Title of the Invention:

Improved Methods for the Treatment of Vascularizing Cancers

Reference to Sequence Listing:

[0001] 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 entirety.

Background of the Invention:

Field of the Invention:

[0002] The invention concerns therapeutic compositions for the treatment of vascularizing cancers, especially, glioblastoma. In particular, the invention is directed to compositions that comprise a molecule having a binding ability that is specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR). The invention is additionally directed to the use of such compositions in the treatment of such cancers, and in particular, in the treatment of glioblastoma.

Description of Related Art:

I. The B7 Superfamily and B7-H3

[0003] 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).

[0004] 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).

[0005] 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).

[0006] 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).

[0007] 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).

[0008] 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).

[0009] 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).

[0010] 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).

[0011] 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).

[0012] 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).

[0013] 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. VEGF and VEGFR

[0014] Vascular endothelial growth factors (VEGFs) belong to the platelet-derived growth factor supergene family, and play central roles in the regulation of angiogenesis and lymphangiogenesis. The VEGF family is divided into five members having a homodimer structure: VEGF-A, VEGF-B, VEGF-C, VEGFD, and placental growth factor (PlGF) (Takahashi, S. (2011) "*Vascular Endothelial Growth Factor (VEGF), VEGF Receptors And Their Inhibitors For Antiangiogenic Tumor Therapy*," Biol. Pharm. Bull. 34(12):1785-1788; Sullivan, L.A. (2010) "*The VEGF Family In Cancer And Antibody-Based Strategies For Their Inhibition*," mAbs 2:2:165-175). These peptides are encoded by individual genes. In addition, VEGF-A exists in four isoforms. VEGF121, VEGF165, VEGF189, and VEGF206 are generated by alternative mRNA splicing (Houck, K.A. et al. (1991) "*The Vascular Endothelial Growth Factor Family: Identification Of A Fourth Molecular Species And Characterization Of Alternative Splicing Of RNA*," Mol. Endocrinol. 5(12):1806-1814; Tischer, E. *et al.* (1991) "*The*

Human Gene for Vascular Endothelial Growth Factor," J. Biol. Chem. 266:11947-11954). VEGF-A is generally called VEGF, because VEGF-A is a key regulator of developmental vasculogenesis, angiogenesis, and differentiation of progenitor endothelial cells. Among VEGF-A isoforms, VEGF165 is dominant from the aspect of amount and biological activity. VEGF165 is overexpressed in a variety of human tumors, and the overexpression is correlated with progression, invasion, and metastasis of tumors (Maeda, K. *et al.* (1996) "*Prognostic Value Of Vascular Endothelial Growth Factor Expression In Gastric Carcinoma*," Cancer 77:858-863; Ishigami, S.I. *et al.* (1998) "*Predictive Value Of Vascular Endothelial Growth Factor (VEGF) In Metastasis And Prognosis Of Human Colorectal Cancer*," Br. J. Cancer 78:1379-1384; Kaya, M. *et al.* (2000) "*Vascular Endothelial Growth Factor Expression In Untreated Osteosarcoma Is Predictive Of Pulmonary Metastasis And Poor Prognosis*," Clin. Cancer Res. 6:572-577).

[0015] VEGF-A binds to two tyrosine kinase (TK) receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), and regulates endothelial cell proliferation, migration, vascular permeability, secretion and other endothelial functions. VEGFR-2 exhibits a strong TK activity towards pro-angiogenic signals, whereas the soluble VEGFR-1 (sFlt-1) functions as an endogenous VEGF inhibitor (Shibuya, M. (2013) "*Vascular Endothelial Growth Factor And Its Receptor System: Physiological Functions In Angiogenesis And Pathological Roles In Various Diseases*," J. Biochem. 153(1):13-19; Koch, S. *et al.* (2011) "*Signal Transduction By Vascular Endothelial Growth Factor Receptor*s," Biochem. J. 437:169-183; Sullivan, L.A. (2010) "*The VEGF Family In Cancer And Antibody-Based Strategies For Their Inhibition*," mAbs 2:2:165-175; Takahashi, S. (2011) "*Vascular Endothelial Growth Factor (VEGF), VEGF Receptors And Their Inhibitors For Antiangiogenic Tumor Therapy*," Biol. Pharm. Bull. 34(12):1785-1788).

III. Immunotherapy

[0016] 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.

[0017] 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).

[0018] 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.

[0019] 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.

[0020] 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.

[0021] 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.

[0022] 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.

[0023] 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).

IV. Glioblastoma

[0024] Glioblastomas are the most common primary tumor of the central nervous system (CNS), and are the deadliest of human cancers (Mrugala, M.M. (2013) "*Advances And Challenges In The Treatment Of Glioblastoma: A Clinician's Perspective*," Discov. Med. 15(83):221-230; Steiner, H.-H. *et al.* (2004) "*Autocrine Pathways Of The Vascular Endothelial Growth Factor (VEGF) In Glioblastoma Multiforme: Clinical Relevance Of Radiation-Induced Increase Of VEGF Levels*," J. Neuro-Oncol. 66:129-138). Effective therapy for patients with malignant glioma remains elusive, with median survival being under 15 months following standard-of-care therapy with surgery, radiation and temozolomide (Reardon, D.A. *et al.* (2008) "*Glioblastoma Multiforme: An Emerging Paradigm Of Anti-VEGF Therapy*," Expert Opin. Biol. Ther. 8(4):541-553). There is no effective therapy following recurrence (Simpson, L. *et al.* (2006) "*Recurrent Glioblastoma Multiforme: Advances In Treatment And Promising Drug Candidates*," Expert Rev. Anticancer Ther. 6(11):1593-607).

[0025] The use of Anti-VEGF antibodies (Bevacizumab, *e.g.*, Avastin®) has been explored as a therapy for glioblastoma (Gerstner, E.R. *et al.* (2012) "*Antiangiogenic Therapy For Glioblastoma*," Cancer J. 18(1):45-50; Pellegatta, S. *et al.* (2011) "*Brain Cancer Immunoediting: Novel Examples Provided By Immunotherapy Of Malignant Gliomas*," Expert Rev. Anticancer Ther. 11(11):1759-1774; Laigle-Donadey, F. *et al.* (2009) "*Association Of* Radiotherapy *And Chemotherapy-Targeted Therapies In Glioblastomas*," Bull. Cancer. 96(3):291-297; Reardon, D.A. *et al.* (2008) "*Glioblastoma Multiforme: An Emerging Paradigm Of Anti-VEGF Therapy*," Expert Opin. Biol. Ther. 8(4):541-553; Narita, Y. (2013) "*Drug Review: Safety And Efficacy Of Bevacizumab For Glioblastoma And Other Brain Tumors*," Jpn. J. Clin. Oncol. 43(6):587-595. Bevacizumab (Avastin®) is described in United States Patent Publications No. US20020032315A1, US20030190317A1, US20050112126A1, US20070059302A1, US20070059312A1, US20070196374A1, US20070248610A1, US20080187534A1, US20080226629A1, US20110052575A1, US20110081342A1, US20130058927A1; United States Patent No. 6,884,879; 7,060,269; 7,169,901; 7,297,334; 7,365,166; 7,375,193; CA Patents No. 2,286,330 and 2,145,985; and PCT

Publication No. WO 1998/045331; Baca, M. *et al.* (1997) "*Antibody Humanization Using Monovalent Phage Display*," J. Biol. Chem. 272(16):10678-10684; Presta, L.G. *et al.* (1997) "*Humanization Of An Anti-Vascular Endothelial Growth Factor Monoclonal Antibody For The Therapy Of Solid Tumors And Other Disorders*," Cancer Res. 57(20):4593-4599; each of which documents is herein incorporated by reference in its entirety.

[0026] Unfortunately, recurrent glioblastomas (rGBM) invariably relapse after initial response to anti-VEGF therapy (di Tomaso, E. *et al.* (2011) "*Glioblastoma Recurrence after Cediranib Therapy in Patients: Lack of "Rebound" Revascularization as Mode of Escape*," Cancer Res. 71:19-28.

[0027] Thus, despite all prior advances, a need remains for improved compositions for treating vascularizing cancers, and glioblastoma, in particular. The present invention is directed to such compositions and to their use in the treatment of glioblastoma and other cancers involving vascularization.

Summary of the Invention:

[0028] The invention concerns therapeutic compositions for the treatment of vascularizing cancers, especially, glioblastoma. In particular, the invention is directed to compositions that comprise a molecule having a binding ability that is specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR). The invention is additionally directed to the use of such compositions in the treatment of such cancers, and in particular, in the treatment of glioblastoma.

[0029] In detail, the invention provides a method of treating a vascularizing cancer comprising administering to a recipient patient in need thereof, a molecule having a binding ability that is specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis.

[0030] The invention particularly concerns the embodiment of such method wherein the molecule having the binding ability that is specific for B7-H3 is:

(A) an anti-B7-H3 antibody;

(B) a B7-H3-binding fragment of (A); or

(C) a diabody that binds B7-H3.

[0031] The invention particularly concerns the embodiment of such methods wherein the molecule having the binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis is:

(A) an anti-VEGF antibody or a VEGF antagonist; or

(B) an anti-VEGFR antibody or a VEGFR antagonist.

[0032] The invention particularly concerns the embodiment of such methods wherein the molecule having the binding ability that is specific for B7-H3:

A. competes for B7-H3 binding with antibodies BRCA84D, BRCA69D, or PRCA157; or

B. has the three heavy chain CDRs and the three light chain CDRs of antibody BRCA84D, of antibody BRCA69D, or of antibody PRCA157.

[0033] The invention additionally concerns the embodiment of such methods wherein the molecule having the binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis:

A. competes for VEGF binding with bevacizumab; or

B. has the three heavy chain CDRs and the three light chain CDRs of bevacizumab.

[0034] The invention additionally concerns the embodiment of such methods wherein the molecule having the binding ability that is specific for B7-H3 is an anti-B7-H3 antibody having:

(A) a light chain variable domain that comprises the CDR1, CDR2 and CDR3 of the light chain of BRCA84D;

(B) a heavy chain variable domain that comprises the CDR1 CDR2 and CDR3 of the heavy chain of BRCA84D; and

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

[0035] The invention concerns the embodiment of such methods wherein the molecule having the binding ability that is specific for B7-H3 is a humanized anti-B7-H3 antibody that comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO:69**); and

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO:79**);

and the molecule having the binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis is bevacizumab.

[0036] The invention additionally concerns the embodiment of such methods wherein the molecule having the B7-H3 binding ability and the molecule having the VEGF-binding ability are administered to the patient, and the two molecules are administered concurrently.

[0037] The invention additionally concerns the embodiment of such methods wherein the second of the administered molecules is administered to the patient within 5 half-lives after the administration of the first of the molecule.

[0038] The invention additionally concerns the embodiment of such methods wherein the vascularizing cancer is glioblastoma.

[0039] The invention additionally concerns the embodiment of such methods wherein the molecule has the binding ability that is specific for B7-H3 and the molecule having the binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis are the same molecule, the molecule being a bi-specific antibody or a bi-specific diabody.

Brief Description of the Drawings:

[0040] **Figure 1** shows MRI scans of glioblastoma Patient NF. The first, second and third columns are, respectively, MRI scans of a first, second and third observed lesion.

Row A shows the status of the patient's lesions at baseline. **Row B** shows the status of the patient's lesions prior to the initiation of a second planned cycle of anti-B7-H3 antibody administration. **Row C** shows the status of the patient's lesions after 3 doses of bevacizumab and one dose of CCNU.

[0041] **Figures 2A-2C** show MRI scans of glioblastoma Patient MR. The first, second, third and fourth columns are, respectively, MRI scans of a first, second, third and fourth observed lesion. **Row A** shows the status of the patient's lesions at baseline. **Row B** shows the status of the patient's lesions prior to the initiation of a second planned cycle of anti-B7-H3 antibody administration. **Row C** shows the state of Lesions 1-5 at the time anti-B7-H3 antibody therapy was stopped. The status post administration of bevacizumab is shown in **Row D** of the Figures.

[0042] **Figure 3** shows MRI scans of glioblastoma Patient GC. **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken prior to the start of cycle 6) shows that the lesions were stable through 5-6 cycles of anti-B7-H3 antibody administration, with a possible minor response. **Row C** (images taken at the end of cycle 6) show signs of progressive disease, including the occurrence of new lesions. The patient experienced cognitive decline and edema was noted in the patient's brain (**Row C**, fourth column). **Row D** shows MRI images taken after 2 doses of bevacizumab. As is evident, lesions and edema were found to decrease in size and extent. Cognition was observed to improve, but was not at baseline.

[0043] **Figure 4** shows MRI scans of glioblastoma Patient DK. **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken in the middle of cycle 2) shows progression and pseudo-progression of the disease. **Row C** (images taken post administration of 2 doses of bevacizumab shows a mild response, with cognition and gait mildly improved.

[0044] **Figure 5** shows MRI scans of glioblastoma Patient JK. **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken prior to cycle 2) shows progression and pseudo-progression of the disease.

[0045] **Figure 6** shows MRI scans of glioblastoma Patient DW. **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken prior to cycle 2) shows progression of the disease.

[0046] **Figure 7** shows the time on study for the 6 treated patients.

[0047] **Figure 8** shows the level of sB7-H3 in all patients receiving anti-B7-H3 antibody at different cycles (C) and days (D) of treatment.

[0048] **Figure 9** shows the level of sB7-H3 in glioblastoma patients receiving anti-B7-H3 antibody at different cycles (C) and days (D) of treatment.

Detailed Description of the Invention:

[0049] The invention concerns therapeutic compositions for the treatment of vascularizing cancers, especially, glioblastoma. In particular, the invention is directed to compositions that comprise a molecule having a binding ability that is specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR). The invention is additionally directed to the use of such compositions in the treatment of such cancers, and in particular, in the treatment of glioblastoma.

[0050] In accordance with the present invention, the B7-H3-specific binding ability is capable of binding to B7-H3 molecules that are present on the surface of cancer cells, and particularly glioblastoma cancer cells, and of facilitating or mediating an immune response against such cells through such binding. The binding ability that is specific for a cell-surface factor (or receptor) involved in promoting tumor angiogenesis (*i.e.*, an "anti-tumor angiogenesis activity") is capable of binding to such factor (or receptor) so as to reduce or suppress, through such binding, the angiogenesis mediated by the interaction between such factor and such receptor. In a first embodiment, the B7-H3-specific binding ability and the binding ability that is specific for a cell-surface factor (or receptor) involved in promoting tumor angiogenesis are present on different molecules, such as for example an anti-B7-H3 antibody and an anti-angiogenesis factor antibody, or the B7-H3-binding fragment and anti-angiogenesis factor-binding

fragment of such antibodies. In a second aspect of the invention, such binding affinities will be possessed by a single molecule (suahc as a bi-specific antibody, a diabody, a BiTE®, or other single-chain or multi-chain variants thereof that are capable of simultaneously binding to both B7-H3 and an anti-angiogenesis factor or receptor. The invention is directed to the use of such compositions in the treatment of cancer, and in particular, in the treatment of glioblastoma.

I. General Techniques

[0051] 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

[0052] 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.

[0053] 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).

[0054] 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.

[0055] 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."

[0056] 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 a non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) "*Reshaping A Human Antibody To Inhibit The Interleukin 6-Dependent Tumor Cell Growth*," Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*,"

Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five or six) which 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.

[0057] 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 such B7-H3 epitope with greater affinity, avidity, more readily, and /or with greater duration than it binds to other B7-H3 epitopes or to a non-B7-H3 epitope. Likewise, an antibody that specifically binds to an epitope of VEGF or VEGFR binds such epitope with greater affinity, avidity, more readily, and /or with greater duration than it binds to other VEGF or VEGFR epitopes or to a non- VEGF or VEGFR epitope. 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.

[0058] 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 or anti-VEGF or anti-VEGFR antibody) to bind to the epitope

under different conditions, for example, after the epitope has been subjected to reducing and denaturing conditions.

[0059] Different biological functions may be 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 inhibit 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. Likewise, different biological functions may be associated with anti-VEGF or anti-VEGFR antibodies including, but not limited to one or more of: an ability to interfere with the binding of VEGF to VEGFR (and in particular VEGFR molecules that are expressed on the surfaces of cells; an ability to competitively inhibit preferential binding of a known anti-VEGF or anti-VEGFR antibody to VEGF or VEGFR, including the ability to preferentially bind to the same epitope to which the original antibody preferentially binds; an ability to bind to a portion of VEGFR that is exposed on the surface of a living cell *in vitro* or *in vivo*; and/or an ability to deliver a chemotherapeutic agent to VEGFR-expressing cells. The polypeptides (including antibodies) of the invention may have any one or more of these characteristics.

[0060] 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. An "anti-VEGF equivalent antibody" or "anti-VEGF equivalent polypeptide" refers to an antibody or a polypeptide having one or more biological functions associated with an

anti-VEGF antibody, such as, for example, an ability to interfere with the binding of VEGF to VEGFR.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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 are discussed below.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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 Nos. 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.*).

[0076] 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.

[0077] 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 chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a glioblastoma, 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 posterior uveal 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. B7-H3 and Anti-B7-H3 Antibodies

[0078] 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.

[0079] 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.

[0080] 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).

[0081] 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.

[0082] 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.

[0083] 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.

A. B7-H3

[0084] 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.

[0085] 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

[0086] A 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

[0087] 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:3**):

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

[0088] A cDNA sequence encoding the "4Ig" form of human B7-H3 is (**SEQ ID NO:4**); 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

[0089] 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).

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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-H3 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.

[0094] 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.

[0095] 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.

[0096] 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.

B. Anti-B7-H3 Antibodies

[0097] 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. Methods for obtaining such antibodies are described in United States Patent Publication No. 2013/0149236 and WO 2011/109400.

[0098] 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.

[0099] 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.

1. Sequences of BRCA69D

(a) BRCA69D Light Chain Sequences

[00100] Amino Acid Sequence of BRCA69D Variable Light Chain (**SEQ ID NO:5**):

DIQMTQTTSS LSASLGDRVT ISCRASQDIS NYLNWYQQKP DGTVKLLIYY
TSRLHSGVPS RFSGSGSGTD YSLTIDNLEQ EDIATYFCQQ GNTLPPTFGG
GTKLEIK

[00101] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain (**SEQ ID NO:6**):

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

[00102] BRCA69D Variable Light Chain CDR_1 (**SEQ ID NO:7**): RASQDISNYLN

[00103] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_1 (**SEQ ID NO:8**): agggcaagtc aggacattag taattattta aac

[00104] BRCA69D Variable Light Chain CDR_2 (**SEQ ID NO:9**): YTSRLHS

[00105] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_2 (**SEQ ID NO:10**): tacacatcac gattacactc a

[00106] BRCA69D Variable Light Chain CDR_3 (**SEQ ID NO:11**): QQGNTLPPT

[00107] Polynucleotide Sequence Encoding BRCA69D Variable Light Chain CDR_3 (**SEQ ID NO:12**): caacagggta atacgcttcc tccgacg

(b) BRCA69D Heavy Chain Sequences

[00108] Amino Acid Sequence of BRCA69D Variable Heavy Chain (**SEQ ID NO:13**):

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

[00109] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain (**SEQ ID NO:14**):

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

[00110] BRCA69D Variable Heavy Chain CDR_1 (**SEQ ID NO:15**): SYWMQ

[00111] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_1 (**SEQ ID NO:16**): agctactgga tgcag

[00112] BRCA69D Variable Heavy Chain CDR_2 (**SEQ ID NO:17**): TIYPGDGDTR YTQKFKG

[00113] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_2 (**SEQ ID NO:18**): actatttatc ctggagatgg tgatactagg tacactcag aagttcaagg gc

[00114] BRCA69D Variable Heavy Chain CDR_3 (**SEQ ID NO:19**): RGIPRLWYFD V

[00115] Polynucleotide Sequence Encoding BRCA69D Variable Heavy Chain CDR_3 (**SEQ ID NO:20**): agagggattc cacggctttg gtacttcgat gtc

2. Sequences of PRCA157

(a) PRCA157 Light Chain Sequences

[00116] Amino Acid Sequence of PRCA157 Variable Light Chain (**SEQ ID NO:21**):

DIQMTQSPAS LSVSVGETVT ITCRASESIY SYLAWYQQKQ GKSPQLLVYN
TKTLPEGVPS RFSGSGSGTQ FSLKINSLQP EDFGRYYCQH HYGTPPWTFG
GGTNLEIK

[00117] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain (**SEQ ID NO:22**):

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

[00118] PRCA157 Variable Light Chain CDR_1 (**SEQ ID NO:23**): RASESIYSYLA

[00119] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_1 (**SEQ ID NO:24**): cgagcaagtg agagtattta cagttattta gca

[00120] PRCA157 Variable Light Chain CDR_2 (**SEQ ID NO:25**): NTKTLPE

[00121] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_2 (**SEQ ID NO:26**): aatacaaaaa ccttaccaga g

[00122] PRCA157 Variable Light Chain CDR_3 (**SEQ ID NO:27**): QHHYGTPPW

[00123] Polynucleotide Sequence Encoding PRCA157 Variable Light Chain CDR_3 (**SEQ ID NO:28**): caacatcatt atggtactcc tccgtgg

(b) PRCA157 Heavy Chain Sequences

[00124] Amino Acid Sequence of PRCA157 Variable Heavy Chain (**SEQ ID NO:29**):

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

[00125] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain (**SEQ ID NO:30**):

gaggtgcagc aggtggagtc gggggagac 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

[00126] PRCA157 Variable Heavy Chain CDR_1 (**SEQ ID NO:31**): SYGMS

[00127] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_1 (**SEQ ID NO:32**): tcctatggca tgtct

[00128] PRCA157 Variable Heavy Chain CDR_2 (**SEQ ID NO:33**): VATINSGGSN TYYPDSLKG

[00129] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_2 (**SEQ ID NO:34**): gtcgcaacca ttaatagtgg tggaagtaac acctactatc cagacagttt gaagggg

[00130] PRCA157 Variable Heavy Chain CDR_3 (**SEQ ID NO:35**): HDGGAMDY

[00131] Polynucleotide Sequence Encoding PRCA157 Variable Heavy Chain CDR_3 (**SEQ ID NO:36**): catgacgggg gagctatgga ctac

2. Sequences of BRCA84D

(a) BRCA84D Light Chain Sequences

[00132] Amino Acid Sequence of BRCA84D Variable Light Chain (**SEQ ID NO:37**):

DIAMTQSQKF MSTSVGDRVS VTCKASQNVD TNVAWYQQKP GQSPKALIYS
ASYRYSGVPD RFTGSGSGTD FTLTINNVQS EDLAEYFCQQ YNNYPFTFGS
GTKLEIK

[00133] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain (**SEQ ID NO:38**):

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

[00134] BRCA84D Variable Light Chain CDR_1 (**SEQ ID NO:39**): KASQNVDTNVA

[00135] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_1 (**SEQ ID NO:40**): aaggccagtc agaatgtgga tactaatgta gcc

[00136] BRCA84D Variable Light Chain CDR_2 (**SEQ ID NO:41**): SASYRYS

[00137] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_2 (**SEQ ID NO:42**): tcggcatcct accggtacag t

[00138] BRCA84D Variable Light Chain CDR_3 (**SEQ ID NO:43**): QQYNNYPFT

[00139] Polynucleotide Sequence Encoding BRCA84D Variable Light Chain CDR_3 (**SEQ ID NO:44**): cagcaatata acaactatcc attcacg

(b) BRCA84D Heavy Chain Sequences

[00140] Amino Acid Sequence of BRCA84D Variable Heavy Chain (**SEQ ID NO:45**):

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

[00141] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain (**SEQ ID NO:46**):

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

[00142] BRCA84D Variable Heavy Chain CDR_1 (**SEQ ID NO:47**): FGMH

[00143] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_1 (**SEQ ID NO:48**): tttggaatgcac

[00144] BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:49**): YISSDSSAIYYADTVK

[00145] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:50**): tacattagta gtgacagtag tgccatctac tatgcagaca cagtgaag

[00146] BRCA84D Variable Heavy Chain CDR_3 (**SEQ ID NO:51**): GRENIYYGSRLDY

[00147] Polynucleotide Sequence Encoding BRCA84D Variable Heavy Chain CDR_3 (**SEQ ID NO:52**): gggagggaaa acatttacta cggtagtagg cttgactac

[00148] 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:

[00149] Humanized BRCA84D-1 Variable Light Chain (**SEQ ID NO:53**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00150] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain (**SEQ ID NO:54**):

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 acccttttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00151] Humanized BRCA84D-1 Variable Light Chain CDR_1 (**SEQ ID NO:55**): KASQNVDTNVA

[00152] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_1 (**SEQ ID NO:56**): aaggccagtc agaatgtgga tactaatgta gcc

[00153] Humanized BRCA84D-1 Variable Light Chain CDR_2 (**SEQ ID NO:57**): SASYRYS

[00154] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_2 (**SEQ ID NO:58**): tcggcatcct accggtacag t

[00155] Humanized BRCA84D-1 Variable Light Chain CDR_3 (**SEQ ID NO:59**): QQYNNYPFT

[00156] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Light Chain CDR_3 (**SEQ ID NO:60**): cagcaatata acaactatcc attcacg

[00157] Amino Acid Sequence of Humanized BRCA84D-1 Variable Heavy Chain (**SEQ ID NO:61**):

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

[00158] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain (**SEQ ID NO:62**):

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

[00159] Humanized BRCA84D-1 Variable Heavy Chain CDR_1 (**SEQ ID NO:63**): FGMH

[00160] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_1 (**SEQ ID NO:64**): tttggaatgcac

[00161] Humanized BRCA84D Variable Heavy Chain CDR_2 (**SEQ ID NO:65**): YISSDSSAIYYADTVK

[00162] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_2 (**SEQ ID NO:66**): tacattagta gtgacagtag tgccatctac tatgcagaca cagtgaag

[00163] Humanized BRCA84D-1 Variable Heavy Chain CDR_3 (**SEQ ID NO:67**): GRENIYYGSRLDY

[00164] Polynucleotide Sequence Encoding Humanized BRCA84D-1 Variable Heavy Chain CDR_3 (**SEQ ID NO:68**): gggagggaaa acatttacta cggtagtagg cttgactac

[00165] 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:

[00166] Amino Acid Sequence of hBRCA84D-2VL (**SEQ ID NO:69**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00167] Polynucleotide Encoding hBRCA84D-2VL (**SEQ ID NO:70**):

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 acccttttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00168] Amino Acid Sequence of hBRCA84D-3VL (SEQ **ID NO:71**):

DIQLTQSPSF LSASVGDRVS VTCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00169] Polynucleotide Encoding hBRCA84D-3VL (**SEQ ID NO:72**):

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 acccttttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00170] Amino Acid Sequence of hBRCA84D-4VL (SEQ **ID NO:73**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GQAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00171] Polynucleotide Encoding hBRCA84D-4VL (**SEQ ID NO:74**):

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

[00172] Amino Acid Sequence of hBRCA84D-5VL (**SEQ ID NO:75**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GQAPKALIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YNNYPFTFGQ
GTKLEIK

[00173] Polynucleotide Encoding hBRCA84D-5VL (**SEQ ID NO:76**):

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 acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00174] Amino Acid Sequence of hBRCA84D-6VL (**SEQ ID NO:77**):

DIQLTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKLLIYS
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYYCQQ YNNYPFTFGQ
GTKLEIK

[00175] Polynucleotide Encoding hBRCA84D-6VL (**SEQ ID NO:78**):

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 acccctttcac cttcggccag
ggcaccaagc tggaaatcaa g

[00176] Amino Acid Sequence of hBRCA84D-2VH (**SEQ ID NO:79**):

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

[00177] Polynucleotide Encoding hBRCA84D-2VH (**SEQ ID NO:80**):

```
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
```

[00178] Amino Acid Sequence of hBRCA84D-3VH (**SEQ ID NO:81**):

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

[00179] Polynucleotide Encoding hBRCA84D-3VH (**SEQ ID NO:82**):

```
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
```

[00180] Amino Acid Sequence of hBRCA84D-4VH (**SEQ ID NO:83**):

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

[00181] Polynucleotide Encoding hBRCA84D-4VH (**SEQ ID NO:84**):

```
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
```

[00182] **Table 1** lists the hBRCA84D variable light chain and variable heavy chain mutations studied; numbers refer to the Kabat numbering system.

Table 1									
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				

[00183] 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. 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. hBRCA84D-2VH (A93G) was found to have the highest binding affinity of the hBRCA84D-VH tested.

[00184] The amino acid and encoding polynucleotide sequences of the chimeric BRCA84D-1 are as follows:

[00185] Amino Acid Sequence of chBRCA84D Light Chain (**SEQ ID NO:85**):

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

[00186] Polynucleotide Encoding chBRCA84D Light Chain (**SEQ ID NO:86**):

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

[00187] Amino Acid Sequence of chBRCA84D Heavy Chain (**SEQ ID NO:87**):

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

[00188] Polynucleotide Encoding chBRCA84D Heavy Chain (**SEQ ID NO:88**):

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 ttccccctgg
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

IV. VEGF and VEGFR and Anti-VEGF and Anti-VEGFR Antibodies

A. VEGF and VEGFR

[00189] As used herein, the term "VEGF" refers to vascular endothelial growth factor A (Leung, D.W. *et al.* (1989) "*Vascular Endothelial Growth Factor Is A Secreted*

Angiogenic Mitogen," Science 246(4935):1306-1309). VEGF exists in multiple isoforms, reflecting alternative promoter usage, alternative splicing and alternative initiation. The initially discovered form is now designated isoform 162 (**SEQ ID NO:89**):

MNFLLSWVHW SLALLLYLHH AKWSQAAPMA EGGGQNHHEV VKFMDVYQRS
YCHPIETLVD IFQEYPDEIE YIFKPSCVPL MRCGGCCNDE GLECVPTEES
NITMQIMRIK PHQGQHIGEM SFLQHNKCEC RPKKDRARQE NPCGPCSERR
KHLFVQDPQT CKCSCKNTDS RCKARQLELN ERTCRCDKPR R

and is encoded by a cDNA sequence (**SEQ ID NO:90**):

cagtgtgctg gcggcccggc gcgagccggc ccggccccgg tcgggcctcc
gaaaccatga actttctgct gtcttgggtg cattggagcc tcgccttgct
gctctacctc caccatgcca agtggtccca ggctgcaccc atggcagaag
gaggagggca gaatcatcac gaagtggtga agttcatgga tgtctatcag
cgcagctact gccatccaat cgagaccctg gtggacatct tccaggagta
ccctgatgag atcgagtaca tcttcaagcc atcctgtgtg cccctgatgc
gatgcggggg ctgctgcaat gacgagggcc tggagtgtgt gcccactgag
gagtccaaca tcaccatgca gattatgcgg atcaaacctc accaaggcca
gcacatagga gagatgagct tcctacagca caacaaatgt gaatgcagac
caaagaaaga tagagcaaga caagaaaatc cctgtgggcc ttgctcagag
cggagaaagc atttgtttgt acaagatccg cagacgtgta aatgttcctg
caaaaacaca gactcgcgtt gcaaggcgag gcagcttgag ttaaacgaac
gtacttgcag atgtgacaag ccgaggcggt gagccgggca ggaggaagga
gcctccctca gggtttcggg aaccagatct ctcaccagga aagactgata
cagaacgatc gatacagaaa ccacgctgcc gccaccacac catcaccatc
gacagaacag tccttaatcc agaaacctga aatgaaggaa gaggagactc
tgcgcagagc actttgggtc cggagggcga gactccggcg gaagcattcc
cgggcgggtg acccagcacg gtccctcttg gaattggatt cgccatttta
tttttcttgc tgctaaatca ccgagcccgg aagattagag agttttattt
ctgggattcc tgtagacaca ccgcggccgc cagcacactg

[00190] The VEGF 206 isoform (Houck, K.A. et al. (1991) "*The Vascular Endothelial Growth Factor Family: Identification Of A Fourth Molecular Species And Characterization Of Alternative Splicing Of RNA*," Mol. Endocrinol. 5(12):1806-1814) has been selected as the canonical sequence (**SEQ ID NO:91**):

MNFLLSWVHW SLALLLYLHH AKWSQAAPMA EGGGQNHHEV VKFMDVYQRS
YCHPIETLVD IFQEYPDEIE YIFKPSCVPL MRCGGCCNDE GLECVPTEES
NITMQIMRIK PHQGQHIGEM SFLQHNKCEC RPKKDRARQE KKSVRGKGKG
QKRKRKKSRY KSWSVYVGAR CCLMPWSLPG PHPCGPCSER RKHLFVQDPQ
TCKCSCKNTD SRCKARQLEL NERTCRCDKP RR

[00191] As indicated above, three VEGF receptors have been described: VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR) and VEGFR-3 (Flt-4). Only VEGFR-1 and VEGFR-2 are capable of binding to VEGF-A (Shibuya, M. (2013) "*Vascular Endothelial Growth Factor And Its Receptor System: Physiological Functions In Angiogenesis And*

Pathological Roles In Various Diseases," J. Biochem. 153(1):13-19; Koch, S. *et al.* (2011) "*Signal Transduction By Vascular Endothelial Growth Factor Receptor*s," Biochem. J. 437:169-183).

[00192] The amino acid sequence of VEGFR-1 is (**SEQ ID NO:92**):

MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKGTQ HIMQAGQTLH
LQCRGEAAHK WSLPEMVSKE SERLSITKSA CGRNGKQFCS TLTLNTAQAN
HTGFYSCKYL AVPTSKKKET ESAIYIFISD TGRPFVEMYS EIPEIIHMTE
GRELVIPCRV TSPNITVTLK KFPLDTLIPD GKRIIWDSRK GFIISNATYK
EIGLLTCEAT VNGHLYKTNY LTHRQTNTII DVQISTPRPV KLLRGHTLVL
NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN IFYSVLTIDK
MQNKDKGLYT CRVRSGPSFK SVNTSVHIYD KAFITVKHRK QQVLETVAGK
RSYRLSMKVK AFPSPEVVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA
GNYTILLSIK QSNVFKNLTA TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ
ILTCTAYGIP QPTIKWFWHP CNHNHSEARC DFCSNNEESF ILDADSNMGN
RIESITQRMA IIEGKNKMAS TLVVADSRIS GIYICIASNK VGTVGRNISF
YITDVPNGFH VNLEKMPTEG EDLKLSCTVN KFLYRDVTWI LLRTVNNRTM
HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN VYTGEEILQK
KEITIRDQEA PYLLRNLSDH TVAISSSTTL DCHANGVPEP QITWFKNNHK
IQQEPGIILG PGSSTLFIER VTEEDEGVYH CKATNQKGSV ESSAYLTVQG
TSDKSNLELI TLTCTCVAAT LFWLLLTLFI RKMKRSSSEI KTDYLSIIMD
PDEVPLDEQC ERLPYDASKW EFARERLKLG KSLGRGAFGK VVQASAFGIK
KSPTCRTVAV KMLKEGATAS EYKALMTELK ILTHIGHHLN VVNLLGACTK
QGGPLMVIVE YCKYGNLSNY LKSKRDLFFL NKDAALHMEP KKEKMEPGLE
QGKKPRLDSV TSSESFASSG FQEDKSLSDV EEEEDSDGFY KEPITMEDLI
SYSFQVARGM EFLSSRKCIH RDLAARNILL SENNVVKICD FGLARDIYKN
PDYVRKGDTR LPLKWMAPES IFDKIYSTKS DVWSYGVLLW EIFSLGGSPY
PGVQMDEDFC SRLREGMRMR APEYSTPEIY QIMLDCWHRD PKERPRFAEL
VEKLGDLLQA NVQQDGKDYI PINAILTGNS GFTYSTPAFS EDFFKESISA
PKFNSGSSDD VRYVNAFKFM SLERIKTFEE LLPNATSMFD DYQGDSSTLL
ASPMLKRFTW TDSKPKASLK IDLRVTSKSK ESGLSDVSRP SFCHSSCGHV
SEGKRRFTYD HAELERKIAC CSPPPDYNSV VLYSTPPI

[00193] The amino acid sequence of VEGFR-2 is (**SEQ ID NO:93**):

MQSKVLLAVA LWLCVETRAA SVGLPSVSLD LPRLSIQKDI LTIKANTTLQ
ITCRGQRDLD WLWPNNQSGS EQRVEVTECS DGLFCKTLTI PKVIGNDTGA
YKCFYRETDL ASVIYVYVQD YRSPFIASVS DQHGVVYITE NKNKTVVIPC
LGSISNLNVS LCARYPEKRF VPDGNRISWD SKKGFTIPSY MISYAGMVFC
EAKINDESYQ SIMYIVVVVG YRIYDVVLSP SHGIELSVGE KLVLNCTART
ELNVGIDFNW EYPSSKHQHK KLVNRDLKTQ SGSEMKKFLS TLTIDGVTRS
DQGLYTCAAS SGLMTKKNST FVRVHEKPFV AFGSGMESLV EATVGERVRI
PAKYLGYPPP EIKWYKNGIP LESNHTIKAG HVLTIMEVSE RDTGNYTVIL
TNPISKEKQS HVVSLVVYVP PQIGEKSLIS PVDSYQYGTT QTLTCTVYAI
PPPHHIHWYW QLEEECANEP SQAVSVTNPY PCEEWRSVED FQGGNKIEVN
KNQFALIEGK NKTVSTLVIQ AANVSALYKC EAVNKVGRGE RVISFHVTRG
PEITLQPDMQ PTEQESVSLW CTADRSTFEN LTWYKLGPQP LPIHVGELPT
PVCKNLDTLW KLNATMFSNS TNDILIMELK NASLQDQGDY VCLAQDRKTK
KRHCVVRQLT VLERVAPTIT GNLENQTTSI GESIEVSCTA SGNPPPQIMW
FKDNETLVED SGIVLKDGNR NLTIRRVRKE DEGLYTCQAC SVLGCAKVEA
FFIIEGAQEK TNLEIIILVG TAVIAMFFWL LLVIILRTVK RANGGELKTG

YLSIVMDPDE LPLDEHCERL PYDASKWEFP RDRLKLGKPL GRGAFGQVIE
ADAFGIDKTA TCRTVAVKML KEGATHSEHR ALMSELKILI HIGHHLNVVN
LLGACTKPGG PLMVIVEFCK FGNLSTYLRS KRNEFVPYKT KGARFRQGKD
YVGAIPVDLK RRLDSITSSQ SSASSGFVEE KSLSDVEEEE APEDLYKDFL
TLEHLICYSF QVAKGMEFLA SRKCIHRDLA ARNILLSEKN VVKICDFGLA
RDIYKDPDYV RKGDARLPLK WMAPETIFDR VYTIQSDVWS FGVLLWEIFS
LGASPYPGVK IDEEFCRRLK EGTRMRAPDY TTPEMYQTML DCWHGEPSQR
PTFSELVEHL GNLLQANAQQ DGKDYIVLPI SETLSMEEDS GLSLPTSPVS
CMEEEEVCDP KFHYDNTAGI SQYLQNSKRK SRPVSVKTFE DIPLEEPEVK
VIPDDNQTDS GMVLASEELK TLEDRTKLSP SFGGMVPSKS RESVASEGSN
QTSGYQSGYH SDDTDTTVYS SEEAELLKLI EIGVQTGSTA QILQPDSGTT
LSSPPV

B. Anti-VEGF and Anti-VEGFR Antibodies

[00194] As indicated above, anti-VEGF antibodies have been considered in the treatment of glioblastomas. VEGR and its treceptor have additionally been considered with regard to numerous other types of vascularizing cancers, such as breast cancer, glioblastoma, uterine cervical carcinoma, metastatic colorectal cancer, gastric cancer, hepatocellular carcinoma, leukemia, lung cancer, metastatic melanoma, vascularizing pancreatic cancer, and metastatic prostate cancer (Roberts, E. *et al.* (2013) "*The Role of Vascular Endothelial Growth Factor in Metastatic Prostate Cancer to the Skeleton*," Prostate Cancer 2013:418340; Vici, P. *et al.* (2014) "*Emerging Biological Treatments for Uterine Cervical Carcinoma*," J. Cancer 5(2):86-97; Dietvorst, M.H. *et al.* (2013) "*Current and Novel Treatment Options for Metastatic Colorectal Cancer: Emphasis on Aflibercept*," Biol. Ther. 3:25-33; Moeini, A. *et al.* (2012) "*Emerging Signaling Pathways in Hepatocellular Carcinoma*," Liver Cancer 1(2):83-93; Cesca, M. *et al.* (2013) "*Tumor Delivery of Chemotherapy Combined with Inhibitors of Angiogenesis and Vascular Targeting Agents*," Front Oncol. 3:259:1-7; Pavlidis, E.T. *et al.* (2013) "*Role Of Bevacizumab In Colorectal Cancer Growth And Its Adverse Effects: A Review*," World J. Gastroenterol. 19(31):5051-5060; Welti, J. *et al.* (2013) "*Recent Molecular Discoveries In Angiogenesis And Antiangiogenic Therapies In Cancer*," J. Clin. Invest. 123(8):3190-3200; Eveno, C. *et al.* (2012) "*VEGF Levels And The Angiogenic Potential Of The Microenvironment Can Affect Surgical Strategy For Colorectal Liver Metastasis*," Cell. Adh. Migr. 6(6):569-573; Miyake, T.M. *et al.* (2013) "*Contemporary Use Of Bevacizumab In Ovarian Cancer*," Expert Opin. Biol. Ther. 13(2):283-294; Plate, K.H. *et al.* (2012) "*Tumor Angiogenesis And Anti-Angiogenic Therapy In Malignant Gliomas Revisited*," Acta Neuropathol. 124(6):763-

775; Liu, L. *et al.* (2012) "*Prognostic Value Of Vascular Endothelial Growth Factor Expression In Resected Gastric Cancer*," Asian Pac. J. Cancer Prev. 13(7):3089-3097; Kim, K.B. (2013) "*Is There A Role For Targeting Vascular Endothelial Growth Factor/Receptor Axis In The Treatment Of Patients With Metastatic Melanoma*?," Cancer 119(3):477-480; Kubota, Y. (2012) "*Tumor Angiogenesis And Anti-Angiogenic Therapy*," Keio J. Med. 61(2):47-56; Wicki, A. *et al.* (2012) "*Targeted Therapies In Breast Cancer*," Swiss Med. Wkly. 142:w13550:1-7; Parente Lamelas, I. *et al.* (2012) "*Directed Therapies In Lung Cancer: New Hope?*," Arch. Bronconeumol. 48(10):367-371; Saintigny, P. *et al.* (2012) "*Recent Advances In Non-Small Cell Lung Cancer Biology And Clinical Management*," Discov. Med. 13(71):287-297; Stevenson, C.E. *et al.* (2012) "*Bevacizumab And Breast Cancer: What Does The Future Hold?*," Future Oncol. 8(4):403-414; Aggarwal, C. *et al.* (2012) "*Antiangiogenic Agents In The Management Of Non-Small Cell Lung Cancer: Where Do We Stand Now And Where Are We Headed?*," Cancer Biol. Ther. 13(5):247-263).

[00195] Particularly preferred is the humanized anti-VEGF antibody (bevacizumab) sold as Avastin® (Genentech US, Inc.). Bevacizumab (Avastin®) is described in United States Patent Publications No. 2002/0032315, 2003/0190317, 2005/0112126, 2007/0059302, 2007/0059312, 2007/0196374, 2007/0248610, 2008/0187531, 2008/0226629, 2011/0052575, 2011/0081342, 2013/0058927; United States Patent No. 6,884,879; 7,060,269; 7,169,901; 7,297,334; 7,365,166; 7,375,193; CA Patents No. 2,286,330 and 2,145,985; and PCT Publication No. WO 1998/045331; Baca, M. *et al.* (1997) "*Antibody Humanization Using Monovalent Phage Display*," J. Biol. Chem. 272(16):10678-10684; Presta, L.G. *et al.* (1997) "*Humanization Of An Anti-Vascular Endothelial Growth Factor Monoclonal Antibody For The Therapy Of Solid Tumors And Other Disorders*," Cancer Res. 57(20):4593-4599; each of which documents is herein incorporated by reference in its entirety.

[00196] The amino acid sequence of the light chain of bevacizumab is (**SEQ ID NO:94**):

DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIYF
TSSLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ
GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV
DNALQSGNSQ ESVTEEDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEC

[00197] The amino acid sequence of the heavy chain of bevacizumab is (**SEQ ID NO:95**):

EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW
INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP
HYYGSSHWYF DVWGQGTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PGK

[00198] A particularly preferred anti-VEGFR antibody is ramucirumab (a humanized antihuman VEGFR-2 neutralizing monoclonal antibody) (Yang, Y. *et al.* (2013) "*Anti-VEGF- And Anti-VEGF Receptor-Induced Vascular Alteration In Mouse Healthy Tissues*," Proc. Natl. Acad. Sci. (U.S.A.) 110(29):12018-12023; Anonymous (2013) "*Ramucirumab Takes Steps Forward In Gastric Cancer*," Cancer Discov. 3(12):OF4; Aprile, G. *et al.* (2013) "*Critical Appraisal Of Ramucirumab (IMC-1121B) For Cancer Treatment: From Benchside To Clinical Use*," Drugs 73(18):2003-2015; *Ramucirumab Monotherapy For Previously Treated Advanced Gastric Or Gastro-Oesophageal Junction Adenocarcinoma (REGARD): An International, Randomised, Multicentre, Placebo-Controlled, Phase 3 Trial*; Fuchs, C.S. *et al.* (2014) "*Ramucirumab Monotherapy For Previously Treated Advanced Gastric Or Gastro-Oesophageal Junction Adenocarcinoma (REGARD): An International, Randomised, Multicentre, Placebo-Controlled, Phase 3 Trial*," Lancet 383(9911):31-39; Clarke, J.M. *et al.* (2013) "*Targeted Inhibition Of VEGF Receptor 2: An Update On Ramucirumab*," Expert Opin. Biol. Ther. 13(8):1187-1196; Wadhwa, R. *et al.* (2013) "Ramucirumab: a novel antiangiogenic agent," Future Oncol. 9(6):789-795).

[00199] Other anti-VEGFR antibodies include the rat anti-mouse VEGFR-1 neutralizing monoclonal antibody (MF-1) and the rat anti-mouse VEGFR-2 neutralizing monoclonal antibody (DC101) (Xue, Y. *et al.* (2008) "*Anti-VEGF Agents Confer Survival Advantages To Tumor-Bearing Mice By Improving Cancer-Associated Systemic Syndrome*," Proc. Natl. Acad. Sci. (U.S.A.) 105(47):18513-18518; Zhang, D. *et al.* (2011) "*Anti-Angiogenic Agents Significantly Improve Survival In Tumor-Bearing Mice By Increasing Tolerance To Chemotherapy-Induced Toxicity*," Proc. Natl. Acad.

Sci. (U.S.A.) 108(10):4117-4122; Cao, R. *et al.* (2010) "*VEGFR1-Mediated Pericyte Ablation Links VEGF And Plgf To Cancer-Associated Retinopathy*," Proc. Natl. Acad. Sci. (U.S.A.) 107(2):856-861).

Alternatively, the invention contemplates the use of a VEGFR antagonist (for example, Aflibercept (VEGF-Trap), a soluble fusion protein of the extracelluar domain of VEGFR-1 and VEGFR-2 (Dietvorst, M.H. *et al.* (2013) "*Current and Novel Treatment Options for Metastatic Colorectal Cancer: Emphasis on Aflibercept*," Biol. Ther. 3:25-33; Moradi, A. *et al.* (2013) "*Vascular Endothelial Growth Factor Trap-Eye (Aflibercept) For The Management Of Diabetic Macular Edema*," World J. Diabetes. 4(6):303-309; Ventrice, P. *et al.* (2013) "Anti-vascular endothelial growth factor drugs safety and efficacy in ophthalmic diseases," J. Pharmacol. Pharmacother. 4(Suppl1):S38-S42; Sophie, R. *et al.* (2012) "*Aflibercept: a Potent Vascular Endothelial Growth Factor Antagonist for Neovascular Age-Related Macular Degeneration and Other Retinal Vascular Diseases*," Biol. Ther. 2:3:1-22) or the pan-VEGFR tyrosine kinase inhibitor, cediranib (AstraZeneca) (Dietrich, J. *et al.* (2009) "*Cediranib: Profile Of A Novel Anti-Angiogenic Agent In Patients With Glioblastoma*," Expert Opin. Investig. Drugs. 18(10):1549-1557).

V. Fc-Engineered Antibodies

[00200] 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:96**, 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:96**):

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.

[00201] The present invention includes anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies (such as an anti-VEGF or anti-VEGFR antibody) 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).

[00202] 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.

[00203] Preferably, the binding properties of the above-described anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies of the invention are characterized by *in vitro* functional assays for determining one or more FcγR mediator effector cell functions. 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*.

[00204] In some embodiments, the above-described includes anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies 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.

[00205] In particularly preferred embodiments, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that comprise 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.

[00206] In particularly preferred embodiments, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies 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.

[00207] 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.

[00208] The invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00209] The invention also encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00210] The invention also encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00211] The invention particularly encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00212] The invention particularly encompasses such antibodies that 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.

[00213] In a specific embodiment, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that comprise a variant Fc region which comprises the substitution: F243L, R292P, and Y300L. In a further specific embodiment, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that comprise a variant Fc region which comprises the substitution: L235V, F243L, R292P, Y300L, and P396L. In yet a further specific embodiment, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that comprise a variant Fc region which comprises the substitution F243L, R292P, Y300L, V305I, and P396L.

[00214] In a further specific embodiment, the invention encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that comprise 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.

[00215] The invention particularly encompasses antibodies that anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00216] The invention particularly encompasses anti-B7-H3 antibodies and/or anti-tumor angiogenesis antibodies that 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.

[00217] 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.

[00218] The invention encompasses molecules comprising variant Fc regions consisting of or comprising any of the mutations listed in the table below in **Table 2**.

Table 2
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

Table 2
Exemplary Fc Modifications

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	
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

Table 2 Exemplary Fc Modifications		
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
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	

Table 2 Exemplary Fc Modifications	
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	
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	

[00219] In specific embodiments, the variant Fc region of such anti-B7-H3 antibodies and/or anti-tumor angiogenesis 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.

[00220] 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.

[00221] 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.

[00222] 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.

[00223] 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).

VI. B7-H3 / Anti-Tumor Angiogenesis Diabodies

[00224] As discussed above, the present invention additionally encompasses diabody (especially, "DART™" (dual affinity retargeting reagent)) molecules that comprise at least two covalently bonded polypeptide chains which form at least two epitope binding sites, one of which specifically binds to B7-H3 and a second of which binds to a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis. Most preferably, such diabodies will bind to B7-H3 and VEGF or to B7-H3 and VEGFR.

[00225] In preferred embodiments, the first polypeptide chain of the diabody comprises:

(i) a domain (A) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope of B7-H3;

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

(iii) optionally, a domain (C).

The second polypeptide chain of such a diabody comprises:

(i) a domain (D) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for such epitope of either VEGF or VEGFR epitope;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for such epitope of B7-H3; and

(iii) optionally, a domain (F).

[00226] The variable domains of the first and second polypeptide chains may alternatively be reversed, such that the first polypeptide chain of the diabody comprises:

(i) a domain (A) comprising a binding region of a light chain variable domain of the second immunoglobulin (VL2) specific for such epitope of either VEGF or VEGFR epitope;

(ii) a domain (B) comprising a binding region of a heavy chain variable domain of the first immunoglobulin (VH1) specific for such epitope of B7-H3; and

(iii) optionally, a domain (C);

and the second polypeptide chain of such a diabody comprises:

(i) a domain (D) comprising a binding region of a light chain variable domain of a first immunoglobulin (VL1) specific for an epitope of B7-H3;

(ii) a domain (E) comprising a binding region of a heavy chain variable domain of a second immunoglobulin (VH2) specific for an epitope of either VEGF or VEGFR; and

(iii) optionally, a domain (F).

[00227] The diabody domains (A) and (B) do not associate with one another to form an epitope binding site. Similarly, the diabody domains (D) and (E) do not associate with one another to form an epitope binding site. Rather, diabody domains (A) and (E) associate to form a binding site that binds the B7-H3 epitope and the diabody domains (B) and (D) associate to form a binding site that binds the VEGF or VEGFR epitope.

[00228] Neither the VH or VL domain of either polypeptide chain is constrained to any position within the polypeptide chain, *i.e.,* restricted to the amino (N) or carboxy (C) terminus, 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 the functional diabody.

[00229] When present, Domains (C) and (F) are covalently associated together. Domains (C) and (F) may be an Fc 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)).

[00230] The Fc domains in the polypeptide chains of the diabody molecules preferentially dimerize, resulting in the formation of a diabody 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 diabody comprising an Fc domain, albeit with a structure distinct from that of an unmodified bivalent antibody. Such diabody molecules will exhibit altered phenotypes relative to a wild-type immunoglobulin, *e.g.*, altered serum half-life, binding properties, *etc.* In other embodiments, diabody 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 diabody is tetravalent.

[00231] 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 decrease 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.

[00232] 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.

[00233] 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.

[00234] Each domain of the polypeptide chain of the diabody, *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:97**) encoded by the nucleic acid sequence ggaggcggat ccggaggcgg aggc (**SEQ ID NO:98**). The polypeptide chains of the diabody 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 diabody to form an inter-chain disulfide bond. Such interchain disulfide bonds serve to stabilize the diabody 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 (**SEQ ID NO:99**). In a specific embodiment, the C-terminus of the polypeptide chains comprising the diabody molecules of the invention comprises the amino acid sequence LGGC (**SEQ ID NO:99**). 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:100**) or ESKYGPPCPS (**SEQ ID NO:101**). In a specific embodiment, the C-terminus of a polypeptide chain of the diabody molecule of the invention comprises the amino acid

sequence of an IgG hinge domain, *e.g.* **SEQ ID NO:100** or **SEQ ID NO:101**. In another embodiment, the C-terminus of a polypeptide chain of a diabody molecule of the invention comprises the amino acid sequence VEPKSC (**SEQ ID NO:102**), which can be encoded by nucleotide sequence gttgagccca aatcttgt (**SEQ ID NO:103**). In other embodiments, the cysteine residue in introduced into the polypeptide chain within the amino acid sequence LGGCFNRGEC **(SEQ ID NO:104**), which can be encoded by the nucleotide sequence ctgggaggct gcttcaacag gggagagtgt **(SEQ ID NO:105)**. In a specific embodiment, the C-terminus of a polypeptide chain comprising a diabody of the invention comprises the amino acid sequence LGGCFNRGEC **(SEQ ID NO:104**). In yet other embodiments, the cysteine residue in introduced into the polypeptide chain within the amino acid sequence FNRGEC **(SEQ ID NO:106)**, which can be encoded by the nucleotide sequence ttcaacaggg gagagtgt **(SEQ ID NO:107)**. In a specific embodiment, the C-terminus of a polypeptide chain comprising a diabody of the invention comprises the amino acid sequence FNRGEC **(SEQ ID NO:106)**.

[00235] In certain embodiments, the diabody molecule comprises at least two polypeptide chains, each of which comprise the amino acid sequence LGGC (**SEQ ID NO:99**) and are covalently linked by a disulfide bond between the cysteine residues in the LGGC (**SEQ ID NO:99**) sequences. In another specific embodiment, the diabody molecule comprises at least two polypeptide chains, one of which comprises the sequence FNRGEC (**SEQ ID NO:106**) 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:106**) 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 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.

[00236] In still another aspect of this embodiment, the Domain (C) of the first polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:102)**, derived from the hinge domain of a human IgG, and which can be encoded by the nucleotide sequence gttgagccca aatcttgt **(SEQ ID NO:103)**. In another aspect of this embodiment, the Domain (F) of the second polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:102)**. 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:106)**; and Domain (F) of the second polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:102)** 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:106)**; and Domain (C) of the first polypeptide chain comprises the amino acid sequence VEPKSC **(SEQ ID NO:102)** or a hinge domain.

[00237] As will be appreciated in view of the foregoing, the individual polypeptides of a bispecific diabody 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 diabody polypeptides. By selecting charged polypeptides of opposite charge for the individual polypeptides of the bispecific diabody, 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.

[00238] Thus, in a preferred embodiment, a positively charged, "E-coil" will be appended to one of the polypeptides being used to form a bispecific diabody and a negatively charged "K-coil" will be appended to the second of the diabody's polypeptides. A particularly preferred E-coil will have the sequence: $(EVAALEK)_4$ [*i.e.* **(SEQ ID NO:108)** EVAALEKEVAALEKEVAALEKEVAALEK]. A particularly preferred K-coil will have the sequence: $(KVAALKE)_4$ [*i.e.* **(SEQ ID NO:109)** KVAALKEKVAALKEKVAALKEKVAALKE].

[00239] A preferred diabody polypeptide possessing such an E-coil will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(EVAALEK)_4]$—GGGNS**,** where VL is the diabody's variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:97**, VH is the diabody's variable heavy Ig domain, $(EVAALEK)_4$ is **SEQ ID NO:108**, and GGGNS is **SEQ ID NO:110**. A preferred diabody polypeptide possessing such a K-coil will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—$[(KVAALKE)_4]$—GGGNS**,** where VL is the diabody's variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:97**, VH is the diabody's variable heavy Ig domain, $(KVAALKE)_4$ is **SEQ ID NO:109**, and GGGNS is **SEQ ID NO:110**.

[00240] In a further embodiment, Fc-regions can be linked to the E and/or K coils of E-coil or K-coli diabodies. Furthering the separation between the Fc regions and the diabody VH domain of an Fc-containing diabody 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 diabody assembly. Although separators of any amino acid sequence may be employed, it is preferable to employ a separator that forms an α helix coil, 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 function to promote heterodimer formation, such molecules are particularly preferred separators. Such coil-containing

Fc-diabody molecules provide benefits similar to those of Fc-diabodies, 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 diabody will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—[(EVAALEK)$_4$]—**GGG**—Fc domain starting with D234 (Kabat numbering), where VL is the diabody's variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:97**, VH is the diabody's variable heavy Ig domain and (EVAALEK)$_4$ is **SEQ ID NO:108**. Similarly, in a preferred embodiment, the K-coil Fc-containing diabody will have the general sequence: [VL Domain]—[**GGGSGGGG**]—[VH Domain]—[(KVAALKE)$_4$]—**GGG**—Fc domain starting with D234 (Kabat numbering), where VL is the diabody's variable light Ig domain, **GGGSGGGG** is **SEQ ID NO:97**, VH is the diabody's variable heavy Ig domain and (KVAALKE)$_4$ is **SEQ ID NO:109**.

[00241] As indicated above, a coil-containing diabody molecule or a coil-containing Fc-containing diabody 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 diabody's polypeptides). By linking the Fc region to such separator molecule(s), the ability to make bivalent, tetravalent, *etc.* versions of the Fc-diabody molecules by chain swapping is enhanced. Fc-diabody molecules can thus be produced that form monomers or dimers depending upon whether the Fc domain is linked to one or both of the diabody VH domains

VII. Therapeutic Methods

[00242] Antibodies (or diabodies) that specifically bind to B7-H3 or a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR) may be used for therapeutic purposes in individuals with cancer or other diseases. In one embodiment, molecule(s) providing such binding activities are administered concurrently. As used herein, such "concurrent" administration is intended to denote:

A. the administration of a single pharmaceutical composition that contains both a molecule having a binding ability specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR). Such molecules may be the same molecule (*e.g.*, a diabody, or may be distinct (*e.g.*, an anti-B7-H3 antibody, or antigen-binding fragment thereof, and an anti-VEGF-antibody, or antigen-binding fragment thereof.

or

B. the separate administration of two or more pharmaceutical compositions, one composition of which contains a molecule having a binding ability specific for B7-H3 and another composition of which contains a molecule having a binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR), wherein the second administered composition is administered within 1 biological half-life after the administration of the first administered molecule, or within 2, 3, 4, 5, 6, 7, 8, 9, or 10 half-lives after the administration of the first administered composition. Bevacizumab has an estimated half-life of approximately 20 days. The estimated half-life of humanized anti-B7-H3 is 2-3 weeks.

[00243] In a second embodiment, two distinct molecules are employed, and the molecules are administered "sequentially" (*e.g.*, an anti-VEGF antibody is administered and, at a later time, an anti-B7-H3 antibody is provided, or *vice versa*). In such sequential administration, the second administered composition is administered at least 10 half-lives after the administration of the first administered composition.

[00244] Therapy with such molecules can involve the formation of complexes both *in vitro* and *in vivo*. In one embodiment, a monoclonal anti-B7-H3 antibody and a monoclonal anti-VEGF antibody (especially chimeric or humanized variants thereof) can be used for immunotherapy directed at vascularizing cancerous cells of different tissues, and particularly cancerous cells such as breast cancer, glioblastoma, uterine cervical carcinoma, metastatic colorectal cancer, gastric cancer, hepatocellular carcinoma, leukemia, lung cancer, metastatic melanoma, vascularizing pancreatic

cancer, and metastatic prostate cancer. Such immunotherapy may, for example, be sufficient to reduce cell division in the cancer cell, delay the development (*e.g.*, onset and extent) of metastasis, or provide palliative treatment for the cancer. 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.*

[00245] It is understood that the antibody is administered at a concentration that promotes binding at physiological (*e.g.*, *in vivo*) conditions. The antibodies (or diabodies) may be administered with additional agents that enhance or direct an individual's own immune response, such as an agent that strengthens ADCC.

[00246] In yet another embodiment, one or more of such antibodies or diabodies may 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 antibody (*e.g.*, 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 and the antibody (or diabody) that specifically binds to a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR) inhibits tumor vascularization.

[00247] . In yet another embodiment, such antibodies or diabodies can be employed as an adjuvant therapy at the time of the surgical removal of a tumor in order to delay, suppress or prevent the development of metastasis. The antibody can also be administered before surgery (neoadjuvant therapy) in order to decrease the size of the tumor and thus enable or simplify surgery, spare tissue during surgery, and /or decrease any resulting disfigurement.

[00248] 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 antibodies of this invention (alone or with additional therapeutic moieties) 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 the antibody (or diabody) that specifically binds to a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis (especially VEGF or its receptor, VEGFR), alone or with an additional therapeutic moiety.

[00249] 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, fluoropyrimidine-irinotecan, fluoropyrimidine-oxaliplatin, 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. Use of one or more of: fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin, temozolomide, procarbazine, carmustine (BCNU), lomustine (CCNU), vincristine, PVC (procarbazine, lomustine and vincristine), irinotecan, cisplatin, carboplatin, methotrexate, etoposide, bleomycin, vinblastine, actinomycin (dactinomycin), cyclophosphamide and ifosfamide is particularly preferred.

[00250] 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.

[00251] 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.

[00252] 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.

[00253] 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.

[00254] 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.

[00255] 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.

[00256] 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.

[00257] 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.*).

[00258] 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.

[00259] 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.

[00260] 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, the anti-B7-H3 antibodies can bind to cancerous cells, and macrophages or other phagocytic cell can opsonize the cancerous cells.

[00261] Various formulations of antibodies or fragments thereof or diabodies may be used for administration. In some embodiments, such molecules 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.

[00262] 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.

[00263] 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.

[00264] The precise dose to be employed in the formulations of the present invention will depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's

circumstances and can be determined by standard clinical techniques. Effective doses (*i.e.*, doses sufficient to be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder) may be extrapolated from dose-response curves derived from in vitro or animal model test systems. The particular dosage regimen, *i.e.*, dose, timing and repetition, will thus depend on the particular individual and that individual's medical history, as well as the route of administration. The dosage and frequency of administration of the molecules of the invention may be reduced or altered by enhancing their uptake and/or tissue penetration, such as, for example, by lipidation.

[00265] The dosage of such molecules administered to a patient is typically at least about 0.01 μg/kg body weight, at least about 0.05 μg/kg body weight, at least about 0.1 μg/kg body weight, at least about 0.2 μg/kg body weight, at least about 0.5 μg/kg body weight, at least about 1 μg/kg body weight, at least about 2 μg/kg body weight, at least about 3 μg/kg body weight, at least about 5 μg/kg body weight, at least about 10 μg/kg body weight, at least about 20 μg/kg body weight, at least about 30 μg/kg body weight, at least about 50 μg/kg body weight, at least about 100 μg/kg body weight, at least about 250 μg/kg body weight, at least about 750 μg/kg body weight, at least about 1.5 mg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, or at least about 10 mg/kg body weight. The calculated dose will be administered based on the patient's body weight at baseline. Significant (≥ 10%) change in body weight from baseline or established plateau weight should prompt recalculation of dose.

[00266] 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.

[00267] Although, as discussed above, various dosing and administration routes may be employed in order to provide anti-B7-H3 antibody therapy to recipient patients, the following dosing and administrative route is preferred. Anti-B7-H3 antibody (preferably humanized, if to be administered to human subjects) is preferably administered by intravenous (IV) infusion on Study Days 1, 8, 15, and 22 of a (50 day) Cycle 1. Additional anti-B7-H3 antibody is administered in subsequent cycles for eligible patients on Study Days 1, 8, and 15 of each subsequent 28-day cycle. The IV infusion is preferably delivered over a period of 120 minutes if the patient does not exhibit signs or symptoms of an infusion reaction. Occurrence of infusion reaction will necessitate rate reduction, interruption, or discontinuation. It is preferred that the anti-B7-H3 antibody not be administered as an IV push or bolus, and that an in-line filter or non-polyolefin IV infusion bags not be used. The antibody should not be mixed or diluted with other drugs. Infusion or allergic reactions may occur. Premedications for the prevention of infusion reactions are recommended and precautions for anaphylaxis should be observed during antibody administration. Supportive measures may include, but are not limited to: epinephrine, antihistamines, corticosteroids, IV fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen. IV saline, acetaminophen and emergency drugs, including epinephrine sub-cutaneous (0.3 to 0.5 mg (0.3 to 0.5 mL of a 1:1000 solution)), diphenhydramine (25 to 50 mg IV), and Decadron (10 mg IV push or equivalent) to treat hypersensitivity reactions should be available. Should symptoms of fever or chills develop, the drug infusion should be stopped and acetaminophen and diphenhydramine hydrochloride should be given.

[00268] Although, as discussed above, various dosing and administration routes may be employed in order to provide anti-VEGF antibody therapy to recipient patients, the following dosing and administrative route is preferred. Anti-VEGF antibody (bevacizumab) is preferably administered by intravenous (IV) infusion. Bevacizumab is preferably not to be administered as an intravenous push or bolus. Bevacizumab treatment should not be initiated until at least 28 days following major surgery, and only after the surgical incision has fully healed. The first IV infusion is preferably

delivered over a period of 90 minutes if the patient does not exhibit signs or symptoms of an infusion reaction. Occurrence of infusion reaction will necessitate rate reduction, interruption, or discontinuation. If the initial infusion is tolerated, the second IV infusion is preferably delivered over a period of 60 minutes. Subsequent infusions may delivered over a period of 30 minutes, if infusion over 60 minutes is tolerated. Patients should continue treatment until disease progression or unacceptable toxicity. The recommended dosages are: 5 mg/kg body weight or 10 mg/kg body weight every 2 weeks when used in combination with intravenous 5-FU-based chemotherapy; 5 mg/kg body weight when used in combination with bolus-IFL (irinotecan, leucovorin (folinic acid), and fluorouracil); 10 mg/kg body weight when used in combination with FOLFOX4 (leucovorin (folinic acid), fluorouracil (5-FU), oxaliplatin (Eloxatin®); 15 mg/kg body weight every 3 weeks in combination with carboplatin and paclitaxel. The composition is preferably administered at a dose of 5 mg/kg body weight every 2 weeks or 7.5 mg/kg body weight every 3 weeks when used in combination with a fluoropyrimidine-irinotecan or fluoropyrimidine-oxaliplatin based chemotherapy regimen in patients who have progressed on a first-line bevacizumab-containing regimen. For the treatment of glioblastoma, the recommended dosage is 10 mg/kg every 2 weeks.

[00269] In one embodiment, dosages for antibodies may be determined empirically in individuals who have been given one or more administration(s). Individuals are given incremental dosages of an 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.

[00270] 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.

[00271] 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.

VIII. Methods of Administration

[00272] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a pharmaceutical composition of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgous monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00273] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) *"Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System,"* J. Biol. Chem.

262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00274] Methods of administering the molecules of the present invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the molecules of the invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00275] The invention also provides that the pharmaceutical compositions of the invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of such molecules. In one embodiment, the antibodies, antibody fragments or diabodies of such pharmaceutical compositions are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the molecules of the invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 μg, more preferably at least 10 μg, at least 15 μg, at least 25 μg, at least 50 μg, at least 100 μg, or at least 200 μg.

[00276] The lyophilized molecules of the invention should be stored at between 2 and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules of the invention are

supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, the liquid form of such bi-specific monovalent diabodies or bi-specific monovalent Fc diabodies is supplied in a hermetically sealed container in which the molecules are present at a concentration of least 1 μg/ml, more preferably at least 2.5 μg/ml, at least 5 μg/ml, at least 10 μg/ml, at least 50 μg/ml, or at least 100 μg/ml.

[00277] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00278] In another embodiment, the compositions can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, pp. 3 17-327; see generally *ibid.*).

[00279] In yet another embodiment, the compositions can be delivered in a controlled release or sustained release system. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more molecules of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local*

Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled release system (*See* Langer, *supra*; Sefton, (1987) *"Implantable Pumps,"* CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) *"Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis,"* Surgery 88:507-516; and Saudek *et al.* (1989) *"A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery,"* N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) *"Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate,"* Science 228:190-192; During *et al.* (1989) *"Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization,"* Ann. Neurol. 25:351-356; Howard *et al.* (1989) *"Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits,"* J. Neurosurg. 7(1):105-112); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). In another embodiment, polymeric compositions useful as controlled release implants are used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled release of the bioactive material from the polymer

system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. In another embodiment, a non-polymeric sustained delivery system is used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See United States Patent No. 5,888,533).

[00280] Controlled release systems are discussed in the review by Langer (1990, *"New Methods Of Drug Delivery,"* Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00281] Treatment of a subject can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with molecules of the invention one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. In other embodiments, the pharmaceutical compositions of the invention are administered once a day, twice a day, or three times a day. In other embodiments, the pharmaceutical compositions are administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year. It will also be

appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

[00282] 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
Treatment of Recurrent Glioblastoma Multiforme (GBM) with Anti-B7-H3 Antibody and Anti-VEGF Antibody

[00283] In order to demonstrate the ability of a combined therapy involving agents that provide a binding ability that is specific for B7-H3 and a second binding ability that is specific for a cell-surface factor (or its receptor) that is involved in promoting tumor angiogenesis, patients suffering from glioblastoma multiforme (GBM) were treated with a humanized anti-B7-H3 antibody and with bevacizumab (Avastin®).

[00284] Overview: Six patients (4 males and 2 females), ranging in age from 26-68 were treated. Four of the patients were experiencing their third recurrence of cancer, one patient was experiencing a second recurrence of cancer, and one patient was experiencing a first recurrence of cancer. Four of the patients received bevacizumab. One patient received an alternative anti-VEGF therapy and one patient was naïve for anti-VEGF therapy. One patient had been on high-dose dexamethasone (8 mg/day) at baseline; one patient had been on a replacement dose prednisone (5 mg/day) for a secondary adrenal insufficiency; and the remaining four patients had not received steroids. Antibody treatment was designed to include one or more 50-day cycles in which antibody would be administered on days 1, 8, 15, and 22. The profiles of the patients are reported in **Table 3**.

Table 3

Patient Code	Age	Gender	Recurrence	Anti-VEGF Therapy at baseline	Dexamethasone
NF	42	M	At 2nd Recurrence; Recurred 15 months after diagnosis	None	No Dexamethasone at Baseline
MR	42	F	At 3rd Recurrence; Recurred 8 months after second recurrence	Bevacizumab + CCNU for 7 months	No Dexamethasone At Baseline
GC	68	M	At 1st Recurrence; Recurred 26 months after diagnosis	Cediranib for 26 months	Baseline Replacement Dose Prednisone

Table 3					
Patient Code	**Age**	**Gender**	**Recurrence**	**Anti-VEGF Therapy at baseline**	**Dexamethasone**
DK	46	M	At 3rd Recurrence; Recurred 5 months after diagnosis	Bevacizumab + CCNU for 3 months	No Dexamethasone At Baseline
JK	45	F	At 3rd Recurrence; Recurred 9 months after diagnosis	Bevacizumab for 1 year	No Dexamethasone At Baseline
DW	60	M	Recurrent disease; Recurred 3 months after diagnosis	Bevacizumab + CCNU for 2 months	High Dexamethasone At Baseline

Patient NF

[00285] Figure 1 shows the clinical progression of Patient NF. MRI scans of the patient's brain revealed 3 lesions. An initial cycle of humanized anti-B7-H3 antibody was administered at baseline. **Row A** shows the status of the patient's lesions at baseline (note that for Lesion 3, the arrow in **Row A** points to the site where the lesion is seen in **Row B** of the Figure; no evidence of Lesion 3 is seen in **Row A** of the figure). **Row B** shows the status of the patient's lesions prior to the initiation of a second planned cycle of anti-B7-H3 antibody administration. **Row B** reveals that the three lesions appear to have increased in size (a phenomenon known as "pseudo-progression"). Concurrent with such pseudo-progression, the patient experienced increased edema in the brain (fourth column), headaches and increased seizures. Anti-B7-H3 antibody therapy was stopped and the patient was provided with 3 doses of bevacizumab and one dose of CCNU (**Row C**).

[00286] Significantly, the administered anti-B7-H3 antibody had a biological half-life of 2-3 weeks. Thus, at the time of the MRI analysis of **Row C**, the patient's level of bevacizumab had decreased to approximately 25% of maximum. As seen in **Row C**, of Figure 1, a dramatic reduction in lesion size and edema was observed, along with the resolution of the patient's headaches and seizures.

[00287] The data are interpreted as indicating that the combination treatment of bevacizumab and anti-B7-H3 antibody acted synergistically to improve the patient's condition. After 1 cycle of anti-B7-H3 antibody treatment, a significant increase in lesion enhancement with headaches and increased seizures was observed. However, after receiving 3 doses of bevacizumab + CCNU, a marked reduction of all MRI

findings was observed. The patient has been clinically stable for more than 10 months after the last dose of anti-B7-H3 antibody. The patient's overall survival is more than 2 years, 7 months from the date of diagnosis.

Patient MR

[00288] Figures 2A-2C show the clinical progression of Patient MR. Patient MR exhibited secondary glioblastoma and possessed glioblastoma characterized by the *IDH1* mutant (Ichimura, K. *et al.* (2009) "*IDH1 Mutations Are Present In The Majority Of Common Adult Gliomas But Rare In Primary Glioblastomas*," Neuro. Oncol. 11(4):341-347). A characteristic of glioblastoma associated with the IDH1 mutation is that such tumors are non-contrast enhanced (Carillo, J.A. *et al.* (2012) "*Relationship between Tumor Enhancement, Edema, IDH1 Mutational Status, MGMT Promoter Methylation, and Survival in Glioblastoma*," Amer. J. Neuroradiol. 10.3174/ajnr.A2950, pages 1-7).

[00289] MRI scans of the patient's brain revealed 5 lesions. MRI scans of lesions 1-4 are shown in **Figures 2A-2B**; MRI scans of lesion 5 are shown in **Figure 2C**. To assess the lesions of Patient MR, MRI imaging was done in the presence of the contrast agent, gadolinium (**Figures 2A-2C**, "GADO") with and without T2 weighting (**Figures 2A-2C**, T2). As will be appreciated, the signal in MRI images is high (bright) or low (dark), depending on the pulse sequence used, and the type of tissue in the image region of interest. Features appearing bright on a T2-weighted image include increased water, as in edema or inflammation. Features appearing dark on a T2-weighted image include calcification, fibrous tissue, protein-rich fluid, and flow void. Tumors of Patient MR had both contrast-enhancing and non-enhancing (T2 bright) components.

[00290] An initial cycle of humanized anti-B7-H3 antibody was administered at baseline. After the second cycle had started, dexamethasone was administered to the patient (2 mg bid). In the middle of Cycle 3, anti-B7-H3 antibody therapy was stopped and the patient was provided with 1 dose of bevacizumab and a ventriculoperitoneal shunt (VPS). The patient had a significant amount of anti-B7-H3 antibody at the time of bevacizumab administration.

[00291] In **Figures 2A-2C**, **Row A** shows the status of the patient's lesions at baseline. **Row B** shows the status of the patient's lesions prior to the initiation of a second planned cycle of anti-B7-H3 antibody administration. **Row B** of **Figures 2A-2B** reveal that Lesions 1-4 increased in size, and that increased edema in the brain (fourth column) was evident. The data is interpreted as showing pseudo-progression. **Row C** of **Figures 2A-2B** reveals the state of Lesions 1-4 at the time anti-B7-H3 antibody therapy was stopped. The patient was then provided with 1 dose of bevacizumab and a ventriculoperitoneal shunt was provided. The status post administration of bevacizumab is shown in **Row D** of the Figures. As seen in **Row D** of **Figures 2A-2B**, a dramatic reduction in lesion size and edema for Lesions 1-4 was observed.

[00292] As shown in **Figure 2C**, Lesion 5 of the patient failed to respond to the therapy. **Row A** shows the status of Lesion 5 at baseline. **Row B** shows the status of Lesion 5 prior to the initiation of a second planned cycle of anti-B7-H3 antibody administration. **Row C** reveals the state of Lesion 5 at the time anti-B7-H3 antibody therapy was stopped. The status post administration of bevacizumab is shown in **Row D**. Typical of IDH-mutant gliomas (see lower right arrow) much of tumor was never previously enhancing. Most of the tumor developed marked enhancement with anti-B7-H3 antibody (cycle 2). Tumor size remained stable to decreased after 1 dose of bevacziumab. Lesion 5 (lower right arrow) never enhanced much with anti-B7-H3 antibody therapy and grew steadily throughout the course of treatment. Lesion 5 growth trapped left ventricle (see upper right arrow), and the patient needed a VP shunt. The patient's cognition and gait improved.

[00293] The data of **Figures 2A-2C** are interpreted as indicating that the combination treatment of bevacizumab and anti-B7-H3 antibody acted synergistically to improve the patient's condition with respect to Lesions 1-4, but that Lesion 5 grew steadily throughout, without any treatment effect. Lesions 1-4 developed marked enhancement after 1 cycle (having previously never had much enhancement, as is typical of IDH1-mutant gliomas). Treatment effect was symptomatic; the patient experienced neurological decline (ataxia and short term memory loss) upon going off study. After 1 dose of bevacizumab, most of tumor was stable to mildly decreased compared to

baseline (see, *e.g.*, Lesion 2). The treatment effect thus eventually caused tumor shrinkage; tumor heterogeneity resulted in mixed response.

[00294] Currently, the patient is neurologically improved from clinical nadir 5 months after the last dose of anti-B7-H3 antibody. The patient has been clinically stable for more than 10 months after the last dose of anti-B7-H3 antibody. The patient's overall survival is more than 3 years, 5 months from the date of diagnosis.

Patient GC

[00295] **Figure 4** shows the clinical progression of Patient GC. MRI scans of the patient's brain revealed 3 lesions. An initial cycle of humanized anti-B7-H3 antibody was administered at baseline. After cycle 1, markedly increased enhancement with cognitive and gait decline were observed.

[00296] **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken prior to the start of cycle 6) shows that the lesions were stable through 5-6 cycles of anti-B7-H3 antibody administration, with a possible minor response. **Row C** (images taken at the end of cycle 6) show signs of progressive disease, including the occurrence of new lesions. The patient experienced cognitive decline and edema was noted in the patient's brain (**Row C**, fourth column). **Row D** shows MRI images taken after 2 doses of bevacizumab. As is evident, lesions and edema were found to decrease in size and extent. Cognition was observed to improve, but was not at baseline.

[00297] The data of **Figure 4** are interpreted as indicating that the combination treatment of bevacizumab and anti-B7-H3 antibody acted synergistically to improve the patient's condition with respect to the observed lesions. The patient responded to bevacizumab treatment after anti-B7-H3 antibody treatment as indicated by the fact that the prior target lesions were smaller after bevacizumab treatment than prior to anti-B7-H3 antibody treatment. The patient's overall survival is more than 3 years from the date of diagnosis.

Patient DK

[00298] **Figure 4** shows the clinical progression of Patient DK. MRI scans of the patient's brain revealed 3 lesions. An initial cycle of humanized anti-B7-H3 antibody

was administered at baseline. On the first day of cycle 2 (C2D1) treatment was amended to include the administration of dexamethasone (2 mg bid). **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken in the middle of cycle 2) shows progression and pseudo-progression of the disease. **Row C** (images taken post administration of 2 doses of bevacizumab shows a mild response, with cognition and gait mildly improved.

[00299] The data of **Figure 4** are interpreted as indicating both pseudo-progression and progression, and that the combination treatment of bevacizumab and anti-B7-H3 antibody provided a mild response and acted synergistically to improve the patient's condition with respect to the observed lesions. The patient responded to bevacizumab treatment after anti-B7-H3 antibody treatment as indicated by the fact that the prior target lesions were smaller after bevacizumab treatment than prior to anti-B7-H3 antibody treatment.

[00300] The patient was in declining health in a hospice 10 weeks after the last dose of anti-B7-H3 antibody. The patient's overall survival is more than 1 year, 10 months from the date of diagnosis.

Patient JK

[00301] Figure 5 shows the clinical progression of Patient JK. MRI scans of the patient's brain revealed 3 lesions. An initial cycle of humanized anti-B7-H3 antibody was administered at baseline. After cycle 1, treatment was amended to include the administration of dexamethasone (2 mg bid). A mild decline in language and right arm dexterity was observed. **Row A** shows the status of the patient's lesions at baseline. **Row B** (images taken prior to cycle 2) shows progression and pseudo-progression of the disease. After administration of bevacizumab and CCNU x 1 month, the patient shows stable language and right arm dexterity. A further MRI scan is pending.

Patient DW

[00302] Figure 6 shows the clinical progression of Patient DW. MRI scans of the patient's brain revealed 3 lesions. An initial cycle of humanized anti-B7-H3 antibody was administered at baseline. **Row A** shows the status of the patient's lesions at

baseline. After cycle 1, disease progression was noted with severe language and right arm and leg decline. The patient received 1 dose of bevacizumab, but continued to experience severe language and right arm and leg decline. **Row B** (images taken prior to cycle 2) shows progression of the disease. The patient succumbed to illness 5 weeks after the last dose of anti-B7-H3 antibody (overall survival was 14 months from diagnosis). The patient evidenced no clinical response to bevacizumab after anti-B7-H3 antibody treatment. The absence of response is possibly due to high baseline steroids or a particularly refractory disease.

[00303] In conclusion, the data show that a treatment effect (increased enhancement) occurs (pseudo-progression). The treatment effect appears to be associated with tumor response and with observable neurological symptoms, which may not be completely reversible. Bevacizumab appears to synergize or perhaps reduce the treatment effect. A high baseline dose of dexamethasone appears to result in resistance. The anti-B7-H3 antibody treatment was well tolerated. **Figure 7** shows the time on study for the 6 treated patients.

Example 2
Evaluation of Patients Receiving Anti-B7-H3 Antibody

[00304] In the course of an ongoing clinical trial to assess the efficacy of anti-B7-H3 antibody therapy in the treatment of cancer, the levels of soluble B7-H3 ("sB7-H3") in the recipient patients was measured. The level of sB7-H3 in recipient patients prior to treatment was zero.

[00305] As shown in **Figure 8** (all patients) and **Figure 9** (glioblastoma patients), the level of sB7-H3 in recipient patients increased throughout the first cycle of treatment, eventually declining as the treatment continues. This increase may reflect the death (and disruption) of tumor cells or the cleavage and release of membrane bound B7-H3, as a consequence of the therapy. The release of the sB7-H3 in recipient patients is a marker of progressive disease.

[00306] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference

in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth

What Is Claimed Is:

Claim 1. A method of treating a vascularizing cancer comprising administering to a recipient patient in need thereof, a molecule having a binding ability that is specific for B7-H3 and a molecule having a binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis.

Claim 2. The method of claim 1, wherein said molecule having said binding ability that is specific for B7-H3 is:

(A) an anti-B7-H3 antibody;

(B) a B7-H3-binding fragment of (A); or

(C) a diabody that binds B7-H3.

Claim 3. The method of any one of claims 1-2, wherein said molecule having said binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis is:

(A) an anti-VEGF antibody or a VEGF antagonist; or

(B) an anti-VEGFR antibody or a VEGFR antagonist.

Claim 4. The method of any of claims 1-3, wherein said molecule having said binding ability that is specific for B7-H3:

A. competes for B7-H3 binding with antibodies BRCA84D, BRCA69D, or PRCA157; or

B. has the three heavy chain CDRs and the three light chain CDRs of antibody BRCA84D, of antibody BRCA69D, or of antibody PRCA157.

Claim 5. The method of any of claims 1-4, wherein said molecule having said binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis:

A. competes for VEGF binding with bevacizumab; or

B. has the three heavy chain CDRs and the three light chain CDRs of bevacizumab.

Claim 6. The method of claim 1, wherein said molecule having said binding ability that is specific for B7-H3 is an anti-B7-H3 antibody having:

(A) a light chain variable domain that comprises the CDR_1, CDR_2 and CDR_3 of the light chain of BRCA84D;

(B) a heavy chain variable domain that comprises the CDR_1 CDR_2 and CDR_3 of the heavy chain of BRCA84D; and

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

Claim 7. The method of claim 6, wherein said molecule having said binding ability that is specific for B7-H3 is a humanized anti-B7-H3 antibody that comprises:

(A) a variable light chain having the amino acid sequence of hBRCA84D-2 VL (**SEQ ID NO:69**); and

(B) a variable heavy chain having the amino acid sequence of hBRCA84D-2 VH (**SEQ ID NO:79**);

and said molecule having said binding ability that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis is bevacizumab.

Claim 8. The method of any of claims 3-7, wherein said molecule having said B7-H3 binding ability and said molecule having said VEGF-binding ability are administered to said patient, and said two molecules are administered concurrently.

Claim 9. The method of claim 8, wherein the second of said administered molecules is administered to said patient within 5 half-lives after the administration of the first of said molecule.

Claim 10. The method of any of claims 1-9, wherein said vascularizing cancer is glioblastoma.

Claim 11. The method of claim 1, wherein said molecule having said binding ability that is specific for B7-H3 and said molecule having said binding ability

that is specific for a cell-surface factor, or a receptor therefor, that promotes tumor angiogenesis are the same molecule, said molecule being a bi-specific antibody or a bi-specific diabody.