibody; (d) a chimeric antibody comprising variable regions homologous or derived from variable regions of a heavy chain and a light chain of such an antibody, and constant regions homologous or derived from constant regions of a heavy chain and a light chain of a human antibody; (e) an antibody comprising one or more of the light chain and /or heavy chain CDRs (at least one, two, three, four, five, or six) of such an antibody; (f) an antibody comprising a heavy and /or a light chain of such an antibody; (g) a human antibody that is equivalent to such an antibody. A humanized form of the antibody may or may not have CDRs identical to that original antibody, or antibody produced by a host cell with a deposit number identified above. Determination of CDR regions is well within the skill of the art. In some embodiments, the invention provides an antibody which

comprises at least one CDR that is substantially homologous to at least one CDR, at least two, at least three, at least four, at least 5 CDRs of an antibody produced by one of the above-identified deposited hybridomas (or, in some embodiments substantially homologous to all 6 CDRs of one of these antibodies, or derived from one of these antibodies), or antibody produced by the host cell with a deposit number identified above. Other embodiments include antibodies that have at least two, three, four, five, or six CDR(s) that are substantially homologous to at least two, three, four, five or six CDRs of an antibody produced from a hybridoma deposited as identified herein, or derived from such an antibody. It is understood that, for purposes of this invention, binding specificity and /or overall activity (which may be in terms of delivering a chemotherapeutic agent to or into cancerous cells to reduce the growth and /or proliferation of cancer cells, to induce apoptotic cell death in the cancer cell, to delay the development of metastasis, and /or treating palliatively) is generally retained, although the extent of activity may vary compared to an antibody produced by a deposited hybridoma (may be greater or lesser). The invention also provides methods of making any of these antibodies. Methods of making antibodies are known in the art and are described herein.

[00182] The invention also provides polypeptides comprising an amino acid sequence of the antibodies of the invention. In some embodiments, the polypeptide comprises one or more of the light chain and /or heavy chain variable regions of the antibody. In some embodiments, the polypeptide comprises one or more of the light chain and /or heavy chain CDRs of the antibody. In some embodiments, the polypeptide comprises three CDRs of the light chain and /or heavy chain of the antibody. In some embodiments, the polypeptide comprises an amino acid sequence of the antibody that has any of the following: at least 5 contiguous amino acids of a sequence of the original antibody, at least 8 contiguous amino acids, at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids, wherein at least 3 of the amino acids are from a variable region of the antibody. In one embodiment, the variable region is from a light chain of the original antibody. In another embodiment, the variable region is from a heavy chain of the antibody. In another embodiment, the 5 (or more) contiguous amino acids are from a complementarity-determining region (CDR) of the antibody.

[00183] In some embodiments of this invention, cells of this invention that express B7-H3, a portion of B7-H3, anti-B7-H3 antibodies or other B7-H3-binding polypeptides of this invention are administered directly to an individual to modulate *in vivo* B7-H3 biological activity.

[00184] The preferred anti-B7-H3 antibodies of the present invention are BRCA84D, BRCA69D and PRCA157, all of which antibodies are murine antibodies reactive toward the human B7-H3 molecule. The amino acid and encoding polynucleotide sequences of the variable light chain and variable heavy chain of BRCA84D, BRCA69D and PRCA157 are shown below along with the

respective CDR_1, CDR_2 and CDR_3 domains of each such chain. Those of skill in the art will therefore be able to construct antibodies having such CDRs, as well as derivatives thereof, capable of binding to the epitopes recognized by BRCA84D, BRCA69D and PRCA157.

A. Sequences of BRCA84D

(1) BRCA84D Light Chain Sequences

[00185] Amino Acid Sequence of BRCA84D Variable Light Chain (**SEQ ID NO:3**):

DIAMTQSQKF MSTSVGDRVS VTCKASQNVD TNVAWYQQKP GQSPKALIYS
ASYRYSGVPD RFTGSGSGTD FTLTINNVQS EDLAEYFCQQ YNNYPFTFGS
GTKLEIK

[00186] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain (**SEQ ID NO:4**):

gacattgcga tgacccagtc tcaaaaattc atgtccacat cagtaggaga cagggtcagc
gtcacctgca aggccagtca gaatgtggat actaatgtag cctggtatca acagaaacca
gggcaatctc ctaaagcact gatttactcg gcatcctacc ggtacagtgg agtccctgat
cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcaacaa tgtgcagtct
gaagacttgg cagagtattt ctgtcagcaa tataacaact atccattcac gttcggctcg
gggacaaagt tggaaataaa a

[00187] BRCA84D Variable Light Chain CDR_1 (**SEQ ID NO:5**): KASQNVDTNVA

[00188] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_1 (**SEQ ID NO:6**): aaggccagtc agaatgtgga tactaatgta gcc

[00189] BRCA84D Variable Light Chain CDR_2 (**SEQ ID NO:7**): SASYRYS

[00190] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_2 (**SEQ ID NO:8**): tcggcatcct accggtacag t

[00191] BRCA84D Variable Light Chain CDR_3 (**SEQ ID NO:9**): QQYNNYPFT

[00192] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_3 (**SEQ ID NO:10**): cagcaatata acaactatcc attcacg

(2) BRCA84D Heavy Chain Sequences

[00193] Amino Acid Sequence of BRCA84D Variable Heavy Chain (**SEQ ID NO:11**):

DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY
ISSDSSAIYY ADTVKGRFTI SRDNPKNTLF LQMTSLRSED TAMYYCGRGR
ENIYYGSRLD YWGQGTTLTV SS

[00194] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain (**SEQ ID NO:12**):

gatgtgcagc tggtggagtc tgggggaggc ttagtgcagc ctggagggtc ccggaaactc
tcctgtgcag cctctggatt cactttcagt agctttggaa tgcactgggt tcgtcaggct
ccagagaagg ggctggagtg ggtcgcatac attagtagtg acagtagtgc catctactat
gcagacacag tgaagggccg attcaccatc tccagagaca atcccaagaa caccctgttc

ctgcaaatga ccagtctaag gtctgaggac acggccatgt attactgtgg aagagggagg gaaaacattt actacggtag taggcttgac tactggggcc aaggcaccac tctcacagtc tcctca

[00195] BRCA84D Variable Heavy Chain CDR_1 (**SEQ ID NO:13**): FGMH

[00196] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_1 (**SEQ ID NO:14**): tttggaatgcac

[00197] BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:15**): YISSDSSAIYYADTVK

[00198] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:16**): tacattagta gtgacagtag tgccatctac tatgcagaca cagtgaag

[00199] BRCA84D Variable Heavy Chain CDR_3 (**SEQ ID NO:17**): GRENIYYGSRLDY

[00200] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_3 (**SEQ ID NO:18**): gggagggaaa acatttacta cggtagtagg cttgactac

B. Sequences of BRCA69D

(1) BRCA69D Light Chain Sequences

[00201] Amino Acid Sequence of BRCA69D Variable Light Chain (**SEQ ID NO:19**):

DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY TSRLHSGVPS RFSGSGSGTD YSLTIDNLEQ EDIATYFCQQ GNTLPPTFGG GTKLEIK

[00202] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain (**SEQ ID NO:20**):

gatatccaga tgacacagac tacatcctcc ctgtctgcct ctctgggaga cagagtcacc atcagttgca gggcaagtca ggacattagt aattatttaa actggtatca gcagaaacca gatggaactg ttaaactcct gatctactac acatcacgat tacactcagg agtcccatca aggttcagtg gcagtgggtc tggaacagat tattctctca ccattgacaa cctggagcaa gaagatattg ccacttactt ttgccaacag ggtaatacgc ttcctccgac gttcggtgga ggcaccaaac tggaaatcaa a

[00203] BRCA69D Variable Light Chain CDR_1 (**SEQ ID NO:21**): RASQDISNYLN

[00204] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_1 (**SEQ ID NO:22**): agggcaagtc aggacattag taattattta aac

[00205] BRCA69D Variable Light Chain CDR_2 (**SEQ ID NO:23**): YTSRLHS

[00206] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_2 (**SEQ ID NO:24**): tacacatcac gattacactc a

[00207] BRCA69D Variable Light Chain CDR_3 (**SEQ ID NO:25**): QQGNTLPPT

[00208] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_3 (**SEQ ID NO:26**): caacagggta atacgcttcc tccgacg

(2) BRCA69D Heavy Chain Sequences

[00209] Amino Acid Sequence of BRCA69D Variable Heavy Chain (**SEQ ID NO:27**):

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

[00210] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain (**SEQ ID NO:28**):

caggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagttg
tcctgcaagg cttctggcta cacctttact agctactgga tgcagtgggt aaaacagagg
cctggacagg gtctggaatg gattgggact atttatcctg gagatggtga tactaggtac
actcagaagt tcaagggcaa ggccacattg actgcagata aatcctccag cacagcctac
atgcaactca gcagcttggc atctgaggac tctgcggtct attactgtgc aagaagaggg
attccacggc tttggtactt cgatgtctgg ggcgcaggga ccacggtcac cgtctcctca

[00211] BRCA69D Variable Heavy Chain CDR_1 (**SEQ ID NO:29**): SYWMQ

[00212] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_1 (**SEQ ID NO:30**): agctactgga tgcag

[00213] BRCA69D Variable Heavy Chain CDR_2 (**SEQ ID NO:31**): TIYPGDGDTR YTQKFKG

[00214] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_2 (**SEQ ID NO:32**): actatttatc ctggagatgg tgatactagg tacactcag aagttcaagg gc

[00215] BRCA69D Variable Heavy Chain CDR_3 (**SEQ ID NO:33**): RGIPRLWYFD V

[00216] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_3 (**SEQ ID NO:34**): agagggattc cacggctttg gtacttcgat gtc

C. Sequences of PRCA157

(1) PRCA157 Light Chain Sequences

[00217] Amino Acid Sequence of PRCA157 Variable Light Chain (**SEQ ID NO:35**):

DIQMTQSPAS LSVSVGETVT ITCRASESIY SYLAWYQQKQ GKSPQLLVYN
TKTLPEGVPS RFSGSGSGTQ FSLKINSLQP EDFGRYYCQH HYGTPPWTFG
GGTNLEIK

[00218] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain (**SEQ ID NO:36**):

gacatccaga tgactcagtc tccagcctcc ctatctgtat ctgtgggaga
aactgtcacc attacatgtc gagcaagtga gagtatttac agttatttag
catggtatca gcagaaacag ggaaaatctc ctcagctcct ggtctataat
acaaaaacct taccagaggg tgtgccatca aggttcagtg gcagtggatc
aggcacacag ttttctctga agatcaacag cctgcagcct gaagattttg

ggagatatta ctgtcaacat cattatggta ctcctccgtg gacgttcggt
ggaggcacca acctggaaat caaa

[00219] PRCA157 Variable Light Chain CDR_1 (**SEQ ID NO:37**): RASESIYSYLA

[00220] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_1 (**SEQ ID NO:38**): cgagcaagtg agagtattta cagttattta gca

[00221] PRCA157 Variable Light Chain CDR_2 (**SEQ ID NO:39**): NTKTLPE

[00222] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_2 (**SEQ ID NO:40**): aatacaaaaa ccttaccaga g

[00223] PRCA157 Variable Light Chain CDR_3 (**SEQ ID NO:41**): QHHYGTPPW

[00224] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_3 (**SEQ ID NO:42**): caacatcatt atggtactcc tccgtgg

(2) PRCA157 Heavy Chain Sequences

[00225] Amino Acid Sequence of PRCA157 Variable Heavy Chain (**SEQ ID NO:43**):

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

[00226] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain (**SEQ ID NO:44**):

gaggtgcagc aggtggagtc ggggggagac ttagtgaagc ctggagggtc
cctgaaactc tcctgtgcag cctctggatt cactttcagt tcctatggca
tgtcttgggt tcgccagact ccagacaaga ggctggagtg ggtcgcaacc
attaatagtg gtggaagtaa cacctactat ccagacagtt tgaaggggcg
attcaccatc tccagagaca atgccaagaa caccctttac ctgcaaatgc
gcagtctgaa gtctgaggac acagccatgt attactgtgc aagacatgac
gggggagcta tggactactg gggtcaagga acctcagtca ccgtctcctc a

[00227] PRCA157 Variable Heavy Chain CDR_1 (**SEQ ID NO:45**): SYGMS

[00228] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_1 (**SEQ ID NO:46**): tcctatggca tgtct

[00229] PRCA157 Variable Heavy Chain CDR_2 (**SEQ ID NO:47**): VATINSGGSN TYYPDSLKG

[00230] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_2 (**SEQ ID NO:48**): gtcgcaacca ttaatagtgg tggaagtaac acctactatc cagacagttt gaagggg

[00231] PRCA157 Variable Heavy Chain CDR_3 (**SEQ ID NO:49**): HDGGAMDY

[00232] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_3 (**SEQ ID NO:50**): `catgacgggg gagctatgga ctac`

D. Fc-Engineered B7-H3 Antibodies

[00233] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody-dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc domain of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. The amino acid sequence of the IgG1 Fc region is shown below (as **SEQ ID NO:51**, numbered according to Kabat *et al.*, SEQUENCE OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NIH, MD (1991), expressly incorporated herein by reference, and hereafter referred to as "Kabat EU"):

SEQ ID NO:51

PAPELLGGPS	VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV
230	240	250	260	270
DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP
280	290	300	310	320
APIEKTISKA	KGQPREPQVY	TLPPSREEMT	KNQVSLTCLV	KGFYPSDIAV
330	340	350	360	370
EWESNGQPEN	NYKTTPPVLD	SDGSFFLYSK	LTVDKSRWQQ	GNVFSCSVMH
380	390	400	410	420
EALHNHYTQK	SLSLSPGK			
430	440			

Residues 230-341 are the Fc CH2 region. Residues 342-447 are the Fc CH3 region.

[00234] The present invention includes antibodies that specifically bind to B7-H3 that comprise a variant Fc region having one or more amino acid modifications (*e.g.*, substitutions, deletions, insertions) in one or more portions, which modifications increase the affinity and avidity of the variant Fc region for an FcγR (including activating and inhibitory FcγRs). In some embodiments, said one or more amino acid modifications increase the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA. In another embodiment, the variant Fc region further specifically binds FcγRIIB with a lower affinity than does the Fc region of the comparable parent antibody (*i.e.*, an antibody having the same amino acid sequence as the antibody of the invention except for the one or more amino acid modifications in the Fc region). In some embodiments, such modifications increase the

affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA and also enhance the affinity of the variant Fc region for FcγRIIB relative to the parent antibody. In other embodiments, said one or more amino acid modifications increase the affinity of the variant Fc region for FcγRIIIA and/or FcγRIIA but do not alter the affinity of the variant Fc regions for FcγRIIB relative to the Fc region of the parent antibody. In another embodiment, said one or more amino acid modifications enhance the affinity of the variant Fc region for FcγRIIIA and FcγRIIA but reduce the affinity for FcγRIIB relative to the parent antibody. Increased affinity and/or avidity results in detectable binding to the FcγR or FcγR-related activity in cells that express low levels of the FcγR when binding activity of the parent molecule (without the modified Fc region) cannot be detected in the cells. In other embodiments, the modified molecule exhibits detectable binding in cells which express non-FcγR receptor target antigens at a density of 30,000 to 20,000 molecules/cell, at a density of 20,000 to 10,000 molecules/cell, at a density of 10,000 to 5,000 molecules/cell, at a density of 5,000 to 1,000 molecules/cell, at a density of 1,000 to 200 molecules/cell or at a density of 200 molecules/cell or less (but at least 10, 50, 100 or 150 molecules/cell).

[00235] In another embodiment, said one or more modifications to the amino acids of the Fc region reduce the affinity and avidity of the antibody for one or more FcγR receptors. In a specific embodiment, the invention encompasses antibodies comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild type Fc region, which variant Fc region only binds one FcγR, wherein said FcγR is FcγRIIIA. In another specific embodiment, the invention encompasses antibodies comprising a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild type Fc region, which variant Fc region only binds one FcγR, wherein said FcγR is FcγRIIA.

[00236] Preferably, the binding properties of the molecules of the invention are characterized by *in vitro* functional assays for determining one or more FcγR mediator effector cell functions (*See* Section 5.2.7). The affinities and binding properties of the molecules, *e.g.*, antibodies, of the invention for an FcγR can be determined using *in vitro* assays (biochemical or immunological based assays) known in the art for determining antibody-antigen or Fc-FcγR interactions, *i.e.*, specific binding of an antigen to an antibody or specific binding of an Fc region to an FcγR, respectively, including but not limited to ELISA assay, surface plasmon resonance assay, immunoprecipitation assays. In most preferred embodiments, the molecules of the invention have similar binding properties in *in vivo* models (such as those described and disclosed herein) as those in *in vitro* based assays. However, the present invention does not exclude molecules of the invention that do not exhibit the desired phenotype in *in vitro* based assays but do exhibit the desired phenotype *in vivo*.

[00237] In some embodiments, the molecules of the invention comprising a variant Fc region comprise at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more

amino acid modifications) in the CH3 domain of the Fc region, which is defined as extending from amino acids 342-447. In other embodiments, the molecules of the invention comprising a variant Fc region comprise at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) in the CH2 domain of the Fc region, which is defined as extending from amino acids 231-341. In some embodiments, the molecules of the invention comprise at least two amino acid modifications (for example, possessing 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications), wherein at least one such modification is in the CH3 region and at least one such modification is in the CH2 region. The invention further encompasses amino acid modification in the hinge region. In a particular embodiment, the invention encompasses amino acid modification in the CH1 domain of the Fc region, which is defined as extending from amino acids 216-230.

[00238] In particularly preferred embodiments, the invention encompasses molecules comprising a variant Fc region wherein said variant confers or has an increased ADCC activity and/or an increased binding to FcγRIIA (CD32A), as measured using methods known to one skilled in the art and exemplified herein. The ADCC assays used in accordance with the methods of the invention may be NK dependent or macrophage dependent.

[00239] In particularly preferred embodiments, the invention encompasses molecules comprising a variant Fc region wherein said variant confers or has an increased ADCC activity and/or an increased binding to FcγRIIIA (CD16A), as measured using methods known to one skilled in the art and exemplified herein. The ADCC assays used in accordance with the methods of the invention may be NK dependent or macrophage dependent.

[00240] The Fc variants of the present invention may be combined with other Fc modifications, such as those disclosed in United States Patents Nos. 7,632,497; 7,521,542; 7,425,619; 7,416,727; 7,371,826; 7,355,008; 7,335,742; 7,332,581; 7,183,387; 7,122,637; and 6,737,056; in PCT Publications Nos. WO 2008/105886; WO 2008/002933; WO 2007/021841; WO 2007/106707; WO 06/088494; WO 05/115452; WO 05/110474; WO 04/1032269; and in WO 04/063351; and in Presta, L.G. *et al.* (2002) "*Engineering therapeutic antibodies for improved function*," Biochem. Soc. Trans. 30(4):487-490; Shields, R.L. *et al.* (2002) "*Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity*," J. Biol. Chem. 26;277(30):26733-26740 and Shields, R.L. *et al.* (2001) "*High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R*," J. Biol. Chem. 276(9):6591-6604). The invention encompasses combining an Fc variant of the invention with other Fc modifications to provide additive, synergistic, or novel properties to the modified antibody. Preferably, the Fc variants of the invention enhance the phenotype of the modification with which they are combined. For example, if an Fc variant of the invention is combined with a mutant known to bind FcγRIIIA with a

higher affinity than a comparable wild type Fc region; the combination with a mutant of the invention results in a greater fold enhancement in FcγRIIIA affinity.

[00241] The invention encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region, wherein the variant Fc region comprises at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule has an enhanced effector function relative to a molecule comprising a wild-type Fc region, provided that the variant Fc region does not have or is not solely a substitution at any one or more of positions 243, 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438, 439. In a specific embodiment, the invention encompasses such antibodies comprising a variant Fc region, wherein the variant Fc region comprises at least one amino acid modification (for example, possessing 1, 2, 3, 4, 5, 6, 7, 8, 9, or more amino acid modifications) relative to a wild-type Fc region, such that the molecule binds an FcγR with an altered affinity relative to a molecule comprising a wild-type Fc region, provided that the variant Fc region does not have or is not solely a substitution at any one or more of positions 243, 255, 258, 267, 269, 270, 276, 278, 280, 283, 285, 289, 292, 293, 294, 295, 296, 300, 303, 305, 307, 309, 320, 322, 329, 332, 331, 337, 338, 340, 373, 376, 416, 419, 434, 435, 437, 438, 439 and does not have an alanine at any of positions 256, 290, 298, 312, 326, 333, 334, 359, 360, or 430; an asparagine at position 268; a glutamine at position 272; a glutamine, serine, or aspartic acid at position 286; a serine at position 290; a methionine at position 301; a methionine, glutamine, glutamic acid, or arginine at position 320; a glutamic acid at position 322; an asparagine, serine, glutamic acid, or aspartic acid at position 326; a lysine at position 330; a glutamine at position 334; a glutamic acid at position 334; a methionine at position 334; a histidine at position 334; a valine at position 334; a leucine at position 334; a glutamine at position 335; a lysine at position 335; or a threonine at position 339.

[00242] The invention also encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region, wherein the variant Fc region comprises such antibodies comprising a variant Fc region, wherein the variant Fc region does not have or is not solely a substitution at any one or more of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 320, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and does not have a histidine, glutamine, or tyrosine at position 280; a serine, glycine, threonine or tyrosine at position 290, an asparagine at position 294, a lysine at position 295; a proline at position 296; a proline, asparagine, aspartic acid, or valine at position 298; or a leucine or isoleucine at position 300. In another embodiment, the invention encompasses such antibodies comprising a variant Fc region, wherein the variant Fc region comprises at least one amino acid modification relative to a wild-type

Fc region, such that the molecule binds an FcγR with a reduced affinity relative to molecule comprising a wild-type Fc region provided that the variant Fc region does not have or is not solely a substitution at any one or more of positions 243, 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, or 439. In yet another embodiment, the invention encompasses such antibodies comprising a variant Fc region, wherein the variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, such that the molecule binds an FcγR with an enhanced affinity relative to a molecule comprising a wild-type Fc region provided that the variant Fc region does not have or is not solely a substitution at any one or more of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398, or 430.

[00243] The invention also encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region, wherein the variant Fc region does not include or are not solely a substitution at any one or more of positions 330, 243, 247, 298, 241, 240, 244, 263, 262, 235, 269, or 328 and does not have a leucine at position 243, an asparagine at position 298, a leucine at position 241, and isoleucine or an alanine at position 240, a histidine at position 244, a valine at position 330, or an isoleucine at position 328.

[00244] The invention particularly encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region with enhanced effector function and/or altered affinities for activating and/or inhibitory receptors, wherein the variant Fc region comprises: (a) any 1, 2, 3, 4, 5, or 6 of the following substitutions: S239D, S298A, A330L, I332E, E333A, or K334A; or (b) any of the combinations of substitutions: (1) S298A, E333A, and K334A; (2) S239D and I332E; or (3) S239D, A330L and I332E.

[00245] The invention particularly encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region with enhanced effector function and/or altered affinities for activating and/or inhibitory receptors, wherein the variant Fc region comprises a substitution:

(1) at position 288 with asparagine, at position 330 with serine and at position 396 with leucine;

(2) at position 334 with glutamic acid, at position 359 with asparagine, and at position 366 with serine;

(3) at position 316 with aspartic acid, at position 378 with valine, and at position 399 with glutamic acid;

(4) at position 247 with leucine, and a substitution at position 421 with lysine;

(5) at position 392 with threonine, and at position 396 with leucine;

(6) at position 221 with glutamic acid, at position 270 with glutamic acid, at position 308 with alanine, at position 311 with histidine, at position 396 with leucine, and at position 402 with aspartic acid;

(7) at position 419 with histidine, and a substitution at position 396 with leucine;

(8) at position 240 with alanine, and at position 396 with leucine;

(9) at position 410 with histidine, and at position 396 with leucine;

(10) at position 243 with leucine, at position 305 with isoleucine, at position 378 with aspartic acid, at position 404 with serine, and at position 396 with leucine;

(11) at position 255 with isoleucine, and at position 396 with leucine;

(12) at position 370 with glutamic acid and at position 396 with leucine;

(13) at position 270 with glutamic acid;

or

(14) any combination of the foregoing (1)-(12) substitutions.

[00246] In a specific embodiment, the invention encompasses an antibody that specifically binds B7-H3 that comprises a variant Fc region which comprises the substitution: F243L, R292P, and Y300L. In a further specific embodiment, the invention encompasses an antibody that specifically binds B7-H3 that comprises a variant Fc region which comprises the substitution: L235V, F243L, R292P, Y300L, and P396L. In yet a further specific embodiment, the invention encompasses an antibody that specifically binds B7-H3 that comprises a variant Fc region which comprises the substitution F243L, R292P, Y300L, V305I, and P396L.

[00247] In a further specific embodiment, the invention encompasses an antibody that specifically binds B7-H3 that comprises a variant Fc region which comprises a substitution at position 396 with leucine, at position 270 with glutamic acid and at position 243 with leucine. In another specific embodiment the molecule further comprises one or more amino acid modification such as those disclosed herein.

[00248] The invention particularly encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region with enhanced effector function and/or altered affinities for activating and/or inhibitory receptors, that have an amino acid modification at one or more of the following positions: 119, 125, 132, 133, 141, 142, 147, 149, 162, 166, 185, 192, 202, 205, 210, 214, 215, 216, 217, 218, 219, 221, 222, 223, 224, 225, 227, 229, 231, 232, 233, 235, 240, 241, 242, 243, 244, 246, 247, 248, 250, 251, 252, 253, 254, 255, 256, 258, 261, 262, 263, 268, 269, 270, 272, 274, 275, 276, 279, 280, 281, 282, 284, 287, 288, 289, 290, 291, 292, 293, 295, 298, 301, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 315, 316, 317, 318, 319, 320, 323, 326, 327, 328, 330, 333, 334, 335, 337, 339, 340, 343, 344, 345, 347, 348, 352, 353, 354, 355, 358, 359, 360, 361, 362, 365, 366, 367, 369, 370, 371, 372, 375, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 392,

393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 404, 406, 407, 408, 409, 410, 411, 412, 414, 415, 416, 417, 419, 420, 421, 422, 423, 424, 427, 428, 431, 433, 435, 436, 438, 440, 441, 442, 443, 446, or 447. Preferably such mutations result in molecules that have been conferred an effector cell mediated function and, optionally, have an altered affinity for an FcγR as determined using methods disclosed and exemplified herein and known to one skilled in the art.

[00249] The invention particularly encompasses antibodies that specifically bind to B7-H3 which comprise a variant Fc region with altered effector function and/or altered affinities for activating and/or inhibitory receptors, that have:

(I) an amino acid modification at one or more of the following positions: 235, 240, 241, 243, 244, 247, 262, 263, 269, 298, 328, or 330 and more preferably one or more of the following modifications: V240A, V240I, F241L, F243L, P244H, S298N, L328I, A330V; wherein such antibodies exhibit altered effector function relative to antibodies having a wild-type Fc region that lacks such modification;

(II) an amino acid modification at one or more of the following positions: 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and more preferably one or more of the following modifications: D280H, D280Q, D280Y, K290G, K290S, K290T, K290Y, E294N, Q295K, Y296P, S298D, S298N, S298P, S298V, Y300I, Y300L; wherein such antibodies exhibit altered effector function relative to antibodies having a wild-type Fc region that lacks such modification;

(III) an amino acid modification at one or more of the following positions: 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438, 439, and more preferably one or more of the following modifications: T256A, H268N, E272Q, N286D, N286Q, N286S, K290A, K290S, S298A, R301M, D312A, K320E, K320M, K320Q, K320R, K322E, K326A, K326D, K326E, K326N, K326S, A330K, A339T, E333A, K334A, K334E, K334H, K334L, K334M, K334Q, K334V, T335K, T335Q, T359A, K360A, E430A; wherein such antibodies exhibit altered effector function relative to antibodies having a wild-type Fc region that lacks such modification;

(IV) an amino acid modification at one or more of the following positions: 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, or 439; wherein such antibodies exhibit reduced effector function relative to antibodies having a wild-type Fc region that lacks such modification;

(V) an amino acid modification at one or more of the following positions: 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398, or 430; wherein such antibodies exhibit enhanced effector function relative to antibodies having a wild-type Fc region that lacks such modification;

or

(VI) an amino acid modification at one or more of the following positions: R255A, T256A, E258A, S267A, H268A, H268N, E272A, E272Q, N276A, D280A, E283A, H285A, N286A, N286D, N286Q, N286S, K290A, K290S, R301M, K320E, K320M, K320Q, K320R, K322E, K326A, K326D, K326E, K326S, A330K, P331A, T335Q, S337A, E430A; wherein such antibodies exhibit enhanced effector function relative to antibodies having a wild-type Fc region that lacks such modification.

[00250] In other embodiments, the invention encompasses the use of any Fc variant known in the art, such as those disclosed in Jefferis, B.J. *et al.* (2002) "*Interaction Sites On Human IgG-Fc For FcgammaR: Current Models*," Immunol. Lett. 82:57-65; Presta, L.G. *et al.* (2002) "*Engineering Therapeutic Antibodies For Improved Function*," Biochem. Soc. Trans. 30:487-90; Idusogie, E.E. *et al.* (2001) "*Engineered Antibodies With Increased Activity To Recruit Complement*," J. Immunol. 166:2571-75; Shields, R.L. *et al.* (2001) "*High Resolution Mapping Of The Binding Site On Human IgG1 For Fc Gamma RI, Fc Gamma RII, Fc Gamma RIII, And FcRn And Design Of IgG1 Variants With Improved Binding To The Fc gamma R*," J. Biol. Chem. 276:6591-6604; Idusogie, E.E. *et al.* (2000) "*Mapping Of The C1q Binding Site On Rituxan, A Chimeric Antibody With A Human IgG Fc*," J. Immunol. 164:4178-84; Reddy, M.P. *et al.* (2000) "*Elimination Of Fc Receptor-Dependent Effector Functions Of A Modified IgG4 Monoclonal Antibody To Human CD4*," J. Immunol. 164:1925-1933; Xu, D. *et al.* (2000) "*In Vitro Characterization of Five Humanized OKT3 Effector Function Variant Antibodies*," Cell. Immunol. 200:16-26; Armour, K.L. *et al.* (1999) "*Recombinant human IgG Molecules Lacking Fcgamma Receptor I Binding And Monocyte Triggering Activities*," Eur. J. Immunol. 29:2613-24; Jefferis, R. *et al.* (1996) "*Modulation Of Fc(Gamma)R And Human Complement Activation By IgG3-Core Oligosaccharide Interactions*," Immunol. Lett. 54:101-04; Lund, J. *et al.* (1996) "*Multiple Interactions Of IgG With Its Core Oligosaccharide Can Modulate Recognition By Complement And Human Fc Gamma Receptor I And Influence The Synthesis Of Its Oligosaccharide Chains*," J. Immunol. 157:4963-4969; Hutchins *et al.* (1995) "*Improved Biodistribution, Tumor Targeting, And Reduced Immunogenicity In Mice With A Gamma 4 Variant Of Campath-1H*," Proc. Natl. Acad. Sci. (U.S.A.) 92:11980-84; Jefferis, R. *et al.* (1995) "*Recognition Sites On Human IgG For Fc Gamma Receptors: The Role Of Glycosylation*," Immunol. Lett. 44:111-17; Lund, J. *et al.* (1995) "*Oligosaccharide-Protein Interactions In IgG Can Modulate Recognition By Fc Gamma Receptors*," FASEB J. 9:115-19; Alegre, M.L. *et al.* (1994) "*A Non-Activating "Humanized" Anti-CD3 Monoclonal Antibody Retains Immunosuppressive Properties In Vivo*,"

Transplantation 57:1537-1543; Lund *et al.* (1992) "*Multiple Binding Sites On The CH2 Domain Of IgG For Mouse Fc Gamma R11*," Mol. Immunol. 29:53-59; Lund *et al.* (1991) "*Human Fc Gamma RI And Fc Gamma RII Interact With Distinct But Overlapping Sites On Human IgG*," J. Immunol. 147:2657-2662; Duncan, A.R. *et al.* (1988) "*Localization Of The Binding Site For The Human High-Affinity Fc Receptor On IgG*," Nature 332:563-564; US Patent Nos. 5,624,821; 5,885,573; 6,194,551; 7,276,586; and 7,317,091; and PCT Publications WO 00/42072 and PCT WO 99/58572.

[00251] The invention encompasses molecules comprising variant Fc regions consisting of or comprising any of the mutations listed in the table below in **Table 1**.

Table 1
Exemplary Fc Modifications

Substitutions of a Single Site							
S132I	F241W	D265N	D280Q	Y296T	D312A	L328I	K334E
A162V	F241Y	D265Q	D280Y	N297D	W313F	L328K	K334H
S219Y	F241Y	D265T	G281D	N297E	N315I	L328M	K334I
K222N	F243D	D265V	G281K	N297I	E318K	L328N	K334L
H224L	F243H	D265Y	G281P	N297S	K320E	L328P	K334M
T225S	F243L	V266A	G281Y	S298A	K320M	L328Q	K334N
P228E	F243L	V266I	V282M	S298D	K320Q	L328R	K334Q
P228G	F243Q	V266M	E283A	S298N	K320R	L328S	K334V
P228K	F243R	V266T	V284E	S298N	K322E	L328T	T335K
P228Y	F243W	S267A	V284L	S298N	V323I	L328V	T335Q
P230A	F243Y	H268A	V284N	S298P	N325A	L328W	I336E
P230E	P244H	H268N	V284T	S298V	N325D	L328Y	I336K
P230G	P245A	D270E	V284Y	T299A	N325E	A330I	I336Y
P230Y	P247G	P271A	H285A	T299D	N325F	A330K	S337A
A231E	P247L	P271D	N286A	T299E	N325G	A330L	A339T
A231G	P247V	P271E	N286D	T299F	N325H	A330S	M352L
A231K	K248M	P271F	N286S	T299G	N325I	A330V	T359A
A231P	R255A	P271G	K288N	T299H	N325K	A330Y	T359N
A231Y	T256A	P271H	K290A	T299I	N325L	P331A	K360A
P232E	E258A	P271I	K290G	T299K	N325M	I332A	T366N
P232G	V262A	P271K	K290S	T299L	N325P	I332D	T366S
P232K	V262E	P271L	K290T	T299M	N325R	I332E	F372Y
P232Y	V262F	P271M	K290Y	T299N	N325S	I332F	F372Y
E233D	V262I	P271N	P291D	T299P	N325T	I332G	I377F
E233G	V262T	P271Q	P291E	T299Q	N325V	I332H	I377N
L234I	V263A	P271R	P291G	T299R	N325W	I332K	V379L
L235D	V263I	P271S	P291H	T299S	N325Y	I332L	V379M
S239D	V263M	P271T	P291I	T299V	K326A	I332M	K392R
S239E	V263T	P271V	P291Q	T299W	K326D	I332N	P396H
S239N	V264A	P271W	P291T	T299Y	K326E	I332P	P396L
S239Q	V264E	P271Y	R292G	Y300I	K326E	I332Q	L398V
V240A	V264F	E272A	R292L	Y300L	K326N	I332R	S400P
V240I	V264I	E272Q	E294N	R301M	K326S	I332S	D401V
V240M	V264R	V273I	Q295K	R301M	K326T	I332T	S407I
V240T	V264T	F275L	Y296D	V302I	L328A	I332V	K414N
F241E	V264W	F275W	Y296E	S304D	L328D	I332W	E430A
V241I	D265F	F275Y	Y296H	S304H	L328E	I332Y	

Table 1
Exemplary Fc Modifications

F241L	D265H	N276A	Y296N	S304L	L328F	E333A	
F241R	D265I	D280A	Y296P	S304N	L328G	K334A	
F241S	D265L	D280H	Y296Q	S304T	L328H	K334E	

Substitutions of Two Sites

I332E, A330L	S239N / I332Q	V279L, P395S	P396L, P217S
I332E, L328D	S239Q / I332D	V284A, F372L	P396L, P227S
I332E, L328E	S239Q / I332E	K288N, K326N	P396L, V323I
I332E, L328H	S239Q / I332N	K288N, A330S	P396L, V240A
I332E, L328I	S239Q / I332Q	K290E, L142P	P396L, L242F
I332E, L328M	V240I, V281M	K290E, P227S	P396L, P244H
I332E, L328N	F241L, E258G	K290T, G371D	P396L, T250A
I332E, L328Q	F241L / V262I	P291S, P353Q	P396L, R255L
I332E, L328T	F243L, E318K	R292P, V305I	P396L, E258D
I332E, L328V	F243I, V379L	S298A / I332E	P396L, H268D
I332E, N297D	P243L / V264I	S298N, W381R	P396L, H268N
I332E, N297E	K246T, Y319F	S298N, S407R	P396L, V303I
I332E, N297S	K246T, P396H	K317N, F423-DEL	P396L, K326I
S166N, K409R	P247H, G285E	K326E, K320E	P396L, V305L
P232S, S304G	P247L, I377F	K326E, A330T	P396L, L358P
S239D / I332D	P247L, E389G	K326E, G385E	P396L, K370E
S239D / I332E	P247S, P396L	A330V, Q419H	P396L, S375C
S239D / I332N	P247L, L398Q	K334E, E233D	P396L, V379M
S239D / I332Q	P247L, L406F	K334N, K246I	P396L, N384K
S239E / D265N	P247L, N421K	K334E, K288M	P396L, K392T
S239E / D265Q	L251F, F372L	K334E, R292L	P396L, S400F
S239E / I332D	L251F, S415I	K334E, E308D	P396L, L410H
S239E / I332E	R255L, E318K	K334E, E380D	P396L, Q419H
S239E / I332N	R255Q, K326E	K334N, P396L	P396L, Q419L
S239E / I332Q	E258D, N384K	A339V, Q347H	P396L, V427A
S239N / I332D	V263Q, E272D	K370N, S440N	D399E, G402D
S239N / I332E	V264I / I332E	T394M, V397M	D399E, M428L
S239N / I332N	H268D, E318D	P396L, K210M	

Substitutions of Three Sites

V185M, R292L, D399E	P217S, A378V, S408R	K218R, G281D, G385R
S192T, M252L, R301C	P247L, I253N, K334N	P247L, A330T, S440G
V125L, V215I, S408I	D312E, K327N, I378S	T355N, P387S, H435Q
R292L, T359N, P396L	E216D, E345K, S375I	P247L, A431V, S442F
F275I, K334N, V348M	K288N, A330S, P396L	A378V, N390I, V422I
F243L, R255L, E318K	G316D, A378V, D399E	V282E, V369I, L406F
K334E, T359N, T366S	N315I, V379M, T394M	V397M, T411A, S415N
K288N, A330S, P396L	P247L,W313R,E388G	T223I, T256S, L406F
F243I, V379L, G420V	R301H, K340E,D399E	K246N, P396L, Q419R
A231V, Q386H, V412M	K326I, P396L, S408N	P217A, T359A, P396L
E216D,K334R,S375I	K210M, K261N, P396L	V215I, K290V, P396L
T335N, P387S, H435Q	A330V, G427M, K438R	V263Q, E272D, Q419H
K246I, Q362H, K370E	K222E, V263Q, S298N	N276Y, T393N, W417R
K334E, E380D, G446V	E233G, P247S, L306P	D270E, G316D, R416G
V303I, V369F, M428L	S219T, T225K, D270E	D270E, K392T, P396L
K246E, V284M, V308A	R292P, F243L, V305I	R255L, D270E, P396L
E293V, Q295E, A327T	V284M, R292L, K370N	V240A, D270E, P396L
Y319F, P352L ,P396L	D270E, K370E, P396L	270E, P396L, Q419HD

Table 1 Exemplary Fc Modifications		
K290T, N390I, P396L	P247L, D270E, N421K	S239D, A330L, I332E
N297D, A330Y, I332E	Y296D, N297D, I332E	S239D, A330Y, I332E
N297D, T299L, I332E	Y296E, N297D, I332 E	S239D, I332E, A330I
N297D, T299I, I332E	Y296H, N297D, I332E	S239D, N297D, I332E
N297D, T299L, I332E	Y296N, N297D, I332E	S239D, S298A, I332E
N297D, T299V, I332E	Y296Q, N297I, I332E	S239D, V2641I, I332E
F243L, V262I, V264W	Y296T, N297D, I332E	S239E, N297D, I332E
D265F, N297E, I332E	P230A, E233D, I332E	S239E, V264I, I332 E
D265Y, N297D, I332E	P244H, P245A, P247V	S239N, A330L, I332E
V264E, N297D, I332E	V264I, A330Y, I332E	S239N, A330Y, I332E
V264I, A330L, I332E	V264I, S298A, I332E	S239Q, V264I, I332E
Substitutions of Four Sites		
A141V, H268L, K288E, P291S	T256S, V305I, K334E, N390S	
E258D, T289A, H310Y, Y407V	D280E, S354F, A431D, L441I	
K334E, T359N, T366S, Q386R	P343S, P353L, S375I, S383N	
K326Q, K334E, T359N, T366S	E269K ,K290N, Q311R, H433Y	
K288R, T307A, K344E, P396L	K290E, V369A, T393A, P396L	
V273I, K326E, L328I, P396L	K210N, K222I, K320M, P396L	
F275L, Q362H, N384K, P396L	S219T, T225K, D270E, K360R	
V282L, A330V, H433Y, T436R	P243L, S254T, A330V, N361D	
R255L, D270E,Y300L, P396L,	F243L, D270E, K392N, P396L	
R255L, D270E, R292G, P396L	F243L, R255L, D270E, P396L	
V284M, S298N, K334E, R355W	S239D, D265F, N297D, I332E	
D265Y, N297D, T299L, I332E	S239D, D265H, N297D, I332E	
F241E, F2430, V262T, V264F	S239D, D265I, N297D, I332E	
F241E, F243R, V262E, V264R	S239D, 0265L, N297D, I332E	
F241E, F243Y, V262T, V264R	S239D, D265T, N297D, I332E	
F241L, F243L, V262I, V264I	S239D, D265V, N297D, I332E	
F241R, F2430, V262T, V264R	S239D, D265Y, N297D, I332E	
F241W, F243W, V262A, V264A	S239D, N297D, I332E, A330Y	
F241Y, F243Y, V262T, V264T	S239D, N297D, I332E, K326E	
N297D, I332E, S239D, A330L	S239D, N297D, I332E, L235D	
N297D, S298A, A330Y, I 332E	S239D, V264I, A330L, I332E	
S239D, A330Y, I332E, K326E	S239D, V264I, S298A, I332E	
S239D, A330Y, I332E, K326T	S239E, V264I, A330Y, I332 E	
S239D, A330Y, I332E, L234I	S239D, A330Y, I332E, V264T	
S239D, A330Y, I332E, L235D	S239D, A330Y, I332E, V266I	
S239D, A330Y, I332E, V240I		
Substitutions of Five Sites		
V284M, S298N, K334E, R355W, R416T	K147T, Y202M, F275I, K334N, V348M	
P217S, V305I, I309L, N390H, P396L	T335N, K370E, A378V, T394M, S424L	
F243L, V305I, A378D, P396L, F404S	P244H, L358M, V379M, N384K, V397M	
K222N, T335N, K370E, A378V, T394M	P244A, K326I, C367R, S375I, K447T	
L235P, S304G, V305I, V323I, V382M	C229Y, A287T, V379M, P396L, L443V	
F241E, F2430, V262T, V264E, I332E	F241R, F243Q, V262T, V264R, I332E	
F241E, F243R, V262E, V264R, I332E	S239E, V264I, S298A, A330Y, I332E	
F241E, F243Y, V262T, V264R, I332E		
Substitutions of More Than Five Sites		
D221E, D270E, V308A, Q311H, P396L, G402D		
T215P, K274N, A287G, K334N, L365V, P396L		
F241Y, F243Y, V262T, V264T, N297D, I332E		

Table 1 Exemplary Fc Modifications
N297D, T299F, I332E, N297D, T299H, I332E
D221Y, M252I, A330G, A339T, T359N, V422I, H433L
S239D, N297D, I332E, A330Y, F241S, F243H, V262T, V264T
K133M, F149Y, K205E, R214I, K218E, S383N, N384K, T256N, V262L

[00252] In specific embodiments, the variant Fc region of such antiB7-H3 antibodies has:

(1) a leucine at position 247, a lysine at position 421 and a glutamic acid at position 270;

(2) a threonine at position 392, a leucine at position 396, a glutamic acid at position 270, and a leucine at position 243

(3) a histidine at position 419, a leucine at position 396, and a glutamic acid at position 270;

(4) a histidine at position 419, a leucine at position 396, a glutamic acid at position 270, and a leucine at position 243;

(5) an alanine at position 240, a leucine at position 396, and a glutamic acid at position 270;

(6) a lysine at position 255 and a leucine at position 396;

(7) a lysine at position 255, a leucine at position 396, and a glutamic acid at position 270;

(8) a lysine at position 255, a leucine at position 396, a glutamic acid at position 270, and a lysine at position 300;

(9) a lysine at position 255, a leucine at position 396, a glutamic acid at position 270, and a glycine at position 292;

(10) a lysine at position 255, a leucine at position 396, a glutamic acid at position 270, and a leucine at position 243;

(11) a glutamic acid at position 370, a leucine at position 396, and a glutamic acid at position 270;

(12) a glutamic acid at position 270, an aspartic acid at position 316, and a glycine at position 416;

(13) a leucine at position 243, a proline at position 292, an isoleucine at position 305, and a leucine at position 396;

(14) a leucine at position 243, a glutamic acid at position 270, an asparagine at position 392 and a leucine at position 396;

(15) a leucine at position 243, a leucine at position 255, a glutamic acid at position 270 and a leucine at position 396;

(16) a glutamine at position 297;

or

(17) any combination of the foregoing (1)-(16) substitutions.

[00253] In some embodiments, the molecules of the invention further comprise one or more glycosylation sites, so that one or more carbohydrate moieties are covalently attached to the molecule. Preferably, the molecules of the invention with one or more glycosylation sites and/or one or more modifications in the Fc region confer or have an enhanced antibody mediated effector function, *e.g.*,

enhanced ADCC activity, compared to a parent antibody. In some embodiments, the invention further comprises molecules comprising one or more modifications of amino acids that are directly or indirectly known to interact with a carbohydrate moiety of the antibody, including but not limited to amino acids at positions 241, 243, 244, 245, 245, 249, 256, 258, 260, 262, 264, 265, 296, 299, and 301. Amino acids that directly or indirectly interact with a carbohydrate moiety of an antibody are known in the art, *see, e.g.*, Jefferis *et al.*, 1995 *Immunology Letters*, 44: 111-7, which is incorporated herein by reference in its entirety.

[00254] In another embodiment, the invention encompasses molecules that have been modified by introducing one or more glycosylation sites into one or more sites of the molecules, preferably without altering the functionality of the molecules, *e.g.*, binding activity to target antigen or FcγR. Glycosylation sites may be introduced into the variable and/or constant region of the molecules of the invention. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains are typically linked to the backbone of an antibody via either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. The molecules of the invention may comprise one or more glycosylation sites, including N-linked and O-linked glycosylation sites. Any glycosylation site for N-linked or O-linked glycosylation known in the art may be used in accordance with the instant invention. An exemplary N-linked glycosylation site that is useful in accordance with the methods of the present invention is the amino acid sequence: Asn-X-Thr/Ser, wherein X may be any amino acid and Thr/Ser indicates a threonine or a serine. Such a site or sites may be introduced into a molecule of the invention using methods well known in the art to which this invention pertains (see for example, *IN VITRO* MUTAGENESIS, RECOMBINANT DNA: A SHORT COURSE, J. D. Watson, *et al.* W.H. Freeman and Company, New York, 1983, chapter 8, pp. 106-116, which is incorporated herein by reference in its entirety. An exemplary method for introducing a glycosylation site into a molecule of the invention may comprise: modifying or mutating an amino acid sequence of the molecule so that the desired Asn-X-Thr/Ser sequence is obtained.

[00255] In some embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies are well known in the art and encompassed within the invention, *see, e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In other

embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by deleting one or more endogenous carbohydrate moieties of the molecule. In a specific embodiment, the invention encompasses shifting the glycosylation site of the Fc region of an antibody, by modifying positions adjacent to 297. In a specific embodiment, the invention encompasses modifying position 296 so that position 296 and not position 297 is glycosylated.

[00256] Effector function can also be modified by techniques such as by introducing one or more cysteine residues into the Fc region, thereby allowing interchain disulfide bond formation in this region to occur, resulting in the generation of a homodimeric antibody that may have improved internalization capability and/or increased complement-mediated cell killing and ADCC (Caron, P.C. *et al.* (1992) "*Engineered Humanized Dimeric Forms Of IgG Are More Effective Antibodies*," J. Exp. Med. 176:1191-1195; Shopes, B. (1992) "*A Genetically Engineered Human IgG Mutant With Enhanced Cytolytic Activity*," J. Immunol. 148(9):2918-2922. Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff, E.A. *et al.* (1993) "*Monoclonal Antibody Homodimers: Enhanced Antitumor Activity In Nude Mice*," Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities (Stevenson, G.T. *et al.* (1989) "*A Chimeric Antibody With Dual Fc Regions (bisFabFc) Prepared By Manipulations At The IgG Hinge*," Anti-Cancer Drug Design 3:219-230).

E. B7-H3 DART™ (Dual Affinity Retargeting Reagents)

[00257] As discussed above, the present invention additionally encompasses "DART™" (dual affinity retargeting reagent) molecules that comprise at least two polypeptide chains which form at least two epitope binding sites, at least one of which specifically binds to B7-H3.

[00258] In preferred embodiments, the first polypeptide chain of the DART™ comprises:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope (1);

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope (2); and

(iii) a domain (C).

The second polypeptide chain of such a DART™ comprises:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for epitope (2);

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for epitope (1); and

(iii) a domain (F).

The DART™ domains (A) and (B) do not associate with one another to form an epitope binding site. Similarly, the DART™ domains (D) and (E) do not associate with one another to form an epitope binding site. Rather, DART™ domains (A) and (E) associate to form a binding site that binds epitope (1); said DART™ domains (B) and (D) associate to form a binding site that binds said epitope (2). Domains (C) and (F) are covalently associated together.

[00259] Each polypeptide chain of the DART™ molecule comprises a VL domain and a VH domain, which are covalently linked such that the domains are constrained from self assembly. Interaction of two of the polypeptide chains will produce two VL-VH pairings, forming two eptipoe binding sites, *i.e.*, a bivalent molecule. Neither the VH or VL domain is constrained to any position within the polypeptide chain, *i.e.,* restricted to the amino (N) or carboxy (C) teminus, nor are the domains restricted in their relative positions to one another, *i.e.,* the VL domain may be N-terminal to the VH domain and vice-versa. The only restriction is that a complimentary polypeptide chain be available in order to form functional DART™s. Where the VL and VH domains are derived from the same antibody, the two complimentary polypeptide chains may be identical. For example, where the binding domains are derived from an antibody specific for epitope A (i.e., the binding domain is formed from a VL_A-VH_A interaction), each polypeptide will comprise a VH_A and a VL_A. Homodimerization of two polypeptide chains of the antibody will result in the formation two VL_A-VH_A binding sites, resulting in a bivalent monospecific antibody. Where the VL and VH domains are derived from antibodies specific for different antigens, formation of a functional bispecific DART™ requires the interaction of two different polypeptide chains, *i.e.*, formation of a heterodimer. For example, for a bispecific DART™, one polypeptide chain will comprise a VL_A and a VL_B; homodimerization of said chain will result in the formation of two VL_A-VH_B binding sites, either of no binding or of unpredictable binding. In contrast, where two differing polypeptide chains are free to interact, *e.g.,* in a recombinant expression system, one comprising a VL_A and a VH_B and the other comprising a VL_B and a VH_A, two differing binding sites will form: VL_A-VH_A and VL_B-VH_B. For all DART™ polypeptide chain pairs, the possibly of misalignment or mis-binding of the two chains is a possibility, *i.e.,* interaction of VL-VL or VH-VH domains; however, purification of functional diabodies is easily managed based on the immunospecificity of the properly dimerized binding site using any affinity based method known in the art or exemplified herein, *e.g.*, affinity chromatography.

[00260] One or more of the polypeptide chains of the DART™ may optionally comprise an Fc domain domain or portion thereof (*e.g.* a CH2 domain, or CH3 domain). The Fc domain or portion thereof may be derived from any immunoglobulin isotype or allotype including, but not limited to, IgA, IgD, IgG, IgE and IgM. In preferred embodiments, the Fc domain (or portion thereof) is derived from IgG. In specific embodiments, the IgG isotype is IgG1, IgG2, IgG3 or IgG4 or an allotype thereof. In one embodiment, the diabody molecule comprises an Fc domain, which Fc domain

comprises a CH2 domain and CH3 domain independently selected from any immunoglobulin isotype (i.e. an Fc domain comprising the CH2 domain derived from IgG and the CH3 domain derived from IgE, or the CH2 domain derived from IgG1 and the CH3 domain derived from IgG2, etc.). The Fc domain may be engineered into a polypeptide chain comprising the diabody molecule of the invention in any position relative to other domains or portions of said polypeptide chain (*e.g.*, the Fc domain, or portion thereof, may be c-terminal to both the VL and VH domains of the polypeptide of the chain; may be n-terminal to both the VL and VH domains; or may be N-terminal to one domain and c-terminal to another (*i.e.*, between two domains of the polypeptide chain)).

[00261] The Fc domains in the polypeptide chains of the DART™ molecules preferentially dimerize, resulting in the formation of a DART™ molecule that exhibits immunoglobulin-like properties, *e.g.*, Fc-FcγR, interactions. Fc comprising diabodies may be dimers, *e.g.*, comprised of two polypeptide chains, each comprising a VH domain, a VL domain and an Fc domain. Dimerization of said polypeptide chains results in a bivalent DART™ comprising an Fc domain, albeit with a structure distinct from that of an unmodified bivalent antibody. Such DART™ molecules will exhibit altered phenotypes relative to a wild-type immunoglobulin, *e.g.*, altered serum half-life, binding properties, etc. In other embodiments, DART™ molecules comprising Fc domains may be tetramers. Such tetramers comprise two 'heavier' polypeptide chains, *i.e.*, a polypeptide chain comprising a VL, a VH and an Fc domain, and two 'lighter' polypeptide chains, *i.e.*, polypeptide chain comprising a VL and a VH. The lighter and heavier chains interact to form a monomer, and said monomers interact via their unpaired Fc domains to form an Ig-like molecule. Such an Ig-like DART™ is tetravalent and may be monospecific, bispecific or tetraspecific.

[00262] Formation of a tetraspecific diabody molecule as described *supra* requires the interaction of four differing polypeptide chains. Such interactions are difficult to achieve with efficiency within a single cell recombinant production system, due to the many variants of potential chain mispairings. One solution to increase the probability of mispairings, is to engineer "knobs-into-holes" type mutations into the desired polypeptide chain pairs. Such mutations favor heterodimerization over homodimerization. For example, with respect to Fc-Fc-interactions, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a 'knob', *e.g.*, tryptophan) can be introduced into the CH2 or CH3 domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, 'the hole' (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the 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 is hereby incorporated herein by reference in its entirety.

[00263] The invention also encompasses diabody molecules comprising variant Fc or variant hinge-Fc domains (or portion thereof), which variant Fc domain comprises at least one amino acid modification (*e.g.* substitution, insertion deletion) relative to a comparable wild-type Fc domain or hinge-Fc domain (or portion thereof). Molecules comprising variant Fc domains or hinge-Fc domains (or portion thereof) (*e.g.*, antibodies) normally have altered phenotypes relative to molecules comprising wild-type Fc domains or hinge-Fc domains or portions thereof. The variant phenotype may be expressed as altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function as assayed in an NK dependent or macrophage dependent assay. Fc domain modifications identified as altering effector function are disclosed above.

[00264] The present invention also encompasses molecules comprising a hinge domain. The hinge domain be derived from any immunoglobulin isotype or allotype including IgA, IgD, IgG, IgE and IgM. In preferred embodiments, the hinge domain is derived from IgG, wherein the IgG isotype is IgG1, IgG2, IgG3 or IgG4, or an allotpye thereof. Said hinge domain may be engineered into a polypeptide chain comprising the diabody molecule together with an Fc domain such that the diabody molecule comprises a hinge-Fc domain. In certain embodiments, the hinge and Fc domain are independently selected from any immunoglobulin isotype known in the art or exemplified herein. In other embodiments the hinge and Fc domain are separated by at least one other domain of the polypeptide chain, *e.g.*, the VL domain. The hinge domain, or optionally the hinge-Fc domain, may be engineered in to a polypeptide of the invention in any position relative to other domains or portions of said polypeptide chain. In certain embodiments, a polypeptide chain of the invention comprises a hinge domain, which hinge domain is at the C-terminus of the polypeptide chain, wherein said polypeptide chain does not comprise an Fc domain. In yet other embodiments, a polypeptide chain of the invention comprises a hinge-Fc domain, which hinge-Fc domain is at the C-terminus of the polypeptide chain. In further embodiments, a polypeptide chain of the invention comprises a hinge-Fc domain, which hinge-Fc domain is at the N-terminus of the polypeptide chain.

[00265] Each domain of the polypeptide chain of the DART™, *i.e.*, the VL, VH and Fc domain may be separated by a peptide linker. The peptide linker may be 0, 1, 2, 3, 4, 5, 6, 7, 8, or 9 amino acids in length. In certain embodiments the amino acid linker sequence is GGGSGGGG (**SEQ ID NO:52**)

encoded by the nucleic acid sequence ggaggcggat ccggaggcgg aggc (**SEQ ID NO:53**). The polypeptide chains of the DART™ molecule may be engineered to comprise at least one cysteine residue that will interact with a counterpart cysteine residue on a second polypeptide chain of the DART™ to form an inter-chain disulfide bond. Such interchain disulfide bonds serve to stabilize the DART™ molecule, thereby improving expression and recovery in recombinant systems, resulting in a stable and consistent formulation and improving the stability of the isolated and/or purified product *in vivo*. The cysteine residue may be introduced as a single amino acid or as part of larger amino-acid sequence, *e.g.* a hinge domain, in any portion of the polypeptide chain. In a specific embodiment, the cysteine residue may be engineered to occur at the C-terminus of the polypeptide chain. In some embodiments, the cysteine residue is introduced into the polypeptide chain within the amino acid sequence LGGC. In a specific embodiment, the C-terminus of the polypeptide chains comprising the DART™ molecule of the invention comprises the amino acid sequence LGGC (**SEQ ID NO:54**). In another embodiment, the cysteine residue is introduced into the polypeptide within an amino acid sequence comprising a hinge domain, *e.g.* EPKSCDKTHTCPP (**SEQ ID NO:55**) or ESKYGPPCPS (**SEQ ID NO:56**). In a specific embodiment, the C-terminus of a polypeptide chain of the DART™ molecule of the invention comprises the amino acid sequence of an IgG hinge domain, *e.g.* **SEQ ID NO:55** or **SEQ ID NO:56**. In another embodiment, the C-terminus of a polypeptide chain of a DART™ molecule of the invention comprises the amino acid sequence VEPKSC (**SEQ ID NO:57**), which can be encoded by nucleotide sequence gttgagccca aatcttgt (**SEQ ID NO:58**). In other embodiments, the cysteine residue in introduced into the polypeptide chain within the amino acid sequence LGGCFNRGEC **(SEQ ID NO:59**), which can be encoded by the nucleotide sequence ctgggaggct gcttcaacag gggagagtgt **(SEQ ID NO:60)**. In a specific embodiment, the C-terminus of a polypeptide chain comprising the DART™ of the invention comprises the amino acid sequence LGGCFNRGEC **(SEQ ID NO:59**). In yet other embodiments, the cysteine residue in introduced into the polypeptide chain within the amino acid sequence FNRGEC **(SEQ ID NO:61)**, which can be encoded by the nucleotide sequence ttcaacaggg gagagtgt **(SEQ ID NO:62)**. In a specific embodiment, the C-terminus of a polypeptide chain comprising the DART™ of the invention comprises the amino acid sequence FNRGEC **(SEQ ID NO:61)**.

[00266] In certain embodiments, the diabody molecule comprises at least two polypeptide chains, each of which comprise the amino acid sequence LGGC (**SEQ ID NO:54**) and are covalently linked by a disulfide bond between the cysteine residues in the LGGC (**SEQ ID NO:54**) sequences. In another specific embodiment, the diabody molecule comprises at least two polypeptide chains, one of which comprises the sequence FNRGEC (**SEQ ID NO:61**) while the other comprises a hinge domain (containing at least one cysteine residue), wherein said at least two polypeptide chains are covalently linked by a disulfide bond between the cysteine residue in FNRGEC (**SEQ ID NO:61**) and a cysteine

residue in the hinge domain. In particular aspects, the cysteine residue responsible for the disulfide bond located in the hinge domain is Cys-128 (as numbered according to Kabat EU; located in the hinge domain of an unmodified, intact IgG heavy chain) and the counterpart cysteine residue in SEQ ID NO:23 is Cys-214 (as numbered according to Kabat EU; located at the C-terminus of an unmodified, intact IgG light chain) (Elkabetz *et al.* (2005) "*Cysteines In CH1 Underlie Retention Of Unassembled Ig Heavy Chains*," J. Biol. Chem. 280:14402-14412). In yet other embodiments, the at least one cysteine residue is engineered to occur at the N-terminus of the amino acid chain. In still other embodiments, the at least one cysteine residue is engineered to occur in the linker portion of the polypeptide chain of the diabody molecule. In further embodiments, the VH or VL domain is engineered to comprise at least one amino acid modification relative to the parental VH or VL domain such that said amino acid modification comprises a substitution of a parental amino acid with cysteine.

[00267] In still another aspect of this embodiment, the Domain (C) of the first polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:57)**, derived from the hinge domain of a human IgG, and which can be encoded by the nucleotide sequence gttgagccca aatcttgt **(SEQ ID NO:58)**. In another aspect of this embodiment, the Domain (F) of the second polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:57)**. In certain aspects of this embodiment, Domain (C) of the first polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC **(SEQ ID NO:61)**; and Domain (F) of the second polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:57)** or a hinge domain. In other aspects of this embodiment, Domain (F) of the second polypeptide chain comprises the C-terminal 6 amino acids of the human kappa light chain, FNRGEC **(SEQ ID NO:61)**; and Domain (C) of the first polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:57)** or a hinge domain.

[00268] As will be appreciated in view of the foregoing, the individual polypeptides of a bispecific DART™ can form two species of homodimers and one species of heterodimer. In one embodiment of the present invention, a charged polypeptide can be added to the C-terminus of one, or more preferably, both DART™ polypeptides. By selecting charged polypeptides of opposite charge for the individual polypeptides of the bispecific DART™, the inclusion of such charged polypeptides favors formation of heterodimers and lessens formation of homodimers. Preferably, a positively charged polypeptide will contain a substantial content of arginine, glutamine, histidine and/or lysine (or mixtures of such amino acids) and a negatively charged polypeptide will contain a substantial content of aspartate or glutamate (or a mixture of such amino acids). Positively charged polypeptides containing a substantial content of lysine and negatively charged polypeptides containing a substantial content of glutamate are particularly preferred. In order to maximize the electrostatic attraction

between such opposingly charged polypeptides, it is preferred to employ polypeptides capable of spontaneously assuming a helical conformation.

[00269] Thus, in a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form a bispecific DART™ and a negatively charged "K-coil" will be appended to the second of the DART™'s polypeptides. A particularly preferred E-coil will have the sequence: $(EVAALEK)_4$ [*i.e.* **(SEQ ID NO:63)** EVAALEKEVAALEKEVAALEKEVAALEK]. A particularly preferred K-coil will have the sequence: $(KVAALKE)_4$ [*i.e.* **(SEQ ID NO:64)** KVAALKEKVAALKEKVAALKEKVAALKE].

[00270] A preferred DART™ polypeptide possessing such an E-coil will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(EVAALEK)_4]$—GGGNS, where VL is the DART™'s variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:52**, VH is the DART™'s variable heavy Ig domain, $(EVAALEK)_4$ is **SEQ ID NO:63**, and GGGNS is **SEQ ID NO:65**. A preferred DART™ polypeptide possessing such a K-coil will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(KVAALKE)_4]$—GGGNS, where VL is the DART™'s variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:52**, VH is the DART™'s variable heavy Ig domain, $(KVAALKE)_4$ is **SEQ ID NO:64**, and GGGNS is **SEQ ID NO:65**.

[00271] In a further embodiment, Fc-regions can be linked to the E and/or K coils of E-coil or K-coli DART™s. Furthering the separation between the Fc regions and the DART™ VH domain of an Fc-containing DART™ is desirable in cases in which a less separated arrangement of such domains results in diminished interaction between such domains and their binding ligands or otherwise interferes with DART™ assembly. Although separators of any amino acid sequence may be employed, it is preferable to employ separators that form an α helix coils, so as to maximally extend and project the Fc domain away from the variable domains. Because the above-described coiled polypeptides of opposing charge additionally finction to promote heterodimer formation, such molecules are particularly preferred separators. Such coil-containing Fc-DART™ molecules provide benefits similar to those of Fc-DART™s, including improved serum half-life and effector function recruitment. The above-described E-coil and K-coil polypeptides are particularly preferred for this purpose. Thus, in a preferred embodiment, the E-coil Fc-containing DART™ will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(EVAALEK)_4]$—**GGG**—Fc domain starting with D234 (Kabat numbering), where VL is the DART™'s variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:52**, VH is the DART™'s variable heavy Ig domain and $(EVAALEK)_4$ is **SEQ ID NO:63**. Similarly, in a preferred embodiment, the K-coil Fc-containing DART™ will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(KVAALKE)_4]$—**GGG**—Fc domain starting with D234 (Kabat numbering), where VL is the DART™'s variable light Ig domain,

GGGSGGGG is **SEQ ID NO:51**, VH is the DART™'s variable heavy Ig domain and $(KVAALKE)_4$ is **SEQ ID NO:64**.

[00272] As indicated above, a coil-containing DART™ molecule or a coil-containing Fc-containing DART™ molecule may contain only a single such coil separator, or it may contain more than one such separators (*e.g.*, two separators, preferably of opposite charge, of which one is linked to each of the VH domain of the DART™'s polypeptides). By linking the Fc region to such separator molecule(s), the ability to make bivalent, tetravalent, etc. versions of the Fc-DART™ molecules by chain swapping is enhanced. Fc-DART™ molecules can thus be produced that form monomers or dimers depending upon whether the Fc domain is linked to one or both of the DART™ VH domains

1. Versatility of B7-H3 DART™ Molecules

[00273] The bispecific DART™s of the invention can simultaneously bind two separate and distinct epitopes. In certain embodiments the epitopes are from the same antigen. In other embodiments, the epitopes are from different antigens. In preferred embodiments, at least one epitope binding site is specific for a determinant expressed on an immune effector cell (*e.g.* CD3, CD16, CD32, CD64, T-cell receptor, etc.) which are expressed on T lymphocytes, natural killer (NK) cells or other mononuclear cells. In one embodiment, the DART™ molecule binds to the effector cell determinant and also activates said effector cell. In this regard, DART™ molecules of the invention may exhibit Ig-like functionality independent of whether they further comprise an Fc domain (*e.g.*, as assayed in any effector function assay known in the art or exemplified herein (*e.g.*, ADCC assay). In certain embodiments the bispecific DART™ of the invention binds both a cancer antigen on a tumor cell and an effector cell determinant while activating said cell. In alternative embodiments, the bispecific DART™ or DART™ molecule of the invention may inhibit activation of a target, *e.g.*, effector, cell by simultaneously binding, and thus linking, an activating and inhibitory receptor on the same cell (*e.g.*, bind both CD32A and CD32B, BCR and CD32B, or IgERI and CD32B) as described *supra* (*see,* Background Section). In a further aspect of this embodiment, the bispecific DART™ may exhibit anti-viral properties by simultaneously binding two neutralizing epitopes on a virus (*e.g.,* RSV epitopes; WNV epitopes such as E16 and E53).

2. Universal B7-H3 DART™ Molecules

[00274] In one embodiment, the bispecific DART™ molecules of the invention may be constructed to comprise one epitope binding domain that specifically binds to B7-H3 and a second epitope binding domain that specifically binds a hapten, *e.g.* fluorescein isothiocyanate (also known as fluoroisothiocyanate or FITC). Such a DART™ serves as a universal adaptor ("UDART™"), able to co-ligate B7-H3 with molecules that interact with fluorescein-conjugated binding partners. For example, the FITC-reactive arm of the DART™ may be used to bind to an FITC labeled antibody that

is bound to a non-B7-H3 target involved in intercellular clustering, intercellular recruitment, cell-free recruitment, multiple targets, etc. The chimeric mouse Fv/human Fc version of the anti-fluorescein MAb, 4420 may be employed as a source of FITC-specific CDR domains (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).

3. Cell-Target Specific B7-H3 DART™ Molecules

[00275] The bispecific DART™ molecules of the invention offer unique opportunities to target specific cell types. For example, the bispecific DART™ or DART™ molecule can be engineered to comprise a combination of epitope binding sites that recognize a set of antigens unique to a target cell or tissue type. Additionally, where either or both of the individual antigens is/are fairly common separately in other tissue and/or cell types, low affinity biding domains can be used to construct the DART™ or DART™ molecule. Such low affinity binding domains will be unable to bind to the individual epitope or antigen with sufficient avidity for therapeutic purposes. However, where both epitopes or antigens are present on a single target cell or tissue, the avidity of the DART™ or DART™ molecule for the cell or tissue, relative to a cell or tissue expressing only one of the antigens, will be increased such that said cell or tissue can be effectively targeted by the invention. Such a bispecific molecule can exhibit enhanced binding to one or both of its target antigens on cells expressing both of said antigens relative to a monospecific DART™ or an antibody with a specificity to only one of the antigens.

[00276] For Example, the B7-H3 specific DART™s of the present invention may be constructed to comprise a domain that is a binding ligand for the Natural Killer Group 2D (NKG2D) receptor. The NKG2D receptor is expressed on all human (and other mammalian) Natural Killer cells (Bauer, S. *et al.* (1999) "*Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA*," Science 285(5428):727-729; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29) as well as on all $CD8^+$ T cells (Groh, V. *et al.* (2001) "*Costimulation Of CD8αβ T Cells By NKG2D Via Engagement By MIC Induced On Virus-Infected Cells*," Nat. Immunol. 2(3):255-260; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29). Such binding ligands, and particularly those which are not expressed on normal cells, include the histocompatibility 60 (H60) molecule, the product of the retinoic acid early inducible gene-1 (RAE-1), and the murine UL16-binding proteinlike transcript 1 (MULT1) (Raulet D.H. (2003) "*Roles Of The NKG2D Immunoreceptor And Its Ligands*," Nature Rev. Immunol. 3:781-790; Coudert, J.D. *et al.* (2005) "*Altered NKG2D Function In NK Cells Induced By Chronic Exposure To Altered NKG2D Ligand-Expressing Tumor Cells*," Blood 106:1711-1717). Additional ligands reactive with human NKG2D include the polymorphic MHC class I chain-related molecules MICA and MICB

(Diefenbach, A. *et al.* (1999) "*Natural Killer Cells: Stress Out, Turn On, Tune In*," Curr. Biol. 9(22):R851-R8533; Bauer, S. *et al.* (1999) "*Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA*," Science 285(5428):727-729; Stephens, H.A. (2001) "*MICA And MICB Genes: Can The Enigma Of Their Polymorphism Be Resolved?*" Trends Immunol. 22:378-385. The sequence of MICA is **SEQ ID NO:66**:

MGLGPVFLLL AGIFPFAPPG AAAEPHSLRY NLTVLSWDGS VQSGFLTEVH
LDGQPFLRCD RQKCRAKPQG QWAEDVLGNK TWDRETRDLT GNGKDLRMTL
AHIKDQKEGL HSLQEIRVCE IHEDNSTRSS QHFYYDGELF LSQNLETKEW
TMPQSSRAQT LAMNVRNFLK EDAMKTKTHY HAMHADCLQE LRRYLKSGVV
LRRTVPPMVN VTRSEASEGN ITVTCRASGF YPWNITLSWR QDGVSLSHDT
QQWGDVLPDG NGTYQTWVAT RICQGEEQRF TCYMEHSGNH STHPVPSGKV
LVLQSHWQTF HVSAVAAAAI FVIIIFYVRC CKKKTSAAEG PELVSLQVLD
QHPVGTSDHR DATQLGFQPL MSDLGSTGST EGA

The sequence of MICB is **SEQ ID NO:67**:

PHSLRYNLMV LSQDGSVQSG FLAEGHLDGQ PFLRYDRQKR RAKPQGQWAE
DVLGAKTWDT ETEDLTENGQ DLRRTLTHIK DQKGGLHSLQ EIRVCEIHED
SSTRGSRHFY YDGELFLSQN LETQESTVPQ SSRAQTLAMN VTNFWKEDAM
KTKTHYRAMQ ADCLQKLQLP PMVNVICSEV SEGNITVTCR ASSFYPRNIT
LTWRQDGVSL SHNTQQWGDV LPDGNGTYQT WVATRIRQGE EQRFTCYMEH
SGNHGTHPVP SGKALVLQSQ RTDFPYVSAA MPCFVIIIIL CVPCCKKKTS
AAEGP

[00277] Alternatively, the DART™ molecules of the present invention may be constructed to comprise a domain that is a binding ligand for the T-cell receptor ("TCR") or for CD3 (the T-cell co-receptor). The TCR is natively expressed by CD4+ or CD8+ T-cells, and permits such cells to recognize antigenic peptides that are bound and presented by class I or class II MHC proteins of antigen-presenting cells. Recognition of a pMHC (peptide–MHC) complex by a TCR initiates the propagation of a cellular immune response that leads to the production of cytokines and the lysis of the antigen-presenting cell (see, *e.g.*, Armstrong, K.M. *et al.* (2008) "*Conformational Changes And Flexibility In T-Cell Receptor Recognition Of Peptide–MHC Complexes*," Biochem. J. 415(Pt 2):183–196; Willemsen, R. (2008) "*Selection Of Human Antibody Fragments Directed Against Tumor T-Cell Epitopes For Adoptive T-Cell Therapy*," Cytometry A. 73(11):1093-1099; Beier, K.C. *et al.* (2007) "*Master Switches Of T-Cell Activation And Differentiation*," Eur. Respir. J. 29:804-812; Mallone, R. *et al.* (2005) "*Targeting T Lymphocytes For Immune Monitoring And Intervention In Autoimmune Diabetes*," Am. J. Ther. 12(6):534–550). CD3 is the receptor that binds to the TCR (Thomas, S. *et al.* (2010) "*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*," Immunology 129(2):170-177; Guy, C.S. *et al.* (2009) "*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex*," Immunol. Rev. 232(1):7-21; St. Clair, E.W. (Epub 2009 Oct 12) "*Novel Targeted Therapies For Autoimmunity*," Curr. Opin. Immunol. 21(6):648-657; Baeuerle, P.A. *et al.* (Epub 2009 Jun 9) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res.

69(12):4941-4944; Smith-Garvin, J.E. *et al.* (2009) "*T Cell Activation*," Annu. Rev. Immunol. 27:591-619; Renders, L. *et al.* (2003) "*Engineered CD3 Antibodies For Immunosuppression*," Clin. Exp. Immunol. 133(3):307-309).

[00278] By constructing such DART™ molecules to additionally comprise at least one epitope-binding domain capable of binding to, for example, a receptor present on the surface of a target cell, such DART™ molecules will be DART™ molecules and thus be capable of binding to the target cells and thereby cause the target cells to display the binding ligand for the Natural Killer Group 2D (NKG2D) receptor or to the TCR (whichever is present on the target cell-bound DART™) (see, *e.g.*, Germain, C. *et al.* (2008) "*Redirecting NK Cells Mediated Tumor Cell Lysis By A New Recombinant Bifunctional Protein*," Prot. Engineer. Design Selection 21(11):665-672). Such DART™s can be used to redirect any desired target cell into a cell that is a target of NK cell-mediated cell lysis or T-cell mediated cytotoxicity. In one embodiment, the epitope-binding domain of the DART™ capable of binding to a receptor present on the surface of a target cell is an epitope that binds to a tumor-associated antigen so as to redirect such cancer cells into substrates for NK cell-mediated cell lysis or T-cell mediated cytotoxicity. Of particular interest is a tumor-associated antigens that is a breast cancer antigen, an ovarian cancer antigen, a prostate cancer antigen, a cervical cancer antigen, a pancreatic carcinoma antigen, a lung cancer antigen, a bladder cancer antigen, a colon cancer antigen, a testicular cancer antigen, a glioblastoma cancer antigen, an antigen associated with a B cell malignancy, an antigen associated with multiple myeloma, an antigen associated with non-Hodgkins lymphoma, or an antigen associated with chronic lymphocytic leukemia.

[00279] Suitable tumor-associated antigens for such use 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-α receptor, TNF-ß receptor; or TNF-γ receptor; van Horssen, R. *et al.* 2006 *Oncologist.* 11(4):397-408; Gardnerova, M. *et al.* 2000 *Curr Drug Targets.* 1(4):327-64); or VEGF receptor (O'Dwyer. P.J. 2006 *Oncologist.* 11(9):992-8).

[00280] Additional tumor-associated antigens for such use (and publications disclosing specifically reactive antibodies for such antigens) include 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).

[00281] Also of interest are antigens specific to particular infectious agents, e.g., viral agents including, but not limited to human immunodeficiency virus (HIV), hepatitis B virus (HBV),

influenza, human papilloma virus (HPV), foot and mouth (coxsackieviruses), the rabies virus, herpes simplex virus (HSV), and the causative agents of gastroenteritis, including rotaviruses, adenoviruses, caliciviruses, astroviruses and Norwalk virus; bacterial agents including, but not limited to E. coli, Salmonella thyphimurium, Pseudomonas aeruginosa, Vibrio cholerae, Neisseria gonorrhoeae, Helicobacter pylori, Hemophilus influenzae, Shigella dysenteriae, Staphylococcus aureus, Mycobacterium tuberculosis and Streptococcus pneumoniae, fungal agents and parasites such as Giardi.

[00282] In some embodiments, molecules of the invention are engineered to comprise an altered glycosylation pattern or an altered glycoform relative to the comparable portion of the template molecule. Engineered glycoforms may be useful for a variety of purposes, including, but not limited to, enhancing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example, DI N-acetylglucosaminyltransferase III (GnTI11), by expressing a DART™ of the invention in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the DART™ has been expressed and purified. Methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Umana *et al.* (1999) *"Engineered Glycoforms Of An Antineuroblastoma IgG1 With Optimized Antibody-Dependent Cellular Cytotoxic Activity,"* Nat. Biotechnol 17:176-180; Davies *et al.* (2001) *"Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In Adcc Through Higher Affinity For Fc Gamma RIII,"* Biotechnol Bioeng 74:288-294; Shields *et al.* (2002) *"Lack Of Fucose On Human IgG1 N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity,"* J Biol Chem 277:26733-26740; Shinkawa *et al.* (2003) *"The Absence Of Fucose But Not The Presence Of Galactose Or Bisecting N-Acetylglucosamine Of Human IgG1 Complex-Type Oligosaccharides Shows The Critical Role Of Enhancing Antibody-Dependent Cellular Cytotoxicity,"* J Biol Chem 278:3466-3473) US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/292246A1; PCT WO 02/311140A1; PCT WO 02/30954A1; Potillegent™ technology (Biowa, Inc. Princeton, NJ); GlycoMAb™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); each of which is incorporated herein by reference in its entirety. See, *e.g.*, WO 00061739; EA01229125; US 20030115614; Okazaki *et al.* (2004) *"Fucose Depletion From Human IgG1 Oligosaccharide Enhances Binding Enthalpy And Association Rate Between IgG1 And FcGammaRIIIA,"* JMB, 336: 1239-49 each of which is incorporated herein by reference in its entirety.

[00283] The invention further encompasses incorporation of unnatural amino acids to generate the DART™s of the invention. Such methods are known to those skilled in the art such as those using the

natural biosynthetic machinery to allow incorporation of unnatural amino acids into proteins, see, *e.g.*, Wang *et al.* (2002) *"Expanding The Genetic Code,"* Chem. Comm. 1: 1-11; Wang *et al.* (2001) *"Expanding The Genetic Code Of Escherichia coli,"* Science, 292: 498-500; van Hest *et al.* (2001) *"Protein-Based Materials, Toward A New Level Of Structural Control,"* Chem. Comm. 19: 1897-1904, each of which is incorporated herein by reference in its entirety. Alternative strategies focus on the enzymes responsible for the biosynthesis of amino acyl-tRNA, see, *e.g.*, Tang *et al.* (2001) *"Biosynthesis Of A Highly Stable Coiled-Coil Protein Containing Hexafluoroleucine In An Engineered Bacterial Host,"* J. Am. Chem. Soc. 123(44): 11089-11090; Kiick *et al.* (2001) *"Identification Of An Expanded Set Of Translationally Active Methionine Analogues In Escherichia coli,"* FEBS Lett. 502(1-2):25-30; each of which is incorporated herein by reference in its entirety. In some embodiments, the invention encompasses methods of modifying a VL, VH or Fc domain of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate of proteins are well known in the art and encompassed within the invention, *see*, *e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety.

VIII. Methods Of Using B7-H3 Modulators And Anti-B7-H3 Antibodies For Therapeutic Purposes

[00284] Monoclonal antibodies to B7-H3 may be used for therapeutic purposes in individuals with cancer or other diseases. Therapy with anti-B7-H3 antibodies can involve formation of complexes both *in vitro* and *in vivo* as described above. In one embodiment, monoclonal antibody anti-B7-H3 can bind to and reduce the proliferation of cancerous cells. It is understood that the antibody is administered at a concentration that promotes binding at physiological (e.g., *in vivo*) conditions. In another embodiment, monoclonal antibodies to B7-H3 can be used for immunotherapy directed at cancerous cells of different tissues such as colon, lung, breast, prostate, ovary, pancreas, kidney and other types of cancer such as sarcoma. In another embodiment, monoclonal antibody anti-B7-H3 alone can bind to and reduce cell division in the cancer cell. In another embodiment, monoclonal antibody anti-B7-H3 can bind to cancerous cells and delay the development of metastasis. In yet another embodiment, an individual with cancer is given palliative treatment with anti-B7-H3 antibody. Palliative treatment of a cancer individual involves treating or lessening the adverse symptoms of the disease, or iatrogenic symptoms resulting from other treatments given for the disease without directly affecting the cancer progression. This includes treatments for easing of pain, nutritional support, sexual problems, psychological distress, depression, fatigue, psychiatric disorders, nausea, vomiting, etc.

[00285] In such situations, the anti-B7-H3 antibody may be administered with agents that enhance or direct an individual's own immune response, such as an agent that strengthens ADCC.

[00286] In yet another embodiment, anti-B7-H3 antibody be conjugated to or associated with a radioactive molecule, toxin (e.g., calicheamicin), chemotherapeutic molecule, liposomes or other vesicles containing chemotherapeutic compounds and administered to an individual in need of such treatment to target these compounds to the cancer cell containing the antigen recognized by the antibody and thus eliminate cancerous or diseased cells. Without being limited to any particular theory, the anti-B7-H3 antibody is internalized by the cell bearing B7-H3 at its surface, thus delivering the conjugated moiety to the cell to induce the therapeutic effect. In yet another embodiment, the antibody can be employed as adjuvant therapy at the time of the surgical removal of a cancer expressing the antigen in order to delay the development of metastasis. The antibody can also be administered before surgery (neoadjuvant therapy) in an individual with a tumor expressing the antigen in order to decrease the size of the tumor and thus enable or simplify surgery, spare tissue during surgery, and /or decrease the resulting disfigurement.

[00287] Cell cycle dosing is contemplated in the practice of this invention. In such embodiments, a chemotherapeutic agent is used to synchronize the cell cycle of the tumor or other target diseased cells at a pre-determined stage. Subsequently, administration of the anti-B7-H3 antibody of this invention (alone or with an additional therapeutic moiety) is made. In alternative embodiments, an anti-B7-H3 antibody is used to synchronize the cell cycle and reduce cell division prior to administration of a second round of treatment; the second round may be administration of an anti-B7-H3 antibody and/ or an additional therapeutic moiety.

[00288] Chemotherapeutic agents include radioactive molecules, toxins, also referred to as cytotoxins or cytotoxic agents, which includes any agent that is detrimental to the viability of cancerous cells, agents, and liposomes or other vesicles containing chemotherapeutic compounds. Examples of suitable chemotherapeutic agents include but are not limited to 1-dehydrotestosterone, 5-fluorouracil decarbazine, 6-mercaptopurine, 6-thioguanine, actinomycin D, adriamycin, aldesleukin, alkylating agents, allopurinol sodium, altretamine, amifostine, anastrozole, anthramycin (AMC)), anti-mitotic agents, cis-dichlorodiamine platinum (II) (DDP) cisplatin), diaminodichloroplatinum, anthracyclines, antibiotics, antimetabolites, asparaginase, BCG live (intravesical), betamethasone sodium phosphate and betamethasone acetate, bicalutamide, bleomycin sulfate, busulfan, calcium leucouorin, calicheamicin, capecitabine, carboplatin, lomustine (CCNU), carmustine (BSNU), Chlorambucil, Cisplatin, Cladribine, Colchicin, conjugated estrogens, Cyclophosphamide, Cyclothosphamide, Cytarabine, Cytarabine, cytochalasin B, Cytoxan, Dacarbazine, Dactinomycin, dactinomycin (formerly actinomycin), daunirubicin HCL, daunorucbicin citrate, denileukin diftitox, Dexrazoxane, Dibromomannitol, dihydroxyanthracindione, Docetaxel, dolasetronmesylate,

doxorubicin HCL, dronabinol, *E. coli* L-asparaginase, emetine, epoetin alpha, Erwinia L-asparaginase, esterified estrogens, estradiol, estramustine phosphate sodium, ethidium bromide, ethinyl estradiol, etidronate, etoposide citrororum factor, etoposide phosphate, filgrastim, floxuridine, fluconazole, fludarabine phosphate, fluorouracil, flutamide, folinic acid, gemcitabine HCL, glucocorticoids, goserelin acetate, gramicidin D, granisetron HCL, hydroxyurea, idarubicin HCL, ifosfamide, interferon alpha-2b, irinotecan HCL, letrozole, leucovorin calcium, leuprolide acetate, levamisole HCL, lidocaine, lomustine, maytansinoid, mechlorethamine HCL, medroxyprogesterone acetate, megestrol acetate, melphalan HCL, mercaptipurine, mesna, methotrexate, methyltestosterone, mithramycin, mitomycin C, mitotane, mitoxantrone, nilutamide, octreotide acetate, ondansetron HCL, paclitaxel, pamidronate disodium, pentostatin, pilocarpine HCL, plimycin, polifeprosan 20 (with carmustine implant), porfimer sodium, procaine, procarbazine HCL, propranolol, rituximab, sargramostim, streptozotocin, tamoxifen, taxol, teniposide, tenoposide, testolactone, tetracaine, thioepa chlorambucil, thioguanine, thiotepa, topotecan HCL, toremifene citrate, trastuzumab, tretinoin, valrubicin, vinblastine sulfate, vincristine sulfate, and vinorelbine tartrate.

[00289] In a preferred embodiment, the cytotoxin is especially effective in dividing or rapidly dividing cells, such that non-dividing cells are relatively spared from the toxic effects.

[00290] The antibodies of the invention can be internalized within the diseased or carcinoma cells to which they bind and are therefore particularly useful for therapeutic applications, for example, delivering into the cells toxins that need to be internalized for their adverse activity. Examples of such toxins include, but are not limited to, saporin, calicheamicin, auristatin, and maytansinoid.

[00291] The antibodies or polypeptides of the invention can be associated (including conjugated or linked) to a radioactive molecule, a toxin, or other therapeutic agents, or to liposomes or other vesicles containing therapeutic agents covalently or non- covalently, directly or indirectly. The antibody may be linked to the radioactive molecule, the toxin, or the chemotherapeutic molecule at any location along the antibody so long as the antibody is able to bind its target B7-H3.

[00292] A toxin or a chemotherapeutic agent may be administered concurrently with (before, after, or during administration), or coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group, or, alternatively, via a linking molecule with appropriate attachment sites, such as a platform molecule as described in U.S. Patent No. 5,552,391). The toxin and chemotherapeutic agent of the present invention can be coupled directly to the particular targeting proteins using methods known in the art. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be

capable of reacting with a carbonyl- containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

[00293] The antibodies or polypeptides can also be linked to a chemotherapeutic agent via a microcarrier. The term "microcarrier" refers to a biodegradable or a non-biodegradable particle which is insoluble in water and which has a size of less than about 150 μm, 120 μm or 100 μm in size, more commonly less than about 50-60 μm, preferably less than about 10, 5, 2.5, 2 or 1.5 μm. Microcarriers include "nanocarriers", which are microcarriers have a size of less than about 1 μm, preferably less than about 500 nm. Such particles are known in the art. Solid phase microcarriers may be particles formed from biocompatible naturally occurring polymers, synthetic polymers or synthetic copolymers, which may include or exclude microcarriers formed from agarose or cross-linked agarose, as well as other biodegradable materials known in the art. Biodegradable solid phase microcarriers may be formed from polymers which are degradable (*e.g.*, poly(lactic acid), poly(glycolic acid) and copolymers thereof) or erodible (*e.g.*, poly(orthoesters), such as 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane (DETOSU) or poly(anhydrides), such as poly(anhydrides) of sebacic acid) under mammalian physiological conditions. Microcarriers may also be liquid phase (e.g., oil or lipid based), such as liposomes, iscoms (immune-stimulating complexes, which are stable complexes of cholesterol, and phospholipid, adjuvant-active saponin) without antigen, or droplets or micelles found in oil-in-water or water-in-oil emulsions, provided the liquid phase microcarriers are biodegradable. Biodegradable liquid phase microcarriers typically incorporate a biodegradable oil, a number of which are known in the art, including squalene and vegetable oils. Microcarriers are typically spherical in shape, but microcarriers that deviate from spherical shape are also acceptable (*e.g.*, ellipsoid, rod- shaped, *etc.*). Due to their insoluble nature (with respect to water), microcarriers are filterable from water and water-based (aqueous) solutions.

[00294] The antibody or polypeptide conjugates of the present invention may include a bifunctional linker that contains both a group capable of coupling to a toxic agent or chemotherapeutic agent and a group capable of coupling to the antibody. A linker can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker can be cleavable or non-cleavable. A linker can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible. The bifunctional linker can be coupled to the antibody by means that are known in the art. For example, a linker containing an active ester moiety, such as an N- hydroxysuccinimide ester, can be used for coupling to lysine residues in the antibody via an amide linkage. In another example, a linker containing a nucleophilic amine or hydrazine residue can be coupled to aldehyde groups produced by glycolytic oxidation of antibody carbohydrate residues. In addition to these direct methods of

coupling, the linker can be indirectly coupled to the antibody by means of an intermediate carrier such as an aminodextran. In these embodiments the modified linkage is via either lysine, carbohydrate, or an intermediate carrier. In one embodiment, the linker is coupled site- selectively to free thiol residues in the protein. Moieties that are suitable for selective coupling to thiol groups on proteins are well known in the art. Examples include disulfide compounds, α-halocarbonyl and α-halocarboxyl compounds, and maleimides. When a nucleophilic amine function is present in the same molecule as an α-halo carbonyl or carboxyl group the potential exists for cyclization to occur via intramolecular alkylation of the amine. Methods to prevent this problem are well known to one of ordinary skill in the art, for example by preparation of molecules in which the amine and α-halo functions are separated by inflexible groups, such as aryl groups or trans-alkenes, that make the undesired cyclization stereochemically disfavored. See, for example, U.S. Patent No. 6,441,163 for preparation of conjugates of maytansinoids and antibody via a disulfide moiety.

[00295] One of the cleavable linkers that can be used for the preparation of antibody-drug conjugates is an acid-labile linker based on cis-aconitic acid that takes advantage of the acidic environment of different intracellular compartments such as the endosomes encountered during receptor mediated endocytosis and the lysosomes. See, for example, Shen, W.C. *et al.* (1981) ("*cis-Acontityl Spacer Between Daunomycin And Macromolecular Carriers: A Model Of pH-Sensitive Linkage Releasing Drug From A Lysosomotropic Conjugate*," Biochem. Biophys. Res. Comtnun. 102:1048-1054 (1981)) for the preparation of conjugates of daunorubicin with macromolecular carriers; Yang *et al.* (1988) ("*Pharmacokinetics And Mechanism Of Action Of A Doxorubicin-Monoclonal Antibody 9.2.27 Conjugate Directed To A Human Melanoma Proteoglycan*," J. Natl. Canc. Inst. 80:1154-1159) for the preparation of conjugates of daunorubicin to an anti-melanoma antibody; Dillman *et al.* (1988) ("*Superiority Of An Acid-Labile Daunorubicin-Monoclonal Antibody Immunoconjugate Compared To Free Drug*," Cancer Res. 48:6097-6102) for using an acid-labile linker in a similar fashion to prepare conjugates of daunorubicin with an anti- T cell antibody; and Trouet *et al.* (1982) "*A Covalent Linkage Between Daunorubicin And Proteins That Is Stable In Serum And Reversible By Lysosomal Hydrolases, As Required For A Lysosomotropic Drug-Carrier Conjugate: In Vitro And In Vivo Studies*," Proc. Natl. Acad. Sci. (U.S.A.) 79:626-629) for linking daunorubicin to an antibody via a peptide spacer arm.

[00296] An antibody (or polypeptide) of this invention may be conjugated (linked) to a radioactive molecule or toxin by any method known to the art. For a discussion of methods for radiolabeling antibody (see, CANCER THERAPY WITH MONOCLONAL ANTIBODIES, D.M. Goldenberg (Ed.) CRC Press, Boca Raton, 1995). Suitable toxins include taxanes, maytansinoids, auristatins (e.g., monomethyl auristatin (MMAE), monomethyl auristatin F (MMAF), auristatin E (AE), *etc.*) (such as those disclosed in United States Patent No. 5,208,020; 5,416,064; 6,333,410; 6,340,701; 6,372,738;

6,436,931; 6,441,163; 6,596,757; 7,276,497; 7,585,857; or 7,851,432), calicheamicin, anthracyclines (*e.g.*, doxorubicin), CC-1065 analog, docetaxel,; cathepsin B or E; ricin, gelonin, *Pseudomonas* exotoxin, diphtheria toxin, and RNase; tiuxetan or toxic radioisotope (such as ^{90}Y; ^{131}I, ^{177}Lu, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi, ^{225}Ac, *etc.*).

[00297] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in U.S. Patent No. 4,676,980. The formation of cross-linked antibodies can target the immune system to specific types of cells, for example, cancer or diseased cells expressing B7-H3.

[00298] This invention also provides methods of delaying development of metastasis in an individual with cancer (including, but not limited to, prostate, lung, or kidney cancer) using an anti-B7-H3 antibody or other embodiments that bind to B7-H3 in combination with a chemotherapeutic agent, or linked to a chemotherapeutic agent. In some embodiments, the antibody is a humanized or chimeric form of a non-human anti-B7-H3 antibody.

[00299] In yet another embodiment, the antibody can be employed as adjuvant therapy at the time of the surgical removal of a cancer expressing the antigen in order to delay the development of metastasis. The antibody or antibody associated with a chemotherapeutic agent can also be administered before surgery (neoadjuvant therapy) in an individual with a tumor expressing the antigen in order to decrease the size of the tumor and thus enable or simplify surgery, spare tissue during surgery, and /or decrease the resulting disfigurement.

[00300] In yet another embodiment, any of the B7-H3 binding compositions described herein can bind to B7-H3-expressing cancerous cells and induce an active immune response against the cancerous cells expressing B7-H3. In some cases, the active immune response can cause the death of the cancerous cells (*e.g.*, antibody binding to cancer cells inducing apoptotic cell death), or inhibit the growth (*e.g.*, block cells cycle progression) of the cancerous cells. In other cases, any of the novel antibodies described herein can bind to cancerous cells and antibody dependent cellular cytotoxicity (ADCC) can eliminate cancerous cells to which anti-B7-H3 binds. Accordingly, the invention provides methods of stimulating an immune response comprising administering any of the compositions described herein.

[00301] In some cases, antibody binding can also activate both cellular and humoral immune responses and recruit more natural killer cells or increased production of cytokines (*e.g.*, IL-2, IFN-gamma, IL-12, TNF-alpha, TNF-beta, etc.) that further activate an individual's immune system to destroy cancerous cells. In yet another embodiment, anti-B7-H3 antibodies can bind to cancerous cells, and macrophages or other phagocytic cell can opsonize the cancerous cells.

[00302] Various formulations of anti-B7-H3 antibodies or fragments thereof may be used for administration. In some embodiments, anti-B7-H3 antibodies or fragments thereof may be administered neat. In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well known in the art and are relatively inert substances that facilitate administration of a pharmacologically effective substance or which facilitate processing of the active compounds into preparations that can be used pharmaceutically for delivery to the site of action. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers.

[00303] Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate for oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and /or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

[00304] The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21st Edition, Lippincott Williams & Wilkins Publishing (2005). Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof. Generally, these agents are formulated for administration by injection {e.g., intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.), although other forms of administration (e.g., oral, mucosal, etc) can be also used. Accordingly, anti-B7-H3 antibodies are preferably combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like.

[00305] The particular dosage regimen, i.e.., dose, timing and repetition, will depend on the particular individual and that individual's medical history. Generally, a dose of at least about 100 μg/kg body weight, more preferably at least about 250 μg/kg body weight, even more preferably at least about 750 μg/kg body weight, even more preferably at least about 3 mg/kg body weight, even

more preferably at least about 5 mg/kg body weight, even more preferably at least about 10 mg/kg body weight is administered.

[00306] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Antibodies, which are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is based on reducing the number of cancerous cells, maintaining the reduction of cancerous cells, reducing the proliferation of cancerous cells, or delaying the development of metastasis. Alternatively, sustained continuous release formulations of anti-B7-H3 antibodies may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[00307] In one embodiment, dosages for anti-B7-H3 antibodies may be determined empirically in individuals who have been given one or more administration(s). Individuals are given incremental dosages of an anti-B7-H3 antibody. To assess efficacy of anti-B7-H3 antibodies, a marker of the specific cancer disease state can be followed. These include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (e.g., PSA for prostate cancer), a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival. It will be apparent to one of skill in the art that the dosage will vary depending on the individual, the type of cancer, the stage of cancer, whether the cancer has begun to metastasize to other location in the individual, and the past and concurrent treatments being used.

[00308] Other formulations include suitable delivery forms known in the art including, but not limited to, carriers such as liposomes. See, for example, Mahato *et al.* (1997) "*Cationic Lipid-Based Gene Delivery Systems: Pharmaceutical Perspectives*," Pharm. Res. 14:853-859. Liposomal preparations include, but are not limited to, cytofectins, multilamellar vesicles and unilamellar vesicles.

[00309] In some embodiments, more than one antibody may be present. The antibodies can be monoclonal or polyclonal. Such compositions may contain at least one, at least two, at least three, at least four, at least five different antibodies that are reactive against carcinomas, adenocarcinomas, sarcomas, or adenosarcomas. Anti-B7-H3 antibody can be admixed with one or more antibodies reactive against carcinomas, adenocarcinomas, sarcomas, or adenosarcomas in organs including but not limited to ovary, breast, lung, prostate, colon, kidney, skin, thyroid, bone, upper digestive tract,

and pancreas. In one embodiment, a mixture of different anti-B7-H3 antibodies is used. A mixture of antibodies, as they are often denoted in the art, may be particularly useful in treating a broader range of population of individuals.

[00310] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

Example 1
Immunohistocompatability Investigations

[00311] A panel of 49 mAbs was generated from tumor cell / fetal progenitor cell immunizations. The antibodies were evaluated for their ability to exhibit differential IHC staining of tumor tissue relative to normal, non-cancerous tissue, capability of use in primate (and particularly cynomolgus monkey) models of antibody efficacy, levels of affinity and antigen specificity and levels of immunomodulatory activity and cellular internalization. 21 of the mAbs were initially identified by MS analysis and/or binding to B7-H3-CHO cells. The remaining 28 mAbs were identified by rescreening the library by ELISA with B7-H3 protein. Characteristics of 46 of the 49 members of the panel are provided in **Table 2**.

Table 2

Name	Isotype	IHC	ATCC Array	Internali-zation	U-DART™	BIACORE™ Analysis	Cyno B7-H3 Binding
BRCA84D	IgG1/k	2a	2	+	+	+	++
TDH6	IgG1/k	2a	1	+	+	+/–	+
TES7	IgG1/k	2a	1	+	+	+	–
BRCA68D	IgG1/k	2b	3	+	+	++	++
BRCA69D	IgG1/k	2b	3	+	+	++	++
GB8	IgG1/k	2b	3	+	+	+	++
SG27	IgG2b/k	2b	1		+	+	+
OVCA22	IgG1/k	2c	3	+	+	+/–	+
PRCA157	IgG1/k	2c	2	+	+		++
BLA8	IgG1/k	2c		+/–	+	++	++
KID35	IgG1/k	2c	2				++
LUCA50	IgG2a/k	2c	1			+	++
OVCA21	IgG1/k	2c	1		+	+	+
PRCA135	IgG1/k	2c	3		+		++
SG24	IgG2a/k	2c	3			++	++
TDH5	IgG1/k	2c	3		+	++	++
BCCA66	IgG1/k	2c	2		+		–
RECA13	IgG1/k	2c	3		+		–
RECA9	IgG1/k	2c	3		+		–
PRCA123	IgG1/k	2c/3	3		+		++
BRCA126	IgG1/k	3/F					
BRCA192	IgG1/k	3/F					
BRCA34	IgG1/k	3/F					
KID1	IgG1/k	3/F	ND	+	+		+
KID13	IgG2a/k	3/F	3				++
LU14	IgG2b/k	3/F					–
LUCA1	IgG1/k	3/F	1	+	+	++	++

Table 2							
Name	**Isotype**	**IHC**	**ATCC Array**	**Internali-zation**	**U-DART™**	**BIACORE™ Analysis**	**Cyno B7-H3 Binding**
MCLY42	IgG2a/k	3/F					++
MCLY46	IgG1/k	3/F					++
OVCA40	IgG1/k	3/F					++
PA20	IgG1/k	3/F		+		++	–
PA40	IgG2b/k	3/F	3				–
PA41	IgG1/k	3/F	3				
PRO6	IgG1/k	3/F	2				–
RECA22	IgG1/k	3/F	3		–		+
SAL3	IgG2a/k	3/F			+++		++
SG20	IgG1/k	3/F					+
SG29	IgG1/k	3/F					++
SKIN2	IgG1/k	3/F	3		+++		++
STO5	IgG2b/k	3/F	3		++		+
TDH36	IgG1/k	3/F	2				++
TDH37	IgG1/k	3/F	3				+
TDH4	IgG1/k	3/F			+++	++	++
TDH40	IgG2b/k	3/F	3				++
TDH44	IgG2b/k	3/F					++
OVCA25	IgG1/k	3/F	3				+

[00312] IHC staining confirmed that the panel comprised antibodies that elicited a strong tumor to normal tissue binding differential in many of the identified antibodies, exhibited a range of binding properties by BIACORE™ analysis, exhibited reactivity to range of overlapping and non-overlapping epitopes and exhibited a range of specificity to 4Ig vs. 2Ig B7-H3. The characteristics of the nine best candidates are shown in **Table 3** and **Table 4**.

Table 3					
Name	**Normal Tissue**	**Colon Cancer**	**Lung Cancer**	**Prostate Cancer**	**Breast Cancer**
BRCA84D	Colon 1+ Lung 1+ Liver 1+	1231 *	1130	112	1111
TDH6	Colon 1+ Panc 1+ Kidney 1+ Lung 1+ Liver 1+	1110 *	1010	111	1011
TES7		1.5	1.75	3	3
BRCA68D	Panc 1+ Kidney 1+ Lung 1+ Liver 2+	2321 *	3332	333	3333
BRCA69D	Colon 1+ Panc 1+ Kidney 1+ Liver 1+	2231 *	3231	333	3333
GB8					
SG27	Colon 1+ Panc 1+ Kidney 1+ Liver 1+	1221 *	1120	222	1122

Table 3					
Name	**Normal Tissue**	**Colon Cancer**	**Lung Cancer**	**Prostate Cancer**	**Breast Cancer**
OVCA22	Colon 2+ Panc 2+ Liver 2+	1122	3131 **	222	3233
PRCA157	Colon 2+ Liver 2+ Skin 2+	2231 *	3231	333	2333
* +str also; ** str 3+					

Table 4		
Name	**2Ig/4Ig Specificity**	**Epitope Group**
BRCA84D	4Ig/2Ig	1
TDH6	4Ig/2Ig	2
TES7	4Ig	3
BRCA68D	4Ig/2Ig	4
BRCA69D	4Ig/2Ig	4
GB8	4Ig/2Ig	5
SG27	4Ig/2Ig	6
OVCA22	4Ig	7
PRCA157	4Ig/2Ig	8

[00313] **Table 5** provides a summary of the activity profiles of these antibodies.

Table 5							
Name	**Normal Tissue Staining**	**Tumor/Normal Differential**	**Tumor Tissue Positive**	**Cyno Cross-Reactivity**	**IHC ***	**Binding BIACORE™**	**UDART™ Activity**
BRCA84D	1	1	Tumor Stroma bv	Positive (not 1:1)	78	++	++
TES7	1	1	Tumor Stroma bv	negative	1250	+	++
BRCA68D	3	3	Tumor	Positive (1:1)	20	+++	++
BRCA69D	3	3	Tumor Stroma	Positive (1:1)	20	+++	++
GB8	2/3 (Adrenal ND)	3/4	Tumor Stroma	ND, + recomb.	625	+	+
SG27	2/3	ND	ND	ND, + recomb.	20000	+	+
OVCA22	1	1	Tumor Stroma	Negative + recomb.	2500	+	++
PRCA157	2	3	Tumor Stroma bv	Positive (1:1)	20	ND	++
* Optimal Concentration in ng/ml; ND, Not Determined							

[00314] An analysis of the activities of the antibodies shown in Table 6 revealed that their respective profiles differed and that each antibody was associated with both advantages and disadvantages relative to each other (**Table 6**).

Table 6		
Antibody	**Advantages**	**Disadvantages**
BRCA84D	#1 normal tissue staining #1 tumor/normal differential stain tumor, stroma, BV mid affinity, unique binding site (titratable binding)	Cyno cross-reactivity not 1:1
BRCA68D	#3 normal tissue staining #3 tumor/normal differential Cyno cross-reactivity 1:1 high affinity Potent UDART™ activity	Stain tumor only
BRCA69D	#3 normal tissue staining #3 tumor/normal differential Cyno cross-reactivity 1:1 stain tumor, stroma high affinity Potent UDART™ activity	
PRCA157	#2 normal tissue staining #3 tumor/normal differential Cyno cross-reactivity 1:1 stain tumor, stroma, BV Potent UDART™ activity	BIACORE™
TES7	#1/2 normal tissue staining #1/2 tumor/normal differential stain tumor, stroma, BV 4Ig specific Potent UDART™ activity	No Cyno cross-reactivity low affinity
OVCA22	#1/2 normal tissue staining #1/2 tumor/normal differential low affinity stain tumor, stroma 4Ig specific Potent UDART™ activity	No Cyno cross-reactivity low affinity
GB8	#2/3 normal tissue staining (adrenal not determined) #3/4 tumor/normal differential stain tumor, stroma Modest UDART™ activity	Cyno cross-reactivity not determined low affinity
SG27	#2/3 normal tissue staining tumor/normal differential not determined Modest UDART™ activity	Cyno cross-reactivity not determined low affinity

[00315] Because BRCA84D, BRCA68D, BRCA69D and PRCA157 exhibited cleaner normal tissue IHC profiles, stronger tumor/normal IHC differential, moderate to strong binding (BIACORE™ / IHC), cross-reactivity to B7-H3 of cynomolgus monkeys, and potent UDART™ activity, these antibody species were selected for further development. These antibodies differed from TES7 and OVCA2, which exhibited low affinity (in the BIACORE™ assay), and no cross-reactivity to B7-H3 of cynomolgus monkeys. These antibodies differed from SG27, which exhibited low affinity (in the BIACORE™ assay), poor IHC performance (weak binding) and lower UDART™ activity. These antibodies differed from GB8, which exhibited low affinity (in the BIACORE™ assay), poor tumor/normal IHC differential, and lower UDART™ activity.

[00316] Using Caki-2 and Hs700T positive control cells, IHC investigations revealed that each of the antibodies exhibited a different optimal concentration and a different differential concentration relative to one another (**Table 7**).

Table 7

Antibody	Optimal IHC Concentration	Differential IHC Concentration
BRCA84D	0.625 µg/ml	0.078 µg/ml
BRCA68D	0.156 µg/ml	0.0195 µg/ml
BRCA69D	0.156 µg/ml	0.0195 µg/ml
PRCA157	0.078 µg/ml	0.0195 µg/ml
TES7	5 µg/ml	1.25 µg/ml
OVCA22	10 µg/ml	2.5 µg/ml *
GB8	1.25 µg/ml	0.625 µg/ml
SG27	20 µg/ml	Not Determined **
TDH6	20 µg/ml	Not Determined ***

* OVCA22 only showed binding to caki2 cells, did not show binding to Hs700T cells. Optimization decision based on the binding of caki2 cells.
** Because SG27 did not show consistent titration analysis results between two operators, low affinity and differential concentration were not determined.
*** TDH6 study not done due to too low affinity to positive control cells

[00317] Using the optimal and differential concentrations indicated in **Table 7**, the IHC responses of the B7-H3 antibodies in human tissues were determined. The results of these analyses for Adrenal, Liver, Pancreatic, Kidney Lung and Colon are shown in **Tables 8A-8B** and **Tables 9A-9B** (all antibodies exhibited negative IHC responses for heart tissue).

Table 8A: B7H3 mAb IHC at Optimal Concentration in Human Tissues

mAb	Adrenal	Liver	Pancreas
BRCA84D 0.625 µg/ml	Negative	Sinousoid lining cells ++ Hepatocytes +, 5-10%	Epithelium + 5% Fibre ++
BRCA68D 0.156 µg/ml	Cortex +++	Sinousoid lining cells ++ Hepatocytes ++ (m)	Epithelium + Fibre ++
BRCA69D 0.156 µg/ml	Cortex +++	Hepatocytes ++ (m)	Epithelium + Fibre ++
TES7 5 µg/ml	Cortex +	Sinousoid lining cells +	Epithelium + 5% Fibre ++
OVCA22 10 µg/ml	Cortex +	Sinousoid lining cells ++ Hepatocytes + (m)	Epithelium + 5% Fibre ++
PRCA157 0.078 µg/ml	Cortex ++	Sinousoid lining cells ++ Hepatocytes + (m)	Epithelium + 5% Fibre ++
GB8 1.25 µg/ml	Not Determined	Sinousoid lining cells ++ Hepatocytes + (m)	Epithelium + Fibre ++

Table 8B: B7H3 mAb IHC at Optimal Concentration in Human Tissues

mAb	Kidney	Lung	Colon
BRCA84D 0.625 µg/ml	Negative	Epithelium + (5-10%)	Epithelium +
BRCA68D 0.156 µg/ml	Fibroblast +, rare	Epithelium +	Mucosa ++
BRCA69D 0.156 µg/ml	Fibroblast +, rare	Epithelium +	Mucosa +

Table 8B: B7H3 mAb IHC at Optimal Concentration in Human Tissues			
mAb	**Kidney**	**Lung**	**Colon**
TES7 5 μg/ml	Negative	Negative	Epithelium +
OVCA22 10 μg/ml	Fibroblast +	Negative	Epithelium +
PRCA157 0.078 μg/ml	Negative	Negative	Mucosa +
GB8 1.25 μg/ml	Negative	Epithelium +	Mucosa +

Table 9A: B7H3 mAb IHC at Differential Concentration in Human Tissues			
mAb	**Adrenal**	**Liver**	**Pancreas**
BRCA84D 0.078 μg/ml	Negative	Sinousoid lining cells +	Fibre + (rare)
BRCA68D 0.0195 μg/ml	Cortex ++	Hepatocytes + (m)	Fibre +
BRCA69D 0.0195 μg/ml	Cortex ++	Hepatocytes + (m)	Fibre +
TES7 1.25 μg/ml	Fibroblast +	Sinousoid lining cells +	Epithelium +, 5% Fibre ++
OVCA22 2.5 μg/ml	Fibroblast +	Sinousoid lining cells +	Fibre +
PRCA157 0.0195 μg/ml	Not Determined	Sinousoid lining cells + Hepatocytes + (m)	Fibre +
GB8 0.625 μg/ml	Not Determined	Sinousoid lining cells ++ Hepatocytes + (m)	Fibre +

Table 9B: B7H3 mAb IHC at Differential Concentration in Human Tissues			
mAb	**Kidney**	**Lung**	**Colon**
BRCA84D 0.078 μg/ml	Negative	Negative	Epithelium +
BRCA68D 0.0195 μg/ml	Negative	Fibrin + (rare)	Mucosa +
BRCA69D 0.0195 μg/ml	Negative	Negative	Mucosa +
TES7 1.25 μg/ml	Negative	Negative	Epithelium +
OVCA22 2.5 μg/ml	Negative	Negative	Epithelium +
PRCA157 0.0195 μg/ml	Negative	Negative	Mucosa +
GB8 0.625 μg/ml	Negative	Negative	Mucosa +

[00318] IHC investigations conducted using cancer specimens showed that the B7-H3 antibodies of the present invention could be used to identify and diagnose cancer in multiple tissue sources (**Table 10**). In **Table 10**, the numbers indicate the number of plus signs (1 = +, 2 = ++, 3 = +++); each number referring to a different tested sample.

Table 10					
mAbs	**µg/ml**	**Prostate Cancer**	**Breast Cancer**	**Lung Cancer**	**Colon Cancer**
BRCA84D	0.625 µg/ml	2, 2, 1	3, 3, 3, 3	2, 3, 2(stroma), 1(b.v.)	2(stroma), 3, 3, 3(stroma)
	0.078 µg/ml	0, 2, 3, 2	1(stroma), 1(stroma), 1(stroma), 2, 3	1, 1, 0, 1	2, 2(stroma), 1(stroma), 2(stroma)
BRCA68D	0.156 µg/ml	2, 3, 3, 3	2, 3, 3, 3, 3	3, 3, 2, 2	3, 3, 3, 3
	0.0195 µg/ml	0, 1, 1, 1	0, 0, 2, 2, 1	0, 1, 1, 0	1, 1, 1, 1
BRCA69D	0.156 µg/ml	3, 3, 3	3, 3, 3, 3, 3	3, 3, 2, 2(stroma)	3, 3, 3, 3
		0, 1, 2, 1	1, 2, 1, 1	1, 1, 0, 1	1(b.v.), 2(stroma), 1, 1
GB8	1.25 µg/ml	2, 3, 1	2, 2, 1, 2	3, 3, 0, 0	2(stroma), 2, 2, 2
	0.625 µg/ml	0, 1, 1	0, 0, 0, 0, 1	1, 0, 0, 0	1(stroma), 1(stroma), 0, 0
TES7	5 µg/ml	2, 3, 2, 3	1(stroma), 3, 3, 3, 2	3, 2, 1(stroma), 1(b.v.)	3, 3, 2, 2
	1.25 µg/ml	1, 2, 2, 3	1(stroma), 2, 3, 3, 2	3, 1, 1(stroma), 1(b.v.)	3, 2(stroma), 2(stroma), 2
OVCA22	10 µg/ml	3, 2, 2, 1	1(stroma), 2, 2, 3, 3	3, 2, 1(stroma), 0	1(stroma), 1, 1, 2(stroma)
	2.5 µg/ml	1, 1, 3, 1	1(stroma), 1(stroma), 1(stroma), 3, 2, 2	2, 1, 0, 0	1, 0, 2(stroma), 0
PRCA157	0.078 µg/ml	2, 2, 2, 3	1, 2, 2, 3, 3	2, 2, 1(stroma), 1(b.v.)	3, 3, 2(stroma), 2(stroma)
	0.0195 µg/ml	0, 1, 2, 1	1(stroma), 0, 2, 1(stroma)	0, 1, 0, 0	1, 1, 1(stroma), 1(stroma)

[00319] For prostate, breast, colon and lung cancer cells treated with B7-H3 antibody BRCA84D, tumor sample staining was present in tumor cells and stromal cells, including the tumor vasculature. In some tumor samples, stromal staining was much stronger than tumor cells. When BRCA84D mAb was titrated to lower concentration, some cases showed reduced staining in tumor cells, but still maintained strong stromal staining. Upon staining with BRCA84D at 0.625 µg/ml, prostate cancer cells exhibited an IHC of 3/3+; breast cancer cells exhibited an IHC of 4/4+; colon cancer cells exhibited an IHC of 4/4+ and lung cancer cells exhibited an IHC of 4/4+. Upon staining with BRCA84D at 0.078 µg/ml, prostate cancer cells exhibited an IHC of 3/4+; breast cancer cells

exhibited an IHC of 5/5+; colon cancer cells exhibited an IHC of 4/4+ and lung cancer cells exhibited an IHC of of 3/4+.

[00320] Normal liver was treated with B7-H3 antibody BRCA68D, and staining was seen in hepatocytes and sinousoid lining cells. Normal pancreas stained with B7-H3 antibody BRCA68D exhibited multi-focal staining in collagen fibre and epithelium. Normal adrenal cells treated with B7-H3 antibody BRCA68D, exhibited staining in the cortex. Upon staining with BRCA68D at 0.156 µg/ml, gastric, renal and ovarian cancer cells all exhibited an IHC of 5/5+.

[00321] Additional IHC staining analyses were conducted on samples of gastric, kidney and ovarian cancer tissues. The results of such analyses are shown in Table 12. In **Table 11**, the numbers indicate the number of plus signs (1= +, 2= ++, 3 = +++); each number referring to a different tested sample.

Table 11

mAbs	µg/ml	Gastric Cancer	Kidney Cancer	Ovarian Cancer
BRCA84D	0.625 µg/ml	2,1,2,2,2	1,2,1,1,1	0,3,1,2,2
	0.078 µg/ml	1,0,0,1,0	0,1,0,1,0,1	0,2,0,1,1
BRCA68D	0.156 µg/ml	3,2,3,3,3	3,3,2,3,3,3	2,3,3,2,2
	0.0195 µg/ml	2,1,2,1,1	2,2,2,2,2,2	1,2,2,1,1
OVCA22	10 µg/ml	3,1,3,1,1	3,1,2,3,0,2	2,3,2,1,1
	2.5 µg/ml	2,0,2,1,0	2,1,1,2,0,1	1,2,1,0,1
TES7	5 µg/ml	2,1,3,2,1	2,3,1,2,2,1	1,3,1,2,2
	1.25 µg/ml	2,0,2,1,1	2,2,1,1,1,1	1,3,1,2,2

[00322] In summary, all of the tested mAbs showed various degrees of staining intensity in normal liver, pancreas, colon and lung. **Figure 1A** shows the results of IHC investigations conducted using normal pancreas, liver, lung and colon tissue specimens with BRCA84D at 0.625 µg/ml and 0.078 µg/ml. Liver staining was relatively restricted in sinusoid lining cells (fibroblast and kupffer cells) with BRCA84D and TES7. OVCA22 showed membrane hepatocyte staining besides that of the sinusoid ling cells at the optimal concentration. However, the staining in hepatocytes disappeared at the differential concentration. All of the other mAbs showed staining in hepatocytes including either membrane or cytoplasm staining at both the optimal and differential concentration. Pancreas staining was observed in collagen fiber mainly and a small percentage of the epithelium (acinar cells or/and intercalated duct cells). The staining in epithelium diminished or disappeared at differential concentration. Colon staining was relatively restricted in apical membrane of crypt epithelium and fibroblast in mucosa. No binding was observed in lymphoid nodules of colon. Lung showed very weak and patchy staining in epithelium with BRCA84D, BRCA68D, BRCA69D and GB8. However, the staining disappeared at the differential concentration. No staining was observed in lung with TES7, OVCA22 and PRCA157 at both concentrations. Adrenal cortex staining was observed with almost all of the mAbs at optimal concentration, except BRCA84D. The staining in adrenal obviously

diminished with TES7 and OVCA22 at differential concentration. Heart and kidney did not show obvious staining with all mAbs (**Figure 1B**). In light of these properties, BRCA84D was considered the best of the mAbs, followed in order by (2) TES7, (3) OVCA22, (4) the group BRCA68D, BRCA69D and PRCA157, and lastly (5) GB8.

[00323] All of the mAbs included in the study showed positive staining in 4 cancer types at the optimal concentration. At the differential concentration, BRCA84D still maintained good staining in prostate cancer, breast cancer, and colon cancer. TES7 maintained good staining in 4 study cancer types. The remaining mAbs showed various staining intensities in different tumor types. Tumor sample staining was observed in tumor cells and stromal cells, including vasculature. Some tumor samples showed positive staining only in vasculature, i.e. BRCA84D, BRCA69D, TES7, and PRCA157. Some tumors showed stronger stromal staining than tumor cell staining. When mAbs were titrated to lower concentration on these samples, some cases showed diminished or no staining in tumor cells, but still maintained strong stromal staining. In general, in terms of expression in human normal tissues and differential expression in normal vs. tumor tissues, the mAb order from the best IHC performance to the poorest performance is as follows: (1) BRCA84D, (2) TES7, (3) OVCA22, (4) the group BRCA68D, BRCA69D and PRCA157, and lastly (5) GB8. **Table 12** and **Figure 2** show results for antibody BRCA84D.

Table 12

Cancer Tissue Type	BRCA84D 0.625 µg/ml	BRCA84D 0.078 µg/ml
Prostate	3/3+	3/4+
Breast	4/4+	5/5+
Colon	4/4+	4/4+
Lung	4/4+	3/4+

Example 2
Cynomolgus Monkey B7-H3 Cross-Reactivity

[00324] The sequence of cynomolgus monkey B7-H3 shares approximately 90% homology to its human counterpart, suggesting that the cynomolgus monkey is an excellent model for human B7-H3 interactions. Investigations were conducted to evaluate the cross-reactivity of B7H3 candidates BRCA84D, BRCA68D, BRCA69D, TES7, OVCA22 and PRCA157 with adrenal, liver, kidney, pancreas and lung as well as one case full term placenta of cynomolgus monkey, in order to compare any cross-reactivity with the staining intensity and staining patterns observed for human tissues.

[00325] Staining concentration for each tested Mab is the optimal concentration which was determined in Caki-2 and Hs700T positive control cells (see, **Table 8**). Commercial goat anti human B7-H3 (cross-reacted with cyno) was selected as a positive control antibody to stain cynomolgus

placental tissue. Corresponding isotype controls were applied in each run of the experiments. The results of thes investigations are shown in **Table 13**.

Table 13

mAb	Adrenal(2)	Liver(2)	Pancreas(2)	Kidney(2)	Lung(2)	Placenta(1)
BRCA84D 0.625 µg/ml	Negative	Negative	Negative	Negative	1/2 Epithelium 1+	Decidual cells 2+ Mesenchymal cells negative
BRCA68D 0.156 µg/ml	Cortex 3+	2/2 Hepatocytes 1+ (m) Sinousoid lining cells 1+	1/2 Fiber 2+ Epithelium 1+	Fibroblast 1+	Negative	Decidual cells, villi, Mesenchymal cells 3+
BRCA69D 0.156 µg/ml	Cortex 2+	1/2 Hepatocytes 1+ (m)	1/2 Epithelium 1+	Fibroblast 1+ rare	Negative	Decidual cells 2+, villi, Mesenchymal cells 2+
TES7 5 µg/ml	Negative	Negative	Negative	Negative	Negative	Negative
OVCA22 10 µg/ml	Negative	Negative	Negative	Negative	negative	Negative
PRCA157 0.078 µg/ml	Cortex 1+	1/2 Hepatocytes 1+ (m)	Negative	Negative	Negative	Decidual cells 2+, villi, Mesenchymal cells 1+

Note: BRCA84D showed negative staining in liver and pancreas at up to 5µg/ml. Although OVCA22 did not bind to cyno tissue in IHC, modest binding was observed to recombinant cyno B7H3 on CHO cells. IHC score in normal tissues is negative, 1+, 2+ and 3+ 4 grade system; m=membrane; 2/2= 2 of 2 cases, ½= 1 of 2 cases

[00326] The investigation of BRCA84D (0.625 µg/ml) IHC staining in cynomolgus placenta exhibited staining in decidual cells, but not in villi. No staining was observed in cyno liver and pancreas, however, staining of sinousoid lining cells was observed in human liver and localized fibre and epithellium staining was observed in human pancreas tissue.

[00327] The investigation of BRCA68D (0.156 µg/ml) IHC staining in cynomolgus placenta exhibited staining in decidual cells, mesenchymal cells (endothelium and fibroblasts) and villi. Staining was present in membrane of hepatocytes and cytoplasm of liver fibroblasts, as well as in pancreatic fibre and in the cytoplasm of pancreatic epithelium. Thus, human and cyno liver and pancreatic tissue exhibit similar staining patterns with BRCA68D.

[00328] In summary, BRCA84D, BRCA68D, BRCA69D and PRCA157 all showed cross-reactivity in cyno tissues. BRCA84D did not show staining in monkey liver and pancreas; such staining was observed in human liver and pancreatic tissues. BRCA68D and BRCA69D showed similar staining intensity and staining patterns in monkey tissues. Although BRCA68D, BRCA69D and PRCA157 showed comparable staining pattern with human tissues, the staining intensity is not identical with

human tissues at optimal conditions. TES7 and OVCA22 did not show any staining in monkey tissues at optimal conditions.

[00329] A summary of the comparative results of IHC staining in cynomolgus tissue and human tissue is provided in **Table 14**.

Table 14

mAb	Adrenal	Liver	Pancreas	Kidney	Lung	Placenta
BRCA84D 0.625 µg/ml Cynomolgus	Negative	Negative	Negative	Negative	1/2 Epithelium 1+	Decidual cells 2+ Mesenchymal cells negative
BRCA84D 0.625 µg/ml Human	Negative	2/2 Sinousoid lining cells 2+, Hepatocytes 1+ 5-10%	Epithelium 1+, 5%, fibre2+	Negative	Epithelium 1+, 5-10%	Decidual cells 1+, villi, Mesenchymal cells 1+
BRCA68D 0.156 µg/ml Cynomolgus	Cortex 3+	2/2 Hepatocytes 1+ (m) Sinousoid lining cells 1+	1/2 Fiber 2+ Epithelium 1+	Fibroblast 1+	Negative	Decidual cells, villi, Mesenchymal cells 3+
BRCA68D 0.156 µg/ml Human	Cortex 3+	Sinousoid lining cells 2+, Hepatocytes 2+ (m)	Epithelium 1+ Fibre2+	Fibroblast 1+ rare	Epithelium 1+	Decidual cells 3+, villi, Mesenchymal cells 3+
BRCA69D 0.156 µg/ml Cynomolgus	Cortex 2+	1/2 Hepatocytes 1+ (m)	1/2 Epithelium 1+	Fibroblast 1+ rare	Negative	Decidual cells 2+, villi, Mesenchymal cells 2+
BRCA69D 0.156 µg/ml Human	Cortex 3+	Hepatocytes 2+ (m)	Epithelium 1+ Fibre2+	Fibroblast 1+ rare	Epithelium 1+	Decidual cells 3+, villi, Mesenchymal cells 3+
PRCA157 0.078 µg/ml Cynomolgus	Cortex 1+	1/2 Hepatocytes 1+ (m)	Negative	Negative	Negative	Decidual cells 2+, villi, Mesenchymal cells 1+
PRCA157 0.078 µg/ml Human	Cortex 2+	Sinousoid lining cells 2+, Hepatocytes 1+ (m)	Epithelium 1+ 5% Fibre2+	Negative	Negative	Not Determined

Example 3
B7-H3 mAbs Bind to Multiple ATCC Cancer Cell Lines

[00330] The antibodies of the present invention were found to be capable of binding to multiple cancer cell lines contained in the collections of the American Type Culture Collection. **Table 15** and **Table 16** summarize the binding results.

Table 15					
	Antibody				
Cell Lines	**BLA08**	**BRCA68D**	**BRCA69D**	**BRCA84D**	**PRCA157**
Normal Human Lines					
HMEC	++/+++	+++	+++	++	
HUVEC	ND	++	+/++	+/–	+/++
Human Breast Cancer Lines					
BT474	+++	++	++/+++	+/++	++/+++
MCF7	+++	++	++/+++	+	++
MDA175	ND				
MDA361	ND	++		+/+/–	++
SKBR3	+++		++		
Human Lung Cancer Lines					
A549	+++	+/+/–	+/–		+/–
Calu3	+++	+/++	+		+/++
SKMES1	+++	++	++/+++	+/++	++
Human Ovarian Cancer Lines					
ES-2	+++	+/–			
SKOV3	+++	++	+/++	+/+/–	++
Human Pancreatic Cancer Lines					
Panc-1	++/+++	+/++	+/++	+/+/–	+/++
AsPC-1	+++				
HPAFII	+++				
Hs700T	+++	++/+++		+++	+++
Human Colon Cancer Lines					
Colo205	ND				
HT-29	+++	+	+		+
SW480	+++	+/–		+/–	
SW948	ND	+	+		
Human Kidney Cancer Lines					
293	+++	++	++	+	++/+++
786-0	+++	++	++	+	++/+++
A498	+++	++	++	++	++/+++
Caki2	+++	+++	+++	++	++/+++
Non-Human Cell Lines					
Cos7	+++	+	+/++	+/–	+/++
RL65	–				
SVT2	ND				
Human Prostate Cancer Lines					
22Rv1	+++				
DU145	+++	+	+	+	+/+/–
LNCaP	+++	++	++	+/++	++/+++
PC3	+++	+/+/–	+/–	+/–	+/–
TDH	ND	+/+/–	+/+/–		+
Human Stomach Cancer Lines					
HS746T	ND	+/++	+/++	+	++
N87	ND	+/++	+/++	+/–	+/++

Table 16					
	Antibody				
Cell Lines	**TDH06**	**OVCA22**	**GB8**	**SG27**	**TES7**
Normal Human Lines					
HMEC					
HUVEC	+/+/−		+/−	+/−	
Human Breast Cancer Lines					
BT474	+/++	+	++	+/++	+/++
MCF7	+	+	++	+/+/−	+
MDA175		++			
MDA361	+/+/−				+
SKBR3		++			
Human Lung Cancer Lines					
A549					
Calu3			+		
SKMES1	+/++	+/−	+/++	+	+
Human Ovarian Cancer Lines					
ES-2					
SKOV3	+				+/+/−
Human Pancreatic Cancer Lines					
Panc-1	+/+/−		+	+/−	+/+/−
AsPC-1					
HPAFII		+			
Hs700T	+	+++	+++	+	+++
Human Colon Cancer Lines					
Colo205		+			
HT-29	+	+/+/−			+/+/−
SW480	+/−	+++			
SW948	+/−	+			
Human Kidney Cancer Lines					
293	+/+/−		+	+/+/−	+
786-0	+		+*	+/−	+
A498	+		+/++		+/++
Caki2	++	+	+++	+/++	++
Non-Human Cell Lines					
Cos7	+		+/+/− *	+/−	
RL65					
SVT2					
Human Prostate Cancer					
22Rv1		+			
DU145	+/−	+			
LNCaP	+/+/−	+	+*	+/+/−	+
PC3					
TDH		+++	+/−		+/−
Human Stomach Cancer Lines					
HS746T	+		+/+/−	+/−	+/−
N87		+/+/−	+/−		

Example 4
B7-H3 mAbs Redirect Killing

[00331] The antibodies of the present invention bind to B7-H3 present on the surface of cancer cells. Using conventional methods, such antibodies may be labeled with fluorescein, as described above. When such labeled molecules are incubated in the presence of UDART™ molecules having an epitope binding domain that binds to the T-cell receptor and an epitope binding domain that binds to fluorescein ("TCR-UDART™"), they can bind to the DART™ molecules and thereby localize them to the surface of cells that express B7-H3 and cause redirected killing.

A. Redirect Killing of A498 Renal Carcinoma Cells

[00332] To demonstrate such redirected killing, fluorescein-labeled B7-H3 antibodies were incubated with such TCR-UDART™ molecules and the ability of the molecules to mediate cytotoxicity of A498 renal carcinoma cells was evaluated (**Table 17**). On the basis of the attained results, the top candidates were concluded to be: RECA13, BRCA68D, BRCA69D and TDH6.

Table 17: Redirected Killing of A498 Renal Carcinoma Cells

mAb	No UDART™	With TCR-UDART™	FACS
	Mean	Mean	MFI
BCCA66	-1.04	46.39	43.30
BLA8	1.35	49.19	50
BRCA165	0	5.11	5.46
BRCA52	0	55.53	41.7
BRCA68D	0	36.89	83.7
BRCA69D	0	54.71	84.1
BRCA84D	0	72.40	30.6
GB8	4.00	42.00	17.9
KID1	0.38	52.08	18.5
KID13	26.39	58.20	
KID35	-1.68	7.62	
LUCA1	9.85	52.73	52.9
OVCA21	-0.85	47.59	6.04
OVCA22	0.36	38.66	53.9
OVCA25	-2.86	16.70	
PA40	-0.46	40.54	
PRCA123	0	56	130
PRCA135	0	55	127
PRCA157	0	39.14	58.8
RECA13	0	38.62	39.8
RECA22	-0.24	51.74	99.90
RECA9	0	62	50.1
SAL3	4.94	52.23	60.5
SG24	-2.25	42.00	
SG27	-3.98	0.21	
SKIN2	3.11	56.44	45.8
STO5	2.91	37.84	36.7

Table 17: Redirected Killing of A498 Renal Carcinoma Cells			
mAb	No UDART™	With TCR-UDART™	FACS
	Mean	Mean	MFI
TDH36	-1.03	53.52	155.00
TDH37	0.05	65.21	47.50
TDH4	5.09	50.63	45.9
TDH40	-0.65	44.55	
TDH5	2.92	49.60	28.8
TDH6	0	70.10	19.5
TES7	6.23	52.89	17.5

[00333] A498 renal carcinoma cells were incubated with different concentrations of monoclonal antibodies reactive against B7-H3 in order to determine the dose-dependent redirected killing mediated by the antibodies. The results of the experiments (**Figures 3A-3B**) show that the redirected killing was dose-dependent.

B. Redirect Killing of A549 Lung Cancer Cells

[00334] To further demonstrate such redirected killing, fluorescein-labeled B7-H3 antibodies were incubated with the above-described TCR-UDART™ molecules or with UDART™ molecules having an epitope binding domain that binds to CD16 and an epitope binding domain that binds to fluorescein ("CD16-UDART™"), and the ability of the molecules to mediate cytotoxicity of A549 lung cancer cells was evaluated (**Table 18**). The results of the experiments (**Figures 3C-3D**) show that the redirected killing was dose-dependent. On the basis of the attained results, the top candidates were concluded to be: BLA8, BRCA68D, BRCA69D and BRCA84D.

Table 18: Redirected Killing of A549 Lung Cancer Cells				
mAb	No DART™	With TCR-UDART™	With CD16-UDART™	FACS
	Mean	Mean	Mean	MFI
BCCA66	1.89	25.17	8.22	36.1
BLA8	-7.70	10.97	3.68	34.7
BRCA52	0	27.63		37
BRCA68D	-4.42	13.45	15.95	58.3
BRCA69D	0	24.25		60.5
BRCA84D	0	15.33		25
GB8	-8.68	2.44	-4.65	17
KID1	0	22.93		41
LUCA1	0	14.65		53
OVCA21	-2.43	18.90	7.22	31.5
OVCA22	0	32.90		61
PRCA123	7.68	29.88	17.31	79.4
PRCA135	-6.58	22.72	8.14	75.6
PRCA157	0.02	18.63	18.24	44.3
PSMA	-0.70	5.58	9.94	
RECA13	0.86	17.39	11.90	34.4
RECA22	3.71	20.49	19.35	74.3

Table 18: Redirected Killing of A549 Lung Cancer Cells				
mAb	No DART™	With TCR-UDART™	With CD16-UDART™	FACS
	Mean	Mean	Mean	MFI
RECA9	7.01	26.89	31.80	44.3
SAL3	0	31.80		67.4
SKIN2	-0.08	8.65	9.33	41.9
STO5	-10.36	9.28	1.71	54.7
TDH36	6.79	24.12	24.08	107
TDH37	6.93	22.57	23.37	42.3
TDH4	-6.26	10.07	2.21	32.4
TDH40	4.87	22.01	24.90	53.3
TDH5	-5.08	9.35	-2.85	27.1
TDH6	0	19.09		21.3
TES7	0	19.35		15.7

C. Redirect Killing of LNcap Prostate Cancer Cells

[00335] To further demonstrate such redirected killing, fluorescein-labeled B7-H3 antibodies were incubated with the above-described TCR-UDART™ molecules or with UDART™ molecules having an epitope binding domain that binds to CD16 and an epitope binding domain that binds to fluorescein ("CD16-UDART™"), and the ability of the molecules to mediate cytotoxicity of LNcap prostate cancer cells was evaluated (**Table 19**). On the basis of the attained results, the top candidates were concluded to be: BRCA68D, BRCA69D, BRCA84D and PRCA157.

Table 19: Redirected Killing of LNcap Prostate Cancer Cells				
mAb	No DART™	With TCR-UDART™	With CD16-UDART™	FACS
	Mean	Mean	Mean	MFI
BCCA4	-2.96	13.29	2.47	5.1
BCCA66	-2.13	13.42	16.40	41
BLA8	4.32	14.97	24.00	48.4
BRCA165	3.59	57.26	12.02	7.6
BRCA183D	-4.65	43.09	35.30	7.6
BRCA52	32.34	71.23	48.28	42.5
BRCA68D	-1.40	23.00	21.91	86.9
BRCA69D	40.08	78.02	60.55	92.4
BRCA84D	20.11	78.70	41.27	16.4
GB8	-6.25	14.04	10.76	22
KID1	54.65	91.87	67.86	44.8
KID13	15.86	69.21	47.85	
KID133	27.51	45.65	47.12	120
KID24	-4.26	34.13	41.17	14.5
KID35	14.17	64.01	33.05	
KID47	11.34	39.49	15.02	10.8
KID8	16.98	58.80	34.77	5.5
LUCA1	47.40	89.31	67.15	73
LUCA17	23.18	26.90	35.87	11.1
LUCAT1	8.25	22.36	21.49	6.9

Table 19: Redirected Killing of LNcap Prostate Cancer Cells				
mAb	No DART™	With TCR-UDART™	With CD16-UDART™	FACS
	Mean	Mean	Mean	MFI
LUCAT7	26.50	38.29	44.77	8.7
MCL12	26.62	35.59	46.38	17.6
MEL2	6.57	29.90	31.40	19
OVCA21	12.07	26.81	31.30	41
OVCA22	45.09	96.50	77.30	113
OVCA25	16.14	63.26	32.39	
PA22	1.73	57.70	9.89	8.9
PA33	8.99	34.49	48.14	9.4
PA40	38.42	73.07	63.65	
PRCA123	9.96	14.39	18.38	125
PRCA135	-3.75	8.89	13.64	123
PRCA157	1.05	17.07	15.43	16.4
PSMA	11.52	31.38	34.79	
PSMA	52.82	71.19	66.04	
RECA13	5.86	22.55	15.40	37
RECA22	7.33	24.65	23.54	22.5
RECA9	27.67	52.54	45.14	5.3
SAL1	2.76	17.87	44.52	6.5
SAL2	8.71	30.68	29.17	14.5
SAL3	43.79	92.60	76.46	105
SG24	12.64	66.82	44.99	
SG27	1.37	55.30	16.96	
SKIN2	-2.04	14.81	24.23	73.8
SPL16	9.97	29.90	23.74	5.2
STO5	-1.48	21.11	24.97	61.3
TDH28	-4.23	18.55	15.04	13.3
TDH36	3.58	19.61	19.79	199
TDH37	7.90	18.78	25.22	57.3
TDH4	14.48	37.96	54.64	45.2
TDH40	8.51	44.55	43.87	79.3
TDH5	7.35	48.71	38.15	29.1
TDH6	4.50	54.59	19.73	41.7
TES7	50.15	94.47	73.40	22.4

Example 5
Ability of B7-H3 mAbs to Bind to Soluble B7H3-2Ig and Soluble B7H3-4Ig

[00336] As discussed above, B7-H3 exists in both a 4 Ig domain-containing form (B7H3-4Ig) and a 2 Ig domain-containing form (B7H3-2Ig). The anti-B7-H3 antibodies of the present invention were tested for their abilities to bind to soluble B7H3-2Ig (**Figure 4A**) and soluble B7H3-4Ig B7-H3 (**Figure 4B**). The antibodies were found to exhibit a broad range of binding characteristics. Antibodies PRCA123, TDH5, BLA8, BRCA68D and SG24 were found to exhibit the strongest binding to soluble B7H3-2Ig and antibodies TES7, LUCA50, BRCA165, OVCA22, STO9 and PA20 were found to exhibit the weakest binding to soluble B7H3-2Ig. Antibodies PRCA123, BRCA69A, BLA8 and BRCA68D were found to exhibit the strongest binding to soluble B7H3-4Ig and antibodies

TES7, OVCA21, BRCA165 and STO9 were found to exhibit the weakest binding to soluble B7H3-4Ig.

Example 6
Affinity Binding of Antigens in Solution to Captured Monoclonal Antibodies

[00337] In order to demonstrate the binding affinity between antigens in solution and captured monoclonal antibodies, antibodies were captured on immobilized IgG Fc-specific Fab2 fragments at a level of 100-200 RU. Antigens B7-H3 and B7-H3(4Ig) were injected over the captured antibodies at a concentration of 100nM (flow rate 20 µl/min for 120 sec, and binding was measured. Binding responses were normalized to the same level of captured mAb and the binding response to m2B6 antibody (mIgG1) control was subtracted as blank. The results of this analysis (**Figures 5A-5S**; solid lines; B7-H3(4Ig) 100 nM; dashed lines; B7-H3, 100 nM)) demonstrate that the antibodies of the present invention exhibit strong binding to B7-H3(4Ig).

Example 7
BIACORE ™ Analysis: Titration of B7-H3 mAbs to Immobilized B7-H3

[00338] In order to demonstrate the relative binding affinities of B7-H3-2Ig and B7-H3-4Ig for the antibodies of the present invention, a BIACORE ™ analysis was performed. B7-H3 antibodies of the present invention were permitted to bind to immobilized B7-H3-2Ig or to B7-H3-4Ig and the titration of binding over time was assessed (**Figures 6A-6I**). TDH5, PRCA123, BLA8, BRCA69 were found to have high affinity to both B7-H3-2Ig and B7-H3-4Ig. However their epitope(s) were found to be mostly barred in the B7-H3-4Ig molecule, with just a few being available. OVCA22 was found to have a very low affinity to both B7-H3-2Ig and B7-H3-4Ig with its epitope being equally available on both molecules. However, it is likely that only the B7-H3-4Ig form provides enough proximity for antibody bivalent binding (low off-rate), whereas the B7-H3-2Ig can be bound only monovalently. TDH6 was found to have barely any affinity in this format, with binding to 2Ig likely to be non-specific. TES7 and PA20 were found to be B7-H4-4Ig specific antibodies with low affinity. TES7 probably has a low on-rate and a higher off-rate than PA20. BRCA84D was found to be an intermediate affinity antibody with a possibility of multiple binding sites on both B7-H3-2Ig and B7-H3-4Ig. Based on the BIACORE ™ analysis, BRCA84D due to its unusual binding site was considered a preferred antibody. TES7 and PA20 were considered candidates for specific binding to high density antigen surfaces, and one of high affinity-low specificity antibody (*e.g.*, BRCA69D or another).

[00339] **Figure 7** provides a comparison BIACORE™ analysis of antibodies PRCA157, BRCA69D, BLA8, PA20, BRCA84D, GB8 and SG27, illustrating that the anti-B7-H3 antibodies of the present invention can exhibit a range of binding properties.

[00340] **Figure 8** demonstrates the non-competing specificity of several of the anti-B7-H3 antibodies of the present invention. In the experiment, human B7-H3 molecules were incubated in the presence of antibody BRCA84D and subjected to BIACORE™ analysis. After approximately 3 minutes a second anti-B7-H3 antibody was added to the reaction. If the second antibody competed with BRCA84D, it would find the B7-H3 sites occluded and be unable to bind. The results indicate that antibodies BRCA68D, BRCA69D, and PRCA157 do not compete with BRCA84D for binding to human B7-H3.

Example 8
Anti-B7-H3 mAbs Internalize on CSC and ATCC Cell Lines

[00341] The ability of the anti-B7-H3 antibodies of the present invention to become internalized upon binding to cancer cells was investigated. Prostate CSC cells and Hs700t pancreatic cells were incubated with an anti-B7-H3 antibody. The viability of the cells was determined after incubation in the presence of a saporin-conjugate anti-mouse secondary antibody which will be toxic to the cells if bound to the primary antibody and internalized. The results of this investigation for prostate CSC cells (**Figure 9A**) and for Hs700t pancreatic cells (**Figure 9B**) demonstrate the capacity of the antibodies of the present invention to become internalized into cells.

Example 9
B7-H3 mAb Binding and Cross-Blocking Analysis by ELISA

[00342] In order to explore the cross-reactivity of the antibodies of the present invention and the epitopes recognized by such antibodies, the extent of binding occurring in the presence of a competitor B7-H3 antibody was measured. The results of this analysis are shown in **Figures 10A-10F**, and show that BRCA68D competes with BRCA69D. TES7 and OVCA22 were also found to compete with one another, but TES7 and not OVCA22 was found to also compete with both BRCA68D and BRCA69D. GB8 was found to compete with SG27 for binding to B7-H3-2Ig but not to B7-H3-4Ig. The data are summarized in **Table 20** and show at least four distinct epitopes for B7-H3-4Ig (*i.e.*, the epitope recognized by SG27, the epitope recognized by GB8, the epitope recognized by OVCA22 and TES7, and the epitope recognized by BRCA68D, BRCA69D and TES7) and at least two epitopes for B7-H3-2Ig (*i.e.*, the epitope recognized by SG27 and GB8, and the epitope recognized by BRCA68D and BRCA69D).

Table 20 Summary of B7-H3 mAb Cross-Blocking Analysis by ELISA								
Competitor Antibody	Antibody (Percent Binding vs. MIgG)							
	B7-H3 4Ig					B7-H3-2Ig		
	GB8	BRCA 69D	BRCA 68D	TES7	OVCA 22	GB8	BRCA 69D	BRCA 68D
GB8	50.211	119.105	108.948	87.480	98.142	26.618	84.408	94.710
TES7	111.234	109.390	108.425	1.605	16.268	100.645	90.734	99.515
OVCA22	121.783	112.322	100.813	3.371	2.048	100.423	87.991	102.766
TDH6	105.591	105.065	100.494	99.839	96.701	100.089	66.086	100.728
SG27	101.266	103.021	97.763	78.331	87.789	64.421	89.927	94.225
BRCA68D	105.934	40.284	43.144	4.815	102.655	98.888	7.635	7.425
BRCA69D	102.558	66.291	71.441	4.334	96.928	94.952	17.346	17.059
MIgG	100.000	100.000	100.00	100.000	100.000	100.000	100.000	100.000

[00343] The attributes of the key anti-B7-H3 antibodies of the present invention are shown in **Table 21**. Based on their exhibited differential staining of normal and cancer tissues, their ability to bind B7-H3-4Ig as well as B7-H3-2Ig, their binding affinities as measured by the above-described BIACORE™ analysis and their ability to bind to cynomolgus B7H3, antibodies, BRCA68D, BRCA69D, BRCA84D, and PRCA157 were judged to be the most preferred antibodies

Table 21									
MAb	BRCA 84D	TDH 6	TES7	BRCA 68D	BRCA 69D	GB8	SG 27	OVCA 22	PRCA 157
Isotype	G1/k	G1/k	G1/k	G1/k	G1/k	G1/k	2b/k	G1/k	G1/k
IHC	2a	2a	2a	2b	2b	2b	2b	2c	2c
ATCC Array	2	1	1	3	3	3	1	3	2
Normal Tissue									
Colon	1+	1+			1+		1+	2+	2+
Lung	1+	1+		1+					
Liver	1+	1+		2+	2+		1+	2+	2+
Kidney		1+		1+	1+		1+		
Pancreas		1+		1+			1+	2+	
Skin									2+
Cancerous Tissue									
Colon	1231*	1110*	1.5	2321*	2231*		1221*	1122	2231*
Lung	1130	1010	1.75	3332	3231		1120	3131**	3231
Prostate	112	111	3	333	333		222	222	333
Breast	1111	1011	3	3333	3333		1122	3233	2333
Internali-zation	+	+	+	+	+	+	+	+	+
U-DART™	+	+	+	+	+	+	+	+	+
Specificity	4Ig 2Ig	4Ig 2Ig	4Ig	4Ig 2Ig	4Ig 2Ig	4Ig 2Ig	4Ig 2Ig	4Ig	4Ig 2Ig
Epitope Group	A	B	C	D	D	E	F	G	H
BIACORE™	+	+/-	+	++	++	+	+	+	+/-
Cynomolgus B7-H3 Binding	++	+	-	++	++	++	+	+	++
Notes: * Indicates staining of stroma ** stroma staing 3+									

Example 10
Humanized Anti-B7-H3 Antibodies

[00344] Monoclonal antibody BRCA84D was humanized in order to produce antibodies (generically designated herein as "hBRCA84D") offering improved human therapeutic potential. The sequences of the variable light chain, and the variable heavy chain, and their respective amino acid and polynucleotide sequences of a resulting humanized antibody (designated herein as "hBRCA84D-1") are provided below:

[00345] Humanized BRCA84D-1 Variable Light Chain (**SEQ ID NO:68**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00346] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain (**SEQ ID NO:69**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaagctgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00347] Humanized BRCA84D-1 Variable Light Chain CDR_1 (**SEQ ID NO:70**): KASQNVDTNVA

[00348] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_1 (**SEQ ID NO:71**): aaggccagtc agaatgtgga tactaatgta gcc

[00349] Humanized BRCA84D-1 Variable Light Chain CDR_2 (**SEQ ID NO:72**): SASYRYS

[00350] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_2 (**SEQ ID NO:73**): tcggcatcct accggtacag t

[00351] Humanized BRCA84D-1 Variable Light Chain CDR_3 (**SEQ ID NO:74**): QQYNNYPFT

[00352] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_3 (**SEQ ID NO:75**): cagcaatata acaactatcc attcacg

[00353] Amino Acid Sequence of Humanized BRCA84D-1 Variable Heavy Chain (**SEQ ID NO:80**):

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

[00354] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain (**SEQ ID NO:81**):

gaggtgcagc tggtcgagtc tggcggagga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg cctccggctt caccttctcc agcttcggca
tgcactgggt ccgccaggct ccaggcaagg gactggaatg ggtggcctac
atctcctccg actcctccgc catctactac gccgacaccg tgaagggcag
gttcaccatc tcccgggaca acgccaagaa ctccctgtac ctgcagatga
actccctgcg ggacgaggac accgccgtgt actactgcgc cagaggccgg
gagaatatct actacggctc ccggctggat tattggggcc agggcaccac
cgtgaccgtg tcctct

[00355] Humanized BRCA84D-1 Variable Heavy Chain CDR_1 (**SEQ ID NO:82**): FGMH

[00356] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_1 (**SEQ ID NO:83**): tttggaatgcac

[00357] Humanized BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:84**): YISSDSSAIYYADTVK

[00358] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_2 (**SEQ ID NO:85**): tacattagta gtgacagtag tgccatctac tatgcagaca cagtgaag

[00359] Humanized BRCA84D-1 Variable Heavy Chain CDR_3 (**SEQ ID NO:86**): GRENIYYGSRLDY

[00360] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_3 (**SEQ ID NO:87**): gggagggaaa acatttacta cggtagtagg cttgactac

[00361] **Figures 11A-11B** show the alignment of the amino acid residues of the variable light chains (**Figure 11A**) or variable heavy chains (**Figure 11B**) of BRCA84D and its humanized derivative, hBRCA84D.

[00362] In order to obtain hBRCA84D species that exhibit improved affinity for human B7-H3, polynucleotides encoding the light or heavy chains of hBRCA84D-1 (*i.e.*, hBRCA84D-1VL or hBRCA84D-1VH, respectively) were subjected to mutagenesis, and mutated hBRCA84D-1 light chain derivatives hBRCA84D-2VL, hBRCA84D-3VL, hBRCA84D-4VL, hBRCA84D-5VL, and hBRCA84D-6VL and mutated hBRCA84D-1 heavy chain derivatives hBRCA84D-2VH, hBRCA84D-3VH, and hBRCA84D-4VH were isolated and characterized. The amino acid and polynucleotide sequences of the variable light and heavy chains of these antibodies are presented below:

[00363] hBRCA84D-2VL (**SEQ ID NO:89**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00364] Polynucleotide Encoding hBRCA84D-2VL (**SEQ ID NO:90**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaaggcgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00365] hBRCA84D-3VL (**SEQ ID NO:91**):

DIQLTQSPSF LSASVGDRVS VTCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00366] Polynucleotide Encoding hBRCA84D-3VL (**SEQ ID NO:92**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgtcc gtcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaagctgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00367] hBRCA84D-4VL (**SEQ ID NO:93**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GQAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00368] Polynucleotide Encoding hBRCA84D-4VL (**SEQ ID NO:94**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggccaggccc ctaagctgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00369] hBRCA84D-5VL (**SEQ ID NO:95**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GQAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00370] Polynucleotide Encoding hBRCA84D-5VL (**SEQ ID NO:96**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggccaggccc ctaaggcgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc

tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00371] hBRCA84D-6VL (**SEQ ID NO:97**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYYCQQ YNNYPFTFGQ
GTKLEIK

[00372] Polynucleotide Encoding hBRCA84D-6VL (**SEQ ID NO:98**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaagctgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccgagtacta ctgccagcag tacaacaact acccttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00373] hBRCA84D-2VH (**SEQ ID NO:99**):

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

[00374] Polynucleotide Encoding hBRCA84D-2VH (**SEQ ID NO:100**):

gaggtgcagc tggtcgagtc tggcggagga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg cctccggctt caccttctcc agcttcggca
tgcactgggt ccgccaggct ccaggcaagg gactggaatg ggtggcctac
atctcctccg actcctccgc catctactac gccgacaccg tgaagggcag
gttcaccatc tcccgggaca acgccaagaa ctccctgtac ctgcagatga
actccctgcg ggacgaggac accgccgtgt actactgcgg cagaggccgg
gagaatatct actacggctc ccggctggat tattggggcc agggcaccac
cgtgaccgtg tcctct

[00375] hBRCA84D-3VH (**SEQ ID NO:101**):

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

[00376] Polynucleotide Encoding hBRCA84D-3VH (**SEQ ID NO:102**):

gaggtgcagc tggtcgagtc tggcggagga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg cctccggctt caccttctcc agcttcggca
tgcactgggt ccgccaggct ccaggcaagg gactggaatg ggtggcctac
atctcctccg actcctccgc catctactac gccgacaccg tgaagggcag
gttcaccatc tcccgggaca acgccaagaa ctccctgtac ctgcagatga
actccctgcg ggacgaggac accgccatgt actactgcgg cagaggccgg
gagaatatct actacggctc ccggctggat tattggggcc agggcaccac
cgtgaccgtg tcctct

[00377] hBRCA84D-4VH (**SEQ ID NO:103**):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SFGMHWVRQA PGKGLEWVAY
ISSDSSAIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRSED TAVYYCARGR
ENIYYGSRLD YWGQGTTVTV SS

[00378] Polynucleotide Encoding hBRCA84D-4VH (**SEQ ID NO:104**):

```
gaggtgcagc tggtcgagtc tggcggagga ctggtgcagc ctggcggctc
cctgagactg tcttgcgccg cctccggctt caccttctcc agcttcggca
tgcactgggt ccgccaggct ccaggcaagg gactggaatg ggtggcctac
atctcctccg actcctccgc catctactac gccgacaccg tgaagggcag
gttcaccatc tcccgggaca acgccaagaa ctccctgtac ctgcagatga
actccctgcg gagcgaggac accgccgtgt actactgcgc cagaggccgg
gagaatatct actacggctc ccggctggat tattggggcc agggcaccac
cgtgaccgtg tcctct
```

[00379] **Table 22** lists the hBRCA84D variable light chain and variable heavy chain mutations studied; numbers refer to the Kabat numbering system used in **Figures 11A** and **11B**.

Table 22

Variable Light Chain						Variable Heavy Chain			
Kabat Position	**20**	**21**	**42**	**46**	**85**	**Kabat Position**	**84**	**89**	**93**
BRCA84D	**S**	**V**	**Q**	**A**	**E**	**BRCA84D**	**S**	**M**	**G**
hBRCA84D-1VL	**T**	**I**	**K**	**L**	**T**	**hBRCA84D-1VH**	**D**	**V**	**A**
hBRCA84D-2VL	**T**	**I**	**K**	**A**	**T**	**hBRCA84D-2VH**	**D**	**V**	**G**
hBRCA84D-3VL	**S**	**V**	**K**	**L**	**T**	**hBRCA84D-3VH**	**D**	**M**	**G**
hBRCA84D-4VL	**T**	**I**	**Q**	**L**	**T**	**hBRCA84D-4VH**	**S**	**V**	**A**
hBRCA84D-5VL	**T**	**I**	**Q**	**A**	**T**				
hBRCA84D-6VL	**T**	**I**	**K**	**L**	**E**				

[00380] The relative binding affinities of the hBRCA84D light chain derivatives hBRCA84D-3VL, hBRCA84D-4VL and hBRCA84D-5VL for human B7-H3 were determined by forming antibodies containing these light chain variable regions and a chimeric BRCA84D-1VH heavy chain (**Figure 12**). BRCA84D-5VL (K42Q, L46A) was found to have the highest binding affinity of the hBRCA84D-VL tested. BRCA84D-5VL was therefore used as the light chain to investigate the relative binding affinities of the hBRCA84D heavy chains of hBRCA84D-1VH, hBRCA84D-2VH, hBRCA84D-3VH and hBRCA84D-4VH for human B7-H3 (**Figure 13**). hBRCA84D-2VH (A93G) was found to have the highest binding affinity of the hBRCA84D-VH tested.

[00381] The amino acid and encoding polynucleotide sequences of the chimeric BRCA84D-1 are as follows:

[00382] chBRCA84D Light Chain (**SEQ ID NO: 105**):

```
DIAMTQSQKF MSTSVGDRVS VTCKASQNVD TNVAWYQQKP GQSPKALIYS
ASYRYSGVPD RFTGSGSGTD FTLTINNVQS EDLAEYFCQQ YNNYPFTFGS
GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC
```

[00383] Polynucleotide Encoding chBRCA84D Light Chain (**SEQ ID NO: 106**):

```
gacattgcga tgacccagtc tcaaaaattc atgtccacat cagtaggaga
cagggtcagc gtcacctgca aggccagtca gaatgtggat actaatgtag
cctggtatca acagaaacca gggcaatctc ctaaagcact gatttactcg
```

gcatcctacc ggtacagtgg agtccctgat cgcttcacag gcagtggatc
tgggacagat ttcactctca ccatcaacaa tgtgcagtct gaagacttgg
cagagtattt ctgtcagcaa tataacaact atccattcac gttcggctcg
gggacaaagt tggaaataaa acgtacggtg gctgcaccat ctgtcttcat
cttcccgcca tctgatgagc agttgaaatc tggaactgcc tctgttgtgt
gcctgctgaa taacttctat cccagagagg ccaaagtaca gtggaaggtg
gataacgccc tccaatcggg taactcccag gagagtgtca cagagcagga
cagcaaggac agcacctaca gcctcagcag caccctgacg ctgagcaaag
cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag

[00384] chBRCA84D Heavy Chain (**SEQ ID NO: 107**):

DVQLVESGGG LVQPGGSRKL SCAASGFTFS SFGMHWVRQA PEKGLEWVAY
ISSDSSAIYY ADTVKGRFTI SRDNPKNTLF LQMTSLRSED TAMYYCGRGR
ENIYYGSRLD YWGQGTTLTV SSASTKGPSV FPLAPSSKST SGGTAALGCL
VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT
QTYICNVNHK PSNTKVDKRV EPKSCDKTHT CPPCPAPELL GGPSVFLFPP
KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE
PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
GK

[00385] Polynucleotide Encoding chBRCA84D Heavy Chain (**SEQ ID NO: 108**):

gatgtgcagc tggtggagtc tgggggaggc ttagtgcagc ctggagggtc
ccggaaactc tcctgtgcag cctctggatt cactttcagt agctttggaa
tgcactgggt tcgtcaggct ccagagaagg ggctggagtg ggtcgcatac
attagtagtg acagtagtgc catctactat gcagacacag tgaagggccg
attcaccatc tccagagaca atcccaagaa caccctgttc ctgcaaatga
ccagtctaag gtctgaggac acggccatgt attactgtgg aagagggagg
gaaaacattt actacggtag taggcttgac tactggggcc aaggcaccac
tctcacagtc tcctcagcct ccaccaaggg cccatcggtc ttcccctgg
caccctcctc caagagcacc tctgggggca cagcggccct gggctgcctg
gtcaaggact acttccccga accggtgacg gtgtcgtgga actcaggcgc
cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag tcctcaggac
tctactccct cagcagcgtg gtgaccgtgc cctccagcag cttgggcacc
cagacctaca tctgcaacgt gaatcacaag cccagcaaca ccaaggtgga
caagagagtt gagcccaaat cttgtgacaa aactcacaca tgcccaccgt
gcccagcacc tgaactcctg gggggaccgt cagtcttcct cttccccca
aaacccaagg acaccctcat gatctcccgg acccctgagg tcacatgcgt
ggtggtggac gtgagccacg aagaccctga ggtcaagttc aactggtacg
tggacggcgt ggaggtgcat aatgccaaga caaagccgcg ggaggagcag
tacaacagca cgtaccgtgt ggtcagcgtc ctcaccgtcc tgcaccagga
ctggctgaat ggcaaggagt acaagtgcaa ggtctccaac aaagccctcc
cagccccat cgagaaaacc atctccaaag ccaaagggca gccccgagaa
ccacaggtgt acaccctgcc cccatcccgg gatgagctga ccaagaacca
ggtcagcctg acctgcctgg tcaaaggctt ctatcccagc gacatcgccg
tggagtggga gagcaatggg cagccggaga acaactacaa gaccacgcct

cccgtgctgg actccgacgg ctccttcttc ctctacagca agctcaccgt
ggacaagagc aggtggcagc aggggaacgt cttctcatgc tccgtgatgc
atgaggctct gcacaaccac tacacgcaga agagcctctc cctgtctccg
ggtaaatga

[00386] The relative binding affinities of antibodies containing: (1) hBRCA84D-2VL and hBRCA84D-2VH (two trials), (2) chimeric BRCA84D, (3) antibody containing hBRCA84D-5VL and chimeric BRCA84D-HC, and (4) antibody containing hBRCA84D-5VL and hBRCA84D-2VH were compared. The results are shown in **Figure 14**.

Example 11
Humanized Anti-B7-H3 Antibodies Inhibit Tumor Growth In Xenografts

[00387] In order to demonstrate the ability of humanized anti-B7-H3 antibodies to inhibit tumor growth *in vivo*, tumor growth of HT-1197 urinary bladder carcinoma cells and of A498 renal carcinoma cells was studied in a murine xenograft. Humanized antibody hBRCA84D-2 (hBRCA84D-2 VL chain / hBRCA84D-2 VL chain) was modified to comprise an Fc region having substitutions L235V, F243L, R292P, Y300L, and P396L. The Fc-modified hBRCA84D-2 antibody was administered to the mice (at a dose of 1 μg/kg, 10 μg/kg, or 20 μg/kg) 7 days, 14 days, 21 days and 28 days post implantation of the cancer cells. The results show that at all doses the administered Fc-modified hBRCA84D-2 antibody was capable of inhibiting tumor growth of HT-1197 urinary bladder carcinoma cells (**Figure 15**) and of A498 renal carcinoma cells (**Figure 16**).

Example 12
Dual Affinity Retargeting Reagents (DART™s) Specific For B7-H3 and the T-Cell Receptor Mediate Potent Redirected T-Cell Killing

[00388] Dual affinity retargeting reagents (DART™s) specific for B7-H3 and the T-cell receptor ("TCR") and for the Natural Killer Group 2D (NKG2D) receptor were prepared. Such DART™s have the ability to localize a T-cell (by binding such T-cell to the TCR-binding portion of a TCR-binding DART™) or o localize a NK-cell (by binding such NK cell to the NKG2D-binding portion of an NKG2D -binding DART™) to the location of a cancer cell (by binding such cancer cell to the B7-H3-binding portion of the DART™). The localized T-cell or NK cell can then mediate the killing of the cancer cell in a process termed herein "redirected" killing.

[00389] The dual affinity retargeting reagent (DART™) specific for B7-H3 and the T-cell receptor ("TCR") was constructed having the anti-B7-H3 variable domains of hBRCA84D-2 and anti-TCR variable domains:

[00390] TCR VL x hBRCA84D VH-2-E Coil DART™ Chain (**SEQ ID NO: 109**):

EIVLTQSPAT LSLSPGERAT LSCSATSSVS YMHWYQQKPG KAPKRWIYDT
SKLASGVPSR FSGSGSGTEF TLTISSLQPE DFATYYCQQW SSNPLTFGQG

TKLEIK**GGGS** **GGGG**EVQLVE SGGGLVQPGG SLRLSCAASG FTFSSFGMHW
VRQAPGKGLE WVAYISSDSS AIYYADTVKG RFTISRDNAK NSLYLQMNSL
RDEDTAVYYC GRGRENIYYG SRLDYWGQGT TVTVSS**GGCG** **GG**EVAALEKE
VAALEKEVAA LEKEVAALEK

[00391] Polynucleotide Encoding TCR VL x hBRCA84D VH-2-E Coil DART™ Chain (**SEQ ID NO: 110**):

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga
aagagccacc ctctcctgca gtgccacctc aagtgtaagt tacatgcact
ggtatcagca gaaaccaggg aaagccccta agcgctggat ctatgacaca
tccaaactgg cttctggggt cccatcaagg ttcagcggca gtggatctgg
gacagaattt actctcacaa tcagcagcct gcagcctgaa gattttgcaa
cttattactg tcagcagtgg agtagtaacc cgctcacgtt tggccagggg
accaagcttg agatcaaa**gg** **aggcggatcc** **ggcggcggag** **gc**gaggtgca
gctggtcgag tctggcggag gactggtgca gcctggcggc tccctgagac
tgtcttgcgc cgcctccggc ttcaccttct ccagcttcgg catgcactgg
gtccgccagg ctccaggcaa gggactggaa tgggtggcct acatctcctc
cgactcctcc gccatctact acgccgacac cgtgaagggc aggttcacca
tctcccggga caacgccaag aactccctgt acctgcagat gaactccctg
cgggacgagg acaccgccgt gtactactgc ggcagaggcc gggagaatat
ctactacggc tcccggctgg attattgggg ccagggcacc accgtgaccg
tgtcctcc**gg** **aggatgtggc** **ggtgga**gaag tggccgcact ggagaaagag
gttgctgctt tggagaagga ggtcgctgca cttgaaaagg aggtcgcagc
cctggagaaa

[00392] hBRCA84DVL-2 x TCR VH – K coil Chain (**SEQ ID NO: 111**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK**GGG** **SGGGG**QVQLV QSGAEVKKPG ASVKVSCKAS GYKFTSYVMH
WVRQAPGQGL EWIGYINPYN DVTKYNEKFK GRVTITADKS TSTAYLQMNS
LRSEDTAVHY CARGSYYDYD GFVYWGQGTL VTVSS**GGCGG** **G**KVAALKEKV
AALKEKVAAL KEKVAALKE

[00393] Polynucleotide Encoding hBRCA84DVL-2 x TCR VH – K coil Chain (**SEQ ID NO: 112**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaaggcgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g**gaggcgga** **tccggcggcg** **gaggc**caggt
tcagctggtg cagtctggag ctgaggtgaa gaagcctggg gcctcagtga
aggtctcctg caaggccagc ggttacaagt ttaccagcta cgtgatgcac
tgggtgcgac aggcccctgg acaagggctt gagtggatcg gatatattaa
tccttacaat gatgttacta agtacaatga gaagttcaaa ggcagagtca
cgattaccgc ggacaaatcc acgagcacag cctacctgca gatgaacagc
ctgagatccg aggacacggc cgtgcactac tgtgcgagag ggagctacta
tgattacgac gggtttgttt actggggcca agggactctg gtcactgtga

gctccggagg atgtggcggt ggaaaagtgg ccgcactgaa ggagaaagtt gctgctttga aagagaaggt cgccgcactt aaggaaaagg tcgcagccct gaaagag

[00394] The dual affinity retargeting reagent (DART™) specific for B7-H3 and the Natural Killer Group 2D (NKG2D) receptor was constructed having the anti-B7-H3 variable domains of hBRCA84D-2 and anti-TCR variable domains:

[00395] NKG2D VL x hBRCA84D VH-2-E Coil DART™ Chain (**SEQ ID NO: 113**):

QSALTQPASV SGSPGQSITI SCSGSSSNIG NNAVNWYQQL PGKAPKLLIY YDDLLPSGVS DRFSGSKSGT SAFLAISGLQ SEDEADYYCA AWDDSLNGPV FGGGTKLTVL GGGSGGGGEV QLVESGGGLV QPGGSLRLSC AASGFTFSSF GMHWVRQAPG KGLEWVAYIS SDSSAIYYAD TVKGRFTISR DNAKNSLYLQ MNSLRDEDTA VYYCGRGREN IYYGSRLDYW GQGTTVTVSS GGCGGGEVAA LEKEVAALEKE VAALEKEVA ALEK

[00396] Polynucleotide Encoding NKG2D VL x hBRCA84D VH-2-E Coil DART™ Chain (**SEQ ID NO: 114**):

cagtctgccc tgactcagcc tgcctccgtg tctgggtctc ctggacagtc aatcaccatc tcctgttctg gaagcagctc caacatcgga aataatgctg ttaactggta ccagcagctc ccaggaaagg ctcccaaact cctcatctat tatgatgacc tactgccctc aggggtctct gaccgattct ctggctccaa gtctggcacc tcagccttcc tggccatcag tgggctccag tctgaggatg aggctgatta ttactgtgca gcatgggatg acagcctgaa tggtccagtg ttcggcggag ggaccaagct gaccgtccta ggaggcggat ccggcggcgg aggcgaggtg cagctggtcg agtctggcgg aggactggtg cagcctggcg gctccctgag actgtcttgc gccgcctccg gcttcacctt ctccagcttc ggcatgcact gggtccgcca ggctccaggc aagggactgg aatgggtggc ctacatctcc tccgactcct ccgccatcta ctacgccgac accgtgaagg gcaggttcac catctcccgg gacaacgcca agaactccct gtacctgcag atgaactccc tgcgggacga ggacaccgcc gtgtactact gcggcagagg ccgggagaat atctactacg gctcccggct ggattattgg ggccagggca ccaccgtgac cgtgtcctcc ggaggatgtg gcggtggaga agtggccgca ctggagaaag aggttgctgc tttggagaag gaggtcgctg cacttgaaaa ggaggtcgca gccctggaga aa

[00397] hBRCA84DVL-2 x NKG2D VH – K coil Chain (**SEQ ID NO: 115**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ GTKLEIKGGG SGGGGQVQLV ESGGGLVKPG GSLRLSCAAS GFTFSSYGMH WVRQAPGKGL EWVAFIRYDG SNKYYADSVK GRFTISRDNS KNTLYLQMNS LRAEDTAVYY CAKDRGLGDG TYFDYWGQGT TVTVSSGGCG GGKVAALKEK VAALKEKVAA LKEKVAALKE

[00398] Polynucleotide Encoding hBRCA84DVL-2 x NKG2D VH – K coil Chain (**SEQ ID NO: 116**):

gacatccagc tgacccagtc cccctccttc ctgtctgcct ccgtgggcga
cagagtgacc atcacatgca aggcctccca gaacgtggac accaacgtgg
cctggtatca gcagaagcct ggcaaggccc ctaaggcgct gatctactcc
gcctcctacc ggtactccgg cgtgccttcc aggttctccg gctccggctc
tggcaccgac ttcaccctga ccatctccag cctgcagcct gaggacttcg
ccacctacta ctgccagcag tacaacaact acccttcac cttcggccag
ggcaccaagc tggaaatcaa g**ggaggcgga tccggcggcg gaggc**caggt
acagctggtg gagtctgggg gaggcctggt caagcctgga gggtccctga
gactctcctg tgcagcgtct ggattcacct tcagtagcta tggcatgcac
tgggtccgcc aggctccagg caaggggctg gagtgggtgg catttatacg
gtatgatgga agtaataaat actatgcaga ctccgtgaag ggccgattca
ccatctccag agacaattcc aagaacacgc tgtatctgca aatgaacagc
ctgagagctg aggacacggc tgtgtattac tgtgcgaaag atcgaggttt
gggggatgga acctactttg actactgggg ccaagggacc acggtcaccg
tctcctcc**gg aggatgtggc ggtgga**aaag tggccgcact gaaggagaaa
gttgctgctt tgaaagagaa ggtcgccgca cttaaggaaa aggtcgcagc
cctgaaagag

[00399] In order to demonstrate the ability of DART™s to mediate such redirected killing of cancer cells, the above-described hBRCA84D-2 / anti-TCR DART™ ("T-DART™"), hBRCA84D-2, hBRCA84D-2 (Fc-modified: L235V, F243L, R292P, Y300L, and P396L), and a TCR-DART™ control were incubated at various concentrations with target cancer cells (SK-MES-1 lung cancer cells, A498 renal carcinoma cells, LNCaP prostate cancer cells, or UACC-62 melanoma cells) and effector resting PBMC (E:T ratio = 30:1) and cytotoxicity was determined (LDH Assay). The results of these investigations are shown in **Figures 17A-17D** and demonstrate the ability of the hBRCA84D-2 / anti-TCR DART™ ("T-DART™") to mediate redirected killing of cancer cells.

Example 13
Pharmacokinetic Profile in Tumor-Free Mice

[00400] Anti-B7-H3 antibody (Mab1) was injected into male mCD16-/-, hCD16A_FOXN1 mice (5 mg/kg; IV) and serum was assayed (pre-dose and) at 2, 15, 30 min, and 1, 2, 4, 6 hr, and 1, 2, 3, 6, 8, 14, 21, and 28 days after injection. The antibody was found to have a T ½ of 10.54 days and a C_{max} of 43.493 μg/ml. The concentration of antibody over time was found to be biphasic, fitting into a two-component model (**Figures 18A-18B**). The predicted pharmacokinetic profiles generated using a 2-compartment model with parameters from the 5 mg/kg dose are shown in **Figure 18C**.

Example 14
Ability of Anti-B7-H3 antibody to Bind HT-1197 Urinary Bladder Cancer Cells and Prevent or Inhibit Tumor Development in a Murine Xenograft Model

[00401] The above-described anti-B7-H3 antibody (Mab1) was assessed for its ability to bind HT-1197, a human B7-H3-expressing urinary bladder carcinoma cell line. As shown in **Figure 19**, such cells exhibit greater expression of PRCA135 than HER2, and thus are particularly suitable for assessing the therapeutic potential of the antibodies of the present iinvention in remediating HT-1197 tumors. In accordance with this conclusion, anti-B7-H3 antibody hBRCA84D variants were found to be capable of binding to HT-1197 cells. **Figure 20** shows the binding affinity of Mab1 antibodies to HT-1197 cells.

[00402] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 8 x 10^6 HT-1197 cells. The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x5 using doses of 0.1, 0.5, 1, 5, or 10 mg/kg (eight female mice per dose). Centuximab (anti-EGRF antibody) was administered to a control group of mice at doses of 1, 5, or 15 mg/kg (eight female mice per dose). Eight female mice were also injected with vehicle or with 10 mg/kg IgG control. Tumor measurements were made every 3-4 days. The results of the experiment (**Figure 21A**) show that Mab1 was capable of preventing or inhibiting urinary bladder tumor development in the murine xenograft model. **Figure 21B** shows the results obtained using centimab. A comparison of **Figures 21A** and **21B** demonstrate that the antibodies of the present invention are more effective than centimab in preventing or inhibiting urinary bladder tumor development in the murine xenograft model. **Figure 21C** compares the results obtained at the maximum doses tested.

Example 15
Ability of Anti-B7-H3 Antibody to Bind HT-1376 Urinary Bladder Cancer Cells and Prevent or Inhibit Tumor Development in a Murine Xenograft Model

[00403] The above-described anti-B7-H3 antibody (Mab1) was assessed for its ability to bind HT-1376, a human B7-H3-expressing urinary bladder carcinoma cell line. As shown in **Figures 22A-22B**, such cells exhibit greater expression of PRCA135 than HER2 or PMSA, and thus are particularly suitable for assessing the therapeutic potential of the antibodies of the present iinvention in remediating HT-1376 tumors. In accordance with this conclusion, anti-B7-H3 antibody hBRCA84D variants were found to be capable of binding to HT-1197 cells. **Figures 22A-22B** show the binding affinity of Mab1 antibodies to HT-1197 cells.

[00404] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 5 x 10^6 HT-1376 cells. The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x4

at a dose of 1 mg/kg. The results of the experiment (**Figure 23**) show that Mab1 was capable of preventing or inhibiting urinary bladder tumor development in the murine xenograft model.

Example 16
Ability of Anti-B7-H3 Antibody to Bind Cancer Cells

[00405] Anti-B7-H3 antibody BRCA84D was assessed via FACS analysis for its ability to bind: SW480 and SW620 colorectal cancer cells; AGS gastric cancer cells; M-14 and LOX IMVI melanoma cells; 22rv prostate cancer cells; AsPC-1 and BxPc-3 pancreatic cancer cells; A498 and 786-0 renal cancer cells. The antibody was found to be able to bind to all such cells.

Example 17
Ability of Anti-B7-H3 Antibody Prevent or Inhibit Gastric Tumor Development in a Murine Xenograft Model

[00406] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 5 x 10^6 AGS cells. The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x5 using doses of 0.5, 1, 5, or 10 mg/kg. The results of the experiment (**Figure 24**) show that Mab1 was capable of preventing or inhibiting gastric tumor development in the murine xenograft model.

Example 18
Ability of Anti-B7-H3 Antibody to Bind Lung Cancer Cells and Prevent or Inhibit Tumor Development in a Murine Xenograft Model

[00407] A549 lung cancer cells were incubated in the presence of hBRCA84D, chBRCA84D and hBRCA84 (0264 Fc) variant and the cytotoxic effect of these antibodies was determined. The results of this experiment are shown in **Figure 25**, and indicate that all three of the antibodies were cytotoxic to A549 cells.

[00408] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 8 x 10^6 A549 cells. The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x4 using a dose of 1 mg/kg. The results of the experiment (**Figure 26**) show that Mab1 was capable of preventing or inhibiting of lung cancer tumor development in the murine xenograft model.

[00409] FACS analysis was conducted on CaLu3 lung cancer cells in order to determine whether such cells bind anti-B7-H3 antibodies. The experiment confirmed that such cells express B7-H3 and bind to the antibodies of the present invention. To determine whether the antibodies of the present invention were effective to prevent or inhibit lung cancer tumor development, mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 5 x 10^6 CaLu3 cells. The

tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x4 using a dose of 0.5, 1, or 5 mg/kg. The results of the experiment (**Figure 27**) show that Mab1 was capable of preventing or inhibiting of lung cancer tumor development in the murine xenograft model.

Example 19
Ability of Anti-B7-H3 Antibody to Prevent or Inhibit LOX Melanoma Tumor Development in a Murine Xenograft Model

[00410] Mice (eight female mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with LOX-IMVI melanoma cancer cells and then inoculated iv/Q7D x3 with PBS control, IgG control (5/mg/kg), Mab1 (0.5, 1, 5 or 10 mg/kg), or ip/BIWx2 with Doxetaxel (5, 10 or 20 mg/kg). The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation. The results of the experiment (**Figures 28A-28C**) show that Mab1 was capable of preventing or inhibiting of melanoma cancer tumor development in the murine xenograft model.

Example 20
Ability of Anti-B7-H3 Antibody to Prevent or Inhibit UACC-62 Melanoma Tumor Development in a Murine Xenograft Model

[00411] Mice (eight female mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with UACC-62 melanoma cancer cells and then inoculated iv/Q7D x5 with PBS control, IgG control (5/mg/kg) or Mab1 (0.5, 1, 5 or 10 mg/kg). The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation. The results of the experiment (**Figure 29**) show that Mab1 was capable of preventing or inhibiting of melanoma cancer tumor development in the murine xenograft model.

Example 21
Ability of Anti-B7-H3 Antibody to Prevent or Inhibit 22rv Prostate Tumor Development in a Murine Xenograft Model

[00412] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 6 x 10^6 22rv prostate cancer cells and then inoculated iv/Q7D x4 with PBS control, IgG (10 mg/kg), Mab1 (0.5, 1, 5, or 10 mg/kg; Q7D x5) or trastuzumab (1. 7 or 15 mg/kg). The tumor cells were implanted in 200 µl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation. The results of the experiment (**Figures 30A-30C**) show that Mab1 was capable of preventing or inhibiting of prostate cancer tumor development in the murine xenograft model.

Example 22
Ability of Anti-B7-H3 Antibody to Bind Renal Cancer Cells and Prevent or Inhibit Tumor Development in a Murine Xenograft Model

[00413] A498 renal cancer cells were incubated in the presence of hBRCA84D, chBRCA84D and hBRCA84 (0264 Fc) variant and the cytotoxic effect of these antibodies was determined. The results of this experiment are shown in **Figure 31**, and indicate that all three of the antibodies were cytotoxic to A498 cells.

[00414] A498 renal cancer cells were incubated in the presence of hBRCA84D, chBRCA84D and hBRCA84 (0264 Fc) variant and the cytotoxic effect of these antibodies was determined. The results of this experiment are shown in **Figure 31**, and indicate that all three of the antibodies were cytotoxic to A498 cells.

[00415] IHC analysis of the A498 xenograft tumor tissue was conducted using biotinylated BRCA84D antibody (20 μg/ml), BRCA69D (5 μg/ml) and anti-Her2 antibody (20 μg/ml). BRCA84D antibody was found to bind 20-40% of tumor tissue (weakly to moderately: + or ++); BRCA69D was found to bind 80-100% of tumor tissue (moderately to strongly: ++ or +++). BRCA84D antibody was found to weakly bind 40% of UMUC-3 tumor tissue (+); BRCA69D was found to moderately or strongly bind 70% of such tumor tissue (++ or +++); anti-Her2 antibody was found to variably bind 20% of such tumor tissue (+ -+++). As controls, anti-Her2 antibody was found to bind SKBR-3 cells (+++) and BRCA84D and BRCA69D were found to be able to bind Hs 700T cells (+++)

[00416] Mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 5 x 10^6 A498 renal cancer cells. The tumor cells were implanted in 200 μl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x5 using doses of 0.1, 0.5, 1, 5, or 10 mg/kg. Centuximab (anti-EGRF antibody) was administered to a control group of mice at doses of 1, 7, or 15 mg/kg. Additional control mice were injected with vehicle or with 10 mg/kg IgG control. The results of the experiment (**Figure 32**) show that Mab1 was capable of preventing or inhibiting renalcancer tumor development in the murine xenograft model.

[00417] Mice (mCD16-/-, hCD16A+_FoxN1) were alternatively implanted subcutaneously on their flanks with 5 x 10^6 786-0 renal cancer cells. The tumor cells were implanted in 200 μl of Ham's F12 Medium diluted 1:1 with MATRIGEL™. Treatment with Mab1 was initiated within 7 days of implantation via iv Q7D x5 using doses of 0.1, 0.5, 1, 5, or 10 mg/kg. Centuximab (anti-EGRF antibody) was administered to a control group of mice at doses of 1, 7, or 15 mg/kg. Additional control mice were injected with vehicle or with 10 mg/kg IgG control. The results of the experiment

(**Figures 33A-33B**) show that Mab1 was capable of preventing or inhibiting renal cancer tumor development in the murine xenograft model.

[00418] The activity of Mab1 was compared with that of paclitaxel, a mitotic inhibitor used in cancer chemotherapy. Groups of eight female mice (mCD16-/-, hCD16A+_FoxN1) were implanted subcutaneously on their flanks with 786-0 renal cancer cells and then provided with Mab1 via iv Q7D at doses of 0.1, 0.5, 1, 5, or 10 mg/kg. Paclitaxel was administered to a control group of eight such mice at a dose of 2.5 mg/kg on study day 21, 28, and 35. Additional control mice (seven female per group) were injected with vehicle or with 5 mg/kg IgG control. The results of the experiment (**Figure 34**) show that Mab1 was capable of preventing or inhibiting renal cancer tumor development in the murine xenograft model.

Example 23
Cynomolgous Monkey Toxicology Study

[00419] A cynomolgous monket toxicology study is conducted in order to assess acute toxicology profile after a single dose of Mab1, determine the pharmacokinetic profile for Mab1, establish a time vs. dose relationship for induction of cytokines associated with effector cell activation, and assess the effect of drug treatment on the level of circulating leukocytes (*e.g.*, NK and T-cells).

[00420] Such a study may be designed to involve four groups of 6 monkeys (3 males and 3 females) and to extend 7 weeks from initial tratement to final necropsy. Group 1 would comprise a control group that would receive only vehicle for weeks 1 and 2. Four members of Group 1 (two males and two females) would be sacrificed at week 3. The remaining members of Group 1 would receive additional vehicle at week 3 and be sacrificed for necropsy at week 7. Groups 2-4 are experimental groups that would receive vehicle at week 1, and B7-H3 antibody (1, 30, or 100 mg/kg, respectively) at week 2. Four members of each Group (two males and two females) would be sacrificed at week 3. The remaining members of each Group would receive additional vehicle at week 3 and be sacrificed for necropsy at week 7.

[00421] All infusions are well tolerated and no mortality or significant changes in body weight, clinical signs or serum chemistry are observed. Dose-dependent reductions in circulating NK cells but not in circulating B- and T-cells are observed.

The study provides verification of cynomolgus monkey as a relevant toxicological species. When contacted with normal human tissue, antibody BRCA84D showed various degrees of staining intensity in liver, pancreas, colon, lung and adrenal cortex. Liver staining was relatively restricted to sinusoid lining cells (fibroblast and kupffer cells). Pancreas staining was observed in collagen fiber mainly and a small percentage of the epithelium (acinar cells and/or intercalated duct cells). Colon

staining was relatively restricted in apical membrane of crypt epithelium and fibroblast in mucosa. Lung showed very weak and patchy staining in the epithelium. BRCA84D showed good cross-reactivity in cynomolgus monkey tissues in comparison to the human tissue profile with the exception of the lack of staining in the liver and pancreas, and possible expression of B7-H3 in cynomolgus monkey pituitary cells.

[00422] 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. An isolated antibody or an immunoreactive fragment thereof, wherein said isolated antibody or said fragment comprises a variable domain that specifically binds an extracellular domain of B7-H3, wherein said antibody competes for binding to said B7-H3 with any of antibodies: BRCA69D, BRCA84D, or PRCA157.

Claim 2. The isolated antibody or immunoreactive fragment thereof of claim 1, wherein said antibody or said fragment comprises a variable domain that comprises:

(A) CDR_1 (**SEQ ID NO: 21**), CDR_2 (**SEQ ID NO: 23**) and CDR_3 (**SEQ ID NO: 25**) of the light chain of BRCA69D and CDR_1 (**SEQ ID NO: 29**), CDR_2 (**SEQ ID NO: 31**) and CDR_3 (**SEQ ID NO: 33**) of the heavy chain of BRCA69D;

(B) CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; or

(C) CDR_1 (**SEQ ID NO: 37**), CDR_2 (**SEQ ID NO: 39**) and CDR_3 (**SEQ ID NO: 41**) of the light chain of PRCA157 and CDR_1 (**SEQ ID NO: 45**), CDR_2 (**SEQ ID NO: 47**) and CDR_3 (**SEQ ID NO: 49**) of the heavy chain of PRCA157.

Claim 3. The isolated antibody or immunoreactive fragment thereof of any one of claims 1-2, wherein said antibody binds to B7-H3 that is endogenously expressed on the surface of a cancer cell.

Claim 4. The isolated antibody or immunoreactive fragment thereof of any one of claims 1-2, wherein said antibody binds to B7-H3 that is internalized upon binding to B7-H3 expressed on the surface of a cancer cell.

Claim 5. The isolated antibody or immunoreactive fragment thereof of any of claims 1-4, which is a humanized monoclonal antibody.

Claim 6. The isolated antibody of any of claims 1-5, wherein said antibody is a modified antibody that comprises a variant human IgG1 Fc region, wherein said variant human IgG1 Fc region comprises at least one amino acid modification relative to the Fc region of the parent of said antibody, said amino acid modification(s) comprising amino acid modification(s) that alters the affinity or avidity of said variant Fc region

for binding to an FcγR such that said modified antibody exhibits enhanced effector function relative to said parent antibody.

Claim 7. The antibody of claim 6, wherein said Fc region modification comprises:

(A) at least one substitution selected from the group consisting of:
  (1) F243L;
  (2) D270E;
  (3) R292P;
  (4) S298N;
  (5) Y300L;
  (6) V305I;
  (7) A330V; and
  (8) P396L;

(B) at least one substitution of two amino acid residues, said substitutions being selected from the group consisting of:
  (1) F243L and P396L;
  (2) F243L and R292P; and
  (3) R292P and V305I;

(C) at least one substitution of three amino acid residues, said substitutions being selected from the group consisting of:
  (1) F243L, R292P and Y300L;
  (2) F243L, R292P and V305I;
  (3) F243L, R292P and P396L; and
  (4) R292P, V305I and P396L;

(D) at least one substitution of four amino acid residues, said substitutions being selected from the group consisting of:
  (1) F243L, R292P, Y300L and P396L; and
  (2) F243L, R292P, V305I and P396L;

or

(E) a substitution of at least the five amino acid residues: F243L, R292P, Y300L, V305I and P396.

Claim 8. The antibody of claim 7, wherein said antibody comprises substitutions of:

(A) F243L, R292P, and Y300L;

(B) L235V, F243L, R292P, Y300L, and P396L; or

(C) F243L, R292P, Y300L, V305I, and P396L.

Claim 9. The antibody of claim 8, wherein said antibody comprises comprises:

(A) a variable domain that comprises CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; and

(B) an Fc region modification that comprises the substitutions: L235V, F243L, R292P, Y300L, and P396L.

Claim 10. The antibody of claim 9, wherein said antibody is a chimeric antibody.

Claim 11. The antibody of claim 9, wherein said antibody is a humanized antibody.

Claim 12. The isolated antibody or immunoreactive fragment thereof of claim 1, wherein said antibody comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO: 89**);

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO: 99**); and

(C) an Fc-region having the substitutions: L235V, F243L, R292P, Y300L, and P396L.

Claim 13. A hybridoma that secretes a monoclonal antibody that specifically binds an extracellular domain of B7-H3, wherein said antibody competes for binding to said B7-H3 with any of antibodies: BRCA69D, BRCA84D, or PRCA157.

Claim 14. A nucleic acid molecule that encodes the isolated antibody of any of claims 1-12 or the immunoreactive fragment of any of claims 1-5.

Claim 15. A dual affinity retargeting reagent (DART™), said reagent comprising:

(A) a polypeptide chain I that comprises an immunoglobulin VL epitope binding domain specific for binding B7-H3 and a VH epitope binding domain specific for binding a molecule other than B7-H3; and

(B) a polypeptide chain II that comprises an immunoglobulin VH epitope binding domain specific for binding B7-H3 and a VL epitope binding domain specific for binding said molecule other than B7-H3;

wherein said polypeptide chains I and II are associated together so as to form functional epitope binding domains capable of binding to B7-H3 and said molecule other than B7-H3.

Claim 16. The dual affinity retargeting reagent (DART™) of claim 15, wherein said molecule other than B7-H3 that may be bound by said DART™ is a hapten.

Claim 17. The dual affinity retargeting reagent (DART™) of claim 16, wherein said hapten is fluorescein isothiocyanate.

Claim 18. The dual affinity retargeting reagent (DART™) of claim 15, wherein said molecule other than B7-H3 that may be bound by said DART™ is a T-Cell Receptor or the NKG2D receptor.

Claim 19. The dual affinity retargeting reagent (DART™) of claim 15, wherein said molecule other than B7-H3 that may be bound by said DART™ is a tumor-associated antigen.

Claim 20. The dual affinity retargeting reagent (DART™) of claim 19, wherein said tumor-associated antigen is selected from the group consisting of A33; ADAM-9; ALCAM; BAGE; beta-catenin; CA125; Carboxypeptidase M; CD103; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD36; CD40/CD154; CD45; CD46; CD5; CD56; CD79a/CD79b; CDK4; CEA; CTLA4; Cytokeratin 8; EGF-R; EphA2; ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; gp100; HER-2/neu; human papillomavirus-E6; human papillomavirus-E7; Integrin Alpha-V-Beta-6; JAM-3; KID3; KID31; KSA (17-1A); LUCA-2; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; N-acetylglucosaminyltransferase; Oncostatin M; p15; PIPA; PSA; PSMA; ROR1; sTn; TNF-ß receptor; TNF-α receptor; TNF-γ receptor; Transferrin Receptor; and VEGF receptor.

Claim 21. A nucleic acid molecule that encodes a polypeptide chain of the dual affinity retargeting reagent (DART™) of any of claims 15-20.

Claim 22. A pharmaceutical composition comprising (i) a therapeutically effective amount of the isolated antibody of any of claims 1-12 or the immunoreactive fragment of any of claims 1-5 or the dual affinity retargeting reagent (DART™) of any of claims 15-20 and (ii) a pharmaceutically acceptable carrier.

Claim 23. The pharmaceutical composition of claim 21, wherein said antibody is a humanized antibody that comprises:

(A) a variable domain that comprises CDR_1 (**SEQ ID NO: 5**), CDR_2 (**SEQ ID NO: 7**) and CDR_3 (**SEQ ID NO: 9**) of the light chain of BRCA84D and CDR_1 (**SEQ ID NO: 13**), CDR_2 (**SEQ ID NO: 15**) and CDR_3 (**SEQ ID NO: 17**) of the heavy chain of BRCA84D; and

(B) an Fc region modification that comprises the substitutions: L235V, F243L, R292P, Y300L, and P396L.

Claim 24. The pharmaceutical composition of claim 21, wherein said antibody is a humanized antibody that comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO: 89**);

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO: 99**); and

(C) an Fc-region having the substitutions: L235V, F243L, R292P, Y300L, and P396L.

Claim 25. The pharmaceutical composition of any of claims 22-24, which further comprises one or more additional anti-cancer agents.

Claim 26. The pharmaceutical composition of any of claims 22-24 wherein said additional anti-cancer agent is a chemotherapeutic agent, a radiation therapeutic agent, a hormonal therapeutic agent, a toxin or an immunotherapeutic agent.

Claim 27. The pharmaceutical composition of any of claims 22-24 wherein said additional anti-cancer agent is a toxin selected from the group consisting of: a taxane, a maytansinoid, an auristatin, a calicheamicin, an anthracycline, a CC-1065 analog, docetaxel, a cathepsin, ricin, gelonin, *Pseudomonas* exotoxin, diphtheria toxin, RNase, and a toxic radioisotope.

Claim 28. The use of the isolated antibody of any of claims 1-12 or the immunoreactive fragment of any of claims 1-5 or the dual affinity retargeting reagent (DART™) of any of claims 15-20 in the diagnosis of cancer, wherein said isolated antibody, immunoreactive fragment, or DART™ is detectably labeled.

Claim 29. The use of claim 28, characterised in that said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of 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 dhordoma, 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, 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 multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 unveal melanoma, a rare hematologic disorder, 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.

Claim 30. The use of the isolated antibody of any of claims 1-12 or the immunoreactive fragment of any of claims 1-5 or the dual affinity retargeting reagent (DART™) of any of claims 15-20 or of the pharmaceutical composition of any of claims 22-27 in the preparation of a medicament for the treatment of cancer in a patient.

Claim 31. The use of any of claims 29-30, characterised in that said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of 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 dhordoma, 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, 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 multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic 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 unveal melanoma, a rare hematologic disorder, 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.

Claim 32. The use of any of claims 29-31, characterised in that the use further comprises administration of one or more additional cancer therapies selected from the group consisting of chemotherapy, immunotherapy, radiation therapy, hormonal therapy, and surgery.