TITLE OF THE INVENTION:

Novel B7-H3-Binding Molecules, Antibody Drug Conjugates Thereof and Methods of Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Patent Applications Serial No. 62/432,314 (filed December 9, 2016; pending), 62/323,249 (filed April 15, 2016; pending), 62/323,228 (filed April 15, 2016; pending), each of which applications are herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in computer-readable media (file name: 1301_0143-0144PCT_ST25.txt, created on March 28, 2017, and having a size of 104,762 bytes), which file is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention is directed to novel B7-H3-binding molecules capable of binding to human and non-human B7-H3, and in particular to such molecules that are cross-reactive with B7-H3 of a non-human primate (*e.g.*, a cynomolgus monkey). The invention additionally pertains to B7-H3-binding molecules that comprise Variable Light Chain and/or Variable Heavy Chain (VH) Domains that have been humanized and/or deimmunized so as to exhibit a reduced immunogenicity upon administration to recipient subjects. The invention particularly pertains to bispecific, trispecific or multispecific B7-H3-binding molecules, including bispecific diabodies, BiTEs, bispecific antibodies, trivalent binding molecules, *etc.* that comprise: (i) such B7-H3-binding Variable Domains and (ii) a domain capable of binding to an epitope of a molecule present on the surface of an effector cell. The invention is also directed to pharmaceutical compositions that contain any of such B7-H3-binding molecules, and to methods involving the use of any of such B7-H3-binding molecules in the treatment of cancer and other diseases and conditions. The invention also particularly pertains to a molecule that comprises the human B7-H3 binding domain of a humanized anti-human B7-H3 antibody conjugated to at least one drug moiety (a "**B7-H3-ADC**"). The invention is also directed to pharmaceutical compositions that

contain such **B7-H3-ADC**s, and to methods involving the use of any of such **B7-H3-ADC**s in the treatment of cancer and other diseases and conditions.

BACKGROUND OF THE INVENTION

[0004] The growth and metastasis of tumors depends to a large extent on their capacity to evade host immune surveillance and overcome host defenses. Most tumors express antigens that can be recognized to a variable extent by the host immune system, but in many cases, an inadequate immune response is elicited because of the ineffective activation of effector T cells (Khawli, L.A. *et al.* (2008) "*Cytokine, Chemokine, and Co-Stimulatory Fusion Proteins for the Immunotherapy of Solid Tumors*," Exper. Pharmacol. 181:291-328).

I. B7 Superfamily and B7-H3

[0005] **B7-H3** is a member of the B7-CD28 Superfamily and is expressed on Antigen-Presenting Cells. It binds to T Cells, however, the B7-H3 counter-receptor on the surface of such T Cells has not yet been fully characterized.

[0006] 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). Although initially thought to comprise only 2 Ig domains (IgV–IgC) a four immunoglobulin extracellular domain variant ("4Ig-B7-H3") has been identified and found to be the more common human form of the protein (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). However, the natural murine form (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 NK-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 reported 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) however, more recent studies point to an inhibitory role of murine and human B7-H3 (Prasad, D.V., *et al.* (2004) "*Murine B7-H3 Is A Negative Regulator Of T Cells*, J Immunol. 173:2500-2506; Leitner, J., *et al.* (2009) "*B7-H3 Is A Potent Inhibitor Of Human*

T-Cell Activation: No Evidence For B7-H3 And TREML2 Interaction." Eur. J. Immunol. 39:1754-1764; Veenstra, R.G., *et al.* (2015) "*B7-H3 expression in Dnor T Cells and Host Cells Negatively Regulates Acute Graft-Versus-Host Disease Lethality*," Blood 125:3335-3346.). B7-H3 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).

[0007] Although B7-H3 is not expressed on resting B or T Cells, monocytes, or dendritic cells, 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 mode of action of B7-H3 is complex, and the protein is reported to mediate 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 an 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 NK-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). 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).

II. B7-H3 Expressing Tumors

[0008] B7-H3 is also known to be expressed on a variety of cancer cells (*e.g.*, neuroblastoma, gastric, ovarian, non-small cell lung cancers, *etc.*, see, *e.g.*, 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 61:4048-54) and cultured cancer stem-like cells. 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; Sun, Y., *et al.* (2006) "*B7-H3 and B7-H4 expression in non-small-cell lung cancer*," Lung Cancer 53:143-51; Tekle, C., *et al.* (2012) "*B7-H3 Contributes To The Metastatic Capacity Of Melanoma Cells By Modulation Of Known Metastasis-Associated Genes*," Int. J. Cancer 130:2282-90; Wang, L., *et al.* (2013) "*B7-H3 Mediated Tumor Immunology: Friend Or Foe?*," Int. J. Cancer 134(12):2764-2771), suggesting that B7-H3 is exploited by tumors as an immune evasion pathway (Hofmeyer, K. *et al.* (2008) "*The Contrasting Role Of B7-H3*," Proc. Natl. Acad. Sci. (U.S.A.) 105(30):10277-10278).

[0009] B7-H3 protein expression has also 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; Mather, J. *et al,* WO 2004/001381; 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).

[0010] The role of B7-H3 in inhibiting the immune system and the increased expression of B7-H3 on human tumors has suggested that this molecule might serve as a therapeutic target for the treatment of cancer. Thus, the use of anti-B7-H3 antibodies and other molecules that modulate B7-H3 expression to treat tumors and/or up-modulate an immune response has been proposed (see, Loo, D. *et al.* (2012) "*Development of an Fc-Enhanced Anti–B7-H3 Monoclonal Antibody with Potent Antitumor Activity*," Clin Cancer Res; 18: 3834-3845; Ahmed, M. *et al.* (2015) "*Humanized Affinity-Matured Monoclonal Antibody 8H9 Has Potent Anti-Tumor Activity and Binds to FG Loop of B7-H3*," J. Biol. Chem. 290: 30018-30029; Nagase-Zembutsu, A. *et al.* (2016) "*Development of DS-5573a:*

A novel afucosylated monoclonal antibody directed at B7-H3 with potent antitumor activity," Cancer Sci. 2016, doi: 10.1111/cas.12915; 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 (9^{0th} 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; see also, United States Patents No. 7,279,567, 7,358,354, 7,368,554, 7,527,969,7,718,774, 8,216,570, 8,779,098, 8,802,091, 9,150,656, US Patent Publication Nos. 2002/0168762; 2005/0202536, 2008/0081346, 2008/0116219, 2009/0018315, 2009/0022747, 2009/0087416, 2013/0078234, 2015/0274838, PCT Publications Nos. WO 2008/066691; WO 2006/016276; WO 2008/116219; WO 04/001381, WO 2001/094413, WO 2002/10187, WO 2002/32375, WO 2004/093894, WO 2006/016276,WO 2008/116219,WO 2011/109400; and EP 1292619B.

[0011] Notwithstanding all such prior success, a need remains for additional therapeutic agents which target and kill tumor cells expressing B7-H3. The present invention is directed to this and other goals.

SUMMARY

[0012] The present disclosure is directed to novel B7-H3-binding molecules capable of binding to human and non-human B7-H3, and in particular to such molecules that are cross-reactive with B7-H3 of a non-human primate (*e.g.*, a cynomolgus monkey). The disclosure additionally pertains to B7-H3-binding molecules that comprise Variable Light Chain and/or Variable Heavy Chain (VH) Domains that have been humanized and/or deimmunized so as to exhibit a reduced immunogenicity upon administration to recipient

subjects. The disclosure particularly pertains to bispecific, trispecific or multispecific B7-H3-binding molecules, including bispecific diabodies, BiTEs, bispecific antibodies, trivalent binding molecules, *etc*. that comprise: (i) such B7-H3-binding Variable Domains and (ii) a domain capable of binding to an epitope of a molecule present on the surface of an effector cell. The disclosure is also directed to pharmaceutical compositions that contain any of such B7-H3-binding molecules, and to methods involving the use of any of such B7-H3-binding molecules in the treatment of cancer and other diseases and conditions. The disclosure also particularly pertains to a molecule that comprises the human B7-H3 binding domain of a humanized anti-human B7-H3 antibody conjugated to at least one drug moiety (a "**B7-H3-ADC**"). The disclosure is also directed to pharmaceutical compositions that contain such **B7-H3-ADC**s, and to methods involving the use of any of such **B7-H3-ADC**s in the treatment of cancer and other diseases and conditions.

[0013] In detail, disclosed is a B7-H3-binding molecule that comprises a Variable Light Chain (VL) Domain and a Variable Heavy Chain (VH) Domain, wherein said Variable Heavy Chain Domain comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, and said Variable Light Chain Domain comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, wherein at least three of said domains, at least four of said domains, at least five of said domains or all of said domains are selected from the group consisting of:

(1) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:27**;

(2) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:28**;

(3) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:29**;

(4) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:23**;

(5) a CDR_L2 Domain comprising the amino acid sequence of **SEQ ID NO:24**; and

(6) a CDR_L3 Domain comprising the amino acid sequence of **SEQ ID NO:25**.

[0014] The disclosure additionally concerns the example of such B7-H3-binding molecule that comprises said Variable Light Chain (VL) Domain that comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, and said Variable Heavy Chain (VH) Domain that comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, wherein:

(1) said CDR_H1 Domain comprises the amino acid sequence of **SEQ ID NO:27**;

(2) said CDR_H2 Domain comprises the amino acid sequence of **SEQ ID NO:28**;

(3) said CDR_H3 Domain comprises the amino acid sequence of **SEQ ID NO:29**.

[0015] The disclosure additionally concerns the example of such B7-H3-binding molecule that comprises said Variable Light Chain (VL) Domain that comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, and said Variable Heavy Chain (VH) Domain that comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, wherein:

(1) said CDR_L1 Domain comprises the amino acid sequence of **SEQ ID NO:23**;
(2) said CDR_L2 Domain comprises the amino acid sequence of **SEQ ID NO:24**; and
(3) said CDR_L3 Domain comprises the amino acid sequence of **SEQ ID NO:25**.

[0016] The disclosure additionally concerns the example of such B7-H3-binding molecules wherein said Variable Heavy Chain (VH) Domain comprises the amino acid sequence of **SEQ ID NO:26** or **SEQ ID NO:31**.

[0017] The disclosure additionally concerns the example of such B7-H3-binding molecules wherein said Variable Light Chain (VL) Domain comprises the amino acid sequence of **SEQ ID NO:22** or **SEQ ID NO:30**.

[0018] The disclosure additionally concerns B7-H3-binding molecules that comprise a VL Domain and a VH Domain, wherein said VL Domain comprises the amino acid sequence of **SEQ ID NO:20**.

[0019] The disclosure additionally concerns B7-H3-binding molecules that comprise a VL Domain and a VH Domain, wherein said VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

[0020] The disclosure additionally concerns B7-H3-binding molecules that comprise a VL Domain and a VH Domain, wherein said VL Domain comprises the amino acid of **SEQ ID NO:20** and said VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

[0020a] In one aspect, the present disclosure refers to a B7-H3 binding molecule, wherein said B7-H3 binding molecule is an antibody, a diabody, or an epitope-binding fragment thereof, which is capable of binding to B7-H3, wherein said B7-H3 binding molecule comprises:

(i) a humanized Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20**; and
(ii) a humanized Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**.

[0021] The disclosure further concerns the example of such B7-H3-binding molecules, wherein the molecule is an antibody or an epitope-binding fragment thereof. The disclosure also concerns the examples of such a B7-H3-binding molecule, wherein the molecule is a bispecific antibody or a diabody, especially a diabody, or diabody complex,

that comprises two, three, four or five polypeptide chains each having an N-terminus and a C-terminus in which such polypeptide chains are associated together via one or more covalent, and especially one or more covalent disulfide, bonds. The disclosure additionally concerns the example of such B7-H3-binding molecules wherein the molecule is a trivalent binding molecule, and especially wherein the trivalent binding molecule is a covalently bonded complex that comprises three, four, five, or more polypeptide chains. The disclosure further concerns the example of such a B7-H3-binding molecule, wherein the molecule comprises an Fc Domain. The disclosure additionally concerns the example of such B7-H3-binding molecules wherein the molecule is a diabody and comprises an Albumin-Binding Domain, and especially a deimmunized Albumin-Binding Domain.

[0022] The disclosure further concerns the examples of all such B7-H3-binding molecules that additionally comprise an Fc Domain, and especially wherein the Fc Domain is a variant Fc Domain that comprises one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR and/or enhances the serum half-life of the B7-H3-binding molecule, and more particularly, wherein the modifications comprise at least one substitution selected from the group consisting of:

(a) L234A;

(b) L235A;

(c) L234A and L235A;

(d) M252Y; M252Y and S254T;

(e) M252Y and T256E;

(f) M252Y, S254T and T256E; and

(g) K288D and H435K;

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

[0023] The disclosure further concerns the example of such B7-H3-binding molecules, wherein the molecule is bispecific, and particularly concerns the example wherein the molecule comprises two epitope-binding sites capable of immunospecific binding to an epitope of B7-H3 and two epitope-binding sites capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell, or the example wherein the molecule comprises one epitope-binding site capable of immunospecific binding to an epitope of B7-H3 and one epitope-binding site capable of

immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

[0024] The disclosure additionally concerns the example of such B7-H3 binding molecules wherein the molecule is a trivalent binding molecule, and particularly concerns the examples wherein the molecule comprises, one epitope-binding site capable of immunospecific binding to an epitope of B7-H3, one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell, wherein such first and second molecules are not B7-H3.

[0025] The disclosure further concerns the example of such a B7-H3-binding molecule, wherein the molecule is capable of simultaneously binding to B7-H3 and to a second epitope, and particularly concerns the example wherein the second epitope is an epitope of a second molecule present on the surface of an effector cell (especially wherein the second epitope is an epitope of CD2, CD3, CD8, CD16, TCR, or NKG2D, and most particularly wherein the second epitope is an epitope of CD3). The disclosure additionally concerns the example of such B7-H3-binding molecules, wherein the effector cells is a cytotoxic T-cell or a Natural Killer (NK) cell. The disclosure additionally concerns the example of such B7-H3-binding molecules, wherein the molecule is also capable of binding a third epitope, and particularly concerns the example wherein the third epitope is an epitope of CD8. The disclosure further concerns the examples of such molecules wherein molecule mediates coordinated binding of a cell expressing B7-H3 and a cytotoxic T cell.

[0026] The disclosure further provides pharmaceutical compositions comprising an effective amount of any of the above-described B7-H3-binding molecules and a pharmaceutically acceptable carrier, excipient or diluent.
[0026a] In another aspect, the present disclosure refers to a pharmaceutical composition that comprises an effective amount of the **B7-H3-ADC** as described herein, or the B7-H3 binding molecule as described herein, and a pharmaceutically acceptable carrier, excipient or diluent.
[0026b] In another aspect, the present disclosure refers to the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein for use as a medicament.
[0026c] In another example, the present disclosure refers to the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein for use in the treatment of cancer.

[0026d] In another aspect, the present disclosure refers to use of the **B7-H3-ADC** as described herein, the B7-H3 binding molecule as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer.

[0027] The disclosure is additionally directed to the use of any of the above-described B7-H3-binding molecules in the treatment of a disease or condition associated with or characterized by the expression of B7-H3, or in a method of treating a disease or condition characterized by the expression of B7-H3, particularly wherein the disease or condition associated with or characterized by the expression of B7-H3 is cancer, and more

particularly, wherein the cancer is selected from the group consisting of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, 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.

[0028] Disclosed is a molecule that comprises the human B7-H3 binding domain of a humanized anti-human B7-H3 antibody conjugated to at least one drug moiety (a "**B7-H3-ADC**"). The disclosure is also directed to pharmaceutical compositions that contain such **B7-H3-ADCs**, and to methods involving the use of any of such **B7-H3-ADCs** in the treatment of cancer and other diseases and conditions.

[0029] In detail, the disclosure provides an anti-B7-H3 antibody drug conjugate (**B7-H3-ADC**) comprising the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n},$$

wherein:

Ab is an antibody that binds to B7-H3 that comprises a humanized Variable Heavy Chain (VH) Domain and a humanized Variable Light Chain (VL) Domain, or is a B7-H3-binding fragment thereof, and;

D is a cytotoxic drug moiety;

LM is a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the ADC.

[0029a] In another aspect, the present disclosure refers to an anti-B7-H3 antibody drug conjugate (**B7-H3-ADC**) that comprises the formula: **Ab-(LM)$_m$-(D)$_n$,** wherein:

Ab is a humanized B7-H3 antibody or B7-H3 binding fragment thereof that binds to B7-H3 and comprises:

(i) the CDR_L1 sequence RASESIYSYLA, the CDR_L2 sequence NTKTLPE and the CDR_L3 sequence QHHYGTPPWT in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYGMS, the CDR_H2 sequence TINSGGSNTYY PDSLKG and the CDR_H3 sequence HDGGAMDY in its Variable Heavy Chain (VH) domain;

D is a cytotoxic drug moiety comprising a duocarmycin cytotoxin;

LM comprises at least one bond or a Linker Molecule that covalently links Ab and D;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**; and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

[0030] The disclosure further provides such **B7-H3-ADCs**, wherein the Linker Molecule **LM** is absent (*i.e.*, **m** = 0), and **B7-H3-ADCs** that possess more than one Linker Molecule **LM** (*i.e.*, **m** is an integer from 2 through **n**), each of which Linker Molecule **LM** covalently links a cytotoxic drug moiety **D** to the **Ab** of such **B7-H3-ADCs**. The disclosure further provides such **B7-H3-ADCs** whose **Ab** are covalently linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are identical. The cytotoxic drug moieties **D** that are covalently linked to the **Ab** of such **B7-H3-ADCs** may all be identical or may include 2, 3, 4, or more non-identical cytotoxic drug moieties **D**. The disclosure further provides such **B7-H3-ADCs** whose **Ab** are covalently linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are not identical. The cytotoxic drug moieties **D** that are covalently linked to the **Ab** of such **B7-H3-ADCs** may all be identical or may include 2, 3, 4, or more non-identical cytotoxic drug moieties **D**.

[0031] The disclosure further provides such **B7-H3-ADCs**, wherein:

(A) (i) the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:99**, and

(ii) the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:104**;

or

(B) (i) the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:20**, and

(ii) the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:21**;

or

(C) (i) the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:30**, and

(ii) the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:31**.

[0032] The disclosure further provides such **B7-H3-ADCs**, wherein the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:99** and the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:104**.

[0033] The disclosure further provides such **B7-H3-ADCs**, wherein the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:20** and the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

[0034] The disclosure further provides such **B7-H3-ADCs**, wherein the humanized the humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:30** and the humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:31**.

[0035] The disclosure further provides such **B7-H3-ADCs**, wherein the **Ab** is an antibody or an antigen binding fragment of an antibody.

[0036] The disclosure further provides such **B7-H3-ADCs**, wherein the **B7-H3-ADC** comprises an Fc Domain of a human IgG (especially a human IgG1, IgG2, IgG3, or IgG4).

[0037] The disclosure further provides such **B7-H3-ADCs**, wherein the **B7-H3-ADC** comprises a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

[0038] The disclosure further provides such **B7-H3-ADCs** that comprise a variant Fc Domain, wherein the modifications that reduces the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

[0039] The disclosure further provides such **B7-H3-ADCs** that comprise a variant Fc Domain, wherein the modifications that that enhances the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E;

M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

[0040] The disclosure further provides such **B7-H3-ADCs** wherein at least one of the **LM** is a Linker Molecule, and particularly wherein the **LM** Linker Molecule is a peptidic linker and/or a cleavable linker.

[0041] The disclosure further provides such **B7-H3-ADCs** wherein the molecule comprises the formula:

$$\mathbf{Ab - [V\text{-}(W)_k\text{-}(X)_1\text{-}A] - D}$$

wherein:

V is the cleavable **LM** Linker Molecule,

$\mathbf{(W)_k\text{-}(X)_1\text{-}A}$ is an elongated, self-eliminating spacer system, that self-eliminates via a 1,(4+2n)-elimination,

W and **X** are each a 1,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula $(\mathbf{Y})_m$, wherein **Y** is a 1,(4+2n) electronic cascade spacer, or a group of formula **U**, being a cyclisation elimination spacer,

k, 1 and m are independently an integer of 0 (included) to 5 (included),

n is an integer of 0 (included) to 10 (included),

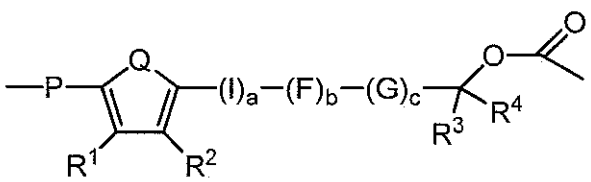
with the provisos that:

when **A** is $(\mathbf{Y})_m$: then $k+l+m \geq 1$, and

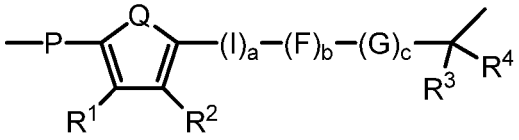
if $k+l+m=1$, then $n>1$;

when **A** is **U**: then $k+1 \geq 1$.

W, **X**, and **Y** are independently selected from compounds having the formula:



or the formula:

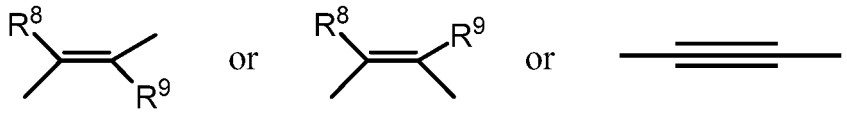


wherein: Q is $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$

P is NR^7, O or S

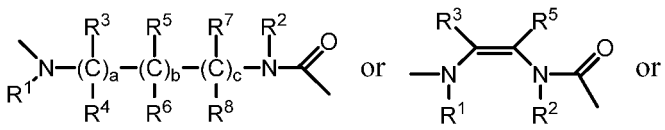
a, b, and c are independently an integer of 0 (included) to 5 (included);

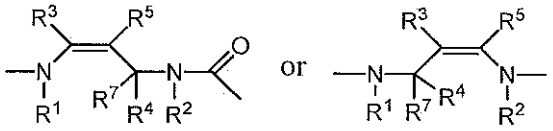
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are independently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures;

U is selected from compounds having the formula:





wherein:

a, b and c are independently selected to be an integer of 0 or 1; provided that a + b + c = 2 or 3;

R^1 and/or R^2 independently represent H, C1-6 alkyl, the alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_X), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_X$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)ORx), sulphinyl (S(=O)Rx), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7 and R^8 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, and two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 are optionally connected to one another to form one or more aliphatic or aromatic cyclic structures.

[0042] The disclosure further provides such **B7-H3-ADCs** wherein the **LM** Linker Molecule comprises:

(1) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(2) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(3) p-ammocinnamyloxycarbonyl;

(4) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl;

(5) p-amino-benzyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(6) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(7) p-aminophenylpentadienyloxycarbonyl;

(8) p-aminophenylpentadienyloxycarbonyl-p-arninocinnamyloxycarbonyl;

(9) p-aminophenylpentadienyloxycarbonyl-paminobenzyloxycarbonyl;

(10) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl;

(11) p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(12) p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(13) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino) ethyl(methylamino)carbonyl;

(14) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(15) p-aminobenzyloxycarbonyl-p-arninocinnamyloxycarbonyl (methylamino)ethyl(methylamino)-carbonyl;

(16) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(17) p-aminobenzyloxycarbonyl-p-aminobenzyl;

(18) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl -p-aminobenzyl;

(19) p-aminocinnamyl;

(20) p-aminocinnamyloxycarbonyl-p-aminobenzyl;

(21) p-aminobenzyloxycarbonyl-p-aminocinnamyl;

(22) p-amino-cinnamyloxycarbonyl-p-aminocinnamyl;

(23) p-aminophenylpentadienyl;

(24) p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl;

(25) p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl;

or

(26) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

[0043] The disclosure further provides such **B7-H3-ADCs** wherein the **LM** Linker Molecule is conjugated to the side chain of an amino acid of a polypeptide chain of **Ab** and binds the **Ab** to a molecule of the cytotoxic drug moiety **D**, and in particular, wherein the cytotoxic drug moiety **D** comprises a cytotoxin, a radioisotope, an immunomodulator, a cytokine, a lymphokine, a chemokine, a growth factor, a tumor necrosis factor, a hormone, a hormone antagonist, an enzyme, an oligonucleotide, a DNA, an RNA, an siRNA, an RNAi, a microRNA, a photoactive therapeutic agent, an anti-angiogenic agent, a pro-apoptotic agent, a peptide, a lipid, a carbohydrate, a chelating agent, or a combinations thereof.

[0044] The disclosure further provides such **B7-H3-ADCs** wherein the **LM** Linker Molecule is conjugated to the side chain of an amino acid of a polypeptide chain of **Ab** and binds the **Ab** to a molecule of the cytotoxic drug moiety **D**, and in particular, wherein the cytotoxic drug moiety **D** comprises a cytotoxin selected from the group consisting of a tubulysin (especially a tubulysin cytotoxin selected from the group consisting of tubulysin A, tubulysin B, tubulysin C, and tubulysin D), an auristatin (especially an auristatin cytotoxin selected from the group consisting of MMAE (N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine) and MMAF (N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine), a maytansinoid (especially a maytansinoid cytotoxin selected from the group consisting of Mytansine, DM1 and DM4), a calicheamicin (especially a calicheamicin cytotoxin selected from the group consisting of calicheamicin γ1, calicheamicin β1Br, calicheamicin γ1Br, calicheamicin α2I, calicheamicin α3I, calicheamicin β1I, calicheamicin γ1I, and calicheamicin Δ1I), a pyrrolobenzodiazepine (especially a pyrrolobenzodiazepine cytotoxin selected from the group consisting of vadastuximab talirine, SJG-136, SG2000, SG2285 and SG2274), and a duocarmycin (especially a duocarmycin cytotoxin and is selected from the group consisting of duocarmycin A, duocarmycin B1, doucarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin (U-80244) and spiro-duocarmycin (DUBA)).

[0045] The disclosure further provides pharmaceutical compositions comprising an effective amount of any of the above-described **B7-H3-ADCs** and a pharmaceutically acceptable carrier, excipient or diluent.

[0046] The disclosure is additionally directed to the use of any of the above-described **B7-H3-ADCs** in the treatment of a disease or condition associated with or characterized by the expression of B7-H3, or in a method of treating a disease or condition characterized by the expression of B7-H3, particularly wherein the disease or condition associated with or characterized by the expression of B7-H3 is cancer, and more particularly, wherein the cancer is selected from the group consisting of: an acute myeloid leukemia, an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, a glioblastoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a malignant mesothelioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a pharyngeal cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] **Figure 1** provides a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting

Domains are provided below). A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0048] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0049] **Figures 3A-3C** provide schematics showing representative covalently bonded tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments wherein the two pairs of polypeptide chains are the same and the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3B**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments wherein the two pairs of polypeptide chains are different and the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown by the different shading and patterns in **Figure 3C**), the resulting molecule possesses four epitope-binding sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc Domain-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc Domain-containing diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue). **Figure 3C**, shows an Fc-Region-Containing diabody, which contains antibody CH1 and CL domains.

[0050] **Figures 4A and 4B** provide schematics of a representative covalently bonded diabody molecule having two epitope-binding sites composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Domain. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0051] **Figure 5** provides the schematics of a representative covalently bonded diabody molecule having four epitope-binding sites composed of five polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Domain that comprises all or part of an Fc Domain. The polypeptide chains comprising the linked VL and VH Domains further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0052] **Figures 6A-6F** provide schematics of representative Fc Domain-containing trivalent binding molecules having three epitope-binding sites. **Figures 6A** and **6B**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains and a Fab-type binding domain having different domain orientations in which the diabody-type binding domains are N-terminal or C-terminal to an Fc Domain. The molecules in **Figures 6A** and **6B** comprise four chains. **Figures 6C** and **6D**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains N-terminal to an Fc Domain, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 6E** and **6F**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains C-terminal to an Fc Domain, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 6C-6F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0053] **Figure 7** shows the results of a screen for anti-B7-H3 antibodies capable of internalizing into Hs700T pancreatic cancer cells.

[0054] **Figures 8A-8J** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vitro* cytotoxicity against B7-H3 expressing JIMT-1 breast cancer cells (**Figure 8A**), MDA-MB-468 breast cancer cells (**Figure 8B**), A375.52 melanoma cells (**Figure 8C**), Calu-6 non-small cell lung cancer cells (**Figure 8D**), NCI-H1703 non-small cell lung cancer cells (**Figure 8E**), NCI-H1975 non-small cell lung cancer cells (**Figure 8F**), PA-1 ovarian cancer cells (**Figure 8G**), Hs700T pancreatic cancer cells (**Figure 8H**), DU145 prostate cancer cells (**Figure 8I**), and B7-H3 negative Raji B Cell lymphoma cells (**Figure 8J**).

[0055] **Figure 9** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vivo* cytotoxicity against MDA-MB-468 breast cancer tumor cells implanted in the mammary fat pad in a CD1 nude mouse model. The tumor growth curves are presented for mice treated intraperitoneally with 10 mg/kg of **chmAb-B-vc-MMAE**, **chmAb-C-vc-MMAE**, and **chmAb-D-vc-MMAE** or vehicle alone on Day 25 (shown by arrow).

[0056] **Figures 10A-10C** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vivo* cytotoxicity against subcutaneously implanted NCI-H1703 non-small cell lung cancer tumor cells in a CD1 nude mouse model. The tumor growth curves are presented for mice treated intraperitoneally with 10 mg/kg (**Figure 10A**), 3 mg/kg (**Figure 10B**), 1 mg/kg (**Figure 10C**) **chmAb-B-vc-MMAE**, **chmAb-C-vc-MMAE**, and **chmAb-D-vc-MMAE** at 10 mg/kg or vehicle alone on Day 52 (shown by arrow).

[0057] **Figures 11A-11C** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vivo* cytotoxicity against subcutaneously implanted PA-1 ovarian cancer tumor cells in a CD1 nude mouse model. The tumor growth curves are presented for mice treated intraperitoneally with 10 mg/kg (**Figure 11A**), 3 mg/kg (**Figure 11B**), 1 mg/kg (**Figure 5C**) **chmAb-B-vc-MMAE**, **chmAb-C-vc-MMAE**, and **chmAb-D-vc-MMAE** at 10 mg/kg or vehicle alone on Day 42 (shown by arrow).

[0058] **Figures 12A-12C** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vivo* cytotoxicity against subcutaneously implanted Calu-6 non-small cell lung cancer tumor cells in a CD1 nude mouse model. The tumor growth curves are presented for mice treated intraperitoneally with 10 mg/kg (**Figure**

12A), 3 mg/kg (**Figure 12B**), 1 mg/kg (**Figure 12C**) **chmAb-B-vc-MMAE**, **chmAb-C-vc-MMAE**, and **chmAb-D-vc-MMAE** at 10 mg/kg or vehicle alone on Day 20 (shown by arrow).

[0059] **Figures 13A-13C** shows the results of a study of the ability of the **B7-H3-ADC** of the present invention to mediate *in vivo* cytotoxicity against subcutaneously implanted A375.S2 melanoma cells in a CD1 nude mouse model. The tumor growth curves are presented for mice treated intraperitoneally with 10 mg/kg (**Figure 13A**), 3 mg/kg (**Figure 13B**), 1 mg/kg (**Figure 13C**) **chmAb-B-vc-MMAE**, **chmAb-C-vc-MMAE**, and **chmAb-D-vc-MMAE** at 10 mg/kg or vehicle alone on Day 30 (shown by arrow).

[0060] **Figures 14A-14C** shows the results of a study of the pharmacokinetic stability of **B7-H3-ADC** molecules. The serum antibody concentration cuves are presented for total antibody (circles) and intact **B7-H3-ADC** (squares) derived from **chmAb-B** (**Figure 14A**), **chmAb-C** (**Figure 14B**), and **chmAb-D** (**Figure 14C**).

[0061] **Figures 15A-15C** show the retention of biological activity by **hmAb-C B7-H3-ADC** having an exemplary duocarmycin moiety (DUBA) linked to an amino acid residue of the **Ab** portion thereof via a cleavable linker ("**hmAb-C-DUBA**"). **Figure 15A**, Calu-6 cells; **Figure 15B**, NCI-H1703 cells; **Figure 15C**, Hs700T cells. The control molecule binds CD20 and is conjugated to DUBA ("Ctrl-DUBA").

[0062] **Figure 16** shows the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** Calu-6 non-small cell lung carcinoma cells. **hmAb-C-DUBA** was introduced into groups of mice (n=5) that had been subcutaneously inoculated with Calu-6 non-small cell lung carcinoma cells. Doses of **hmAb-C-DUBA** (1 mg/kg x 3, 3 mg/kg x 3, or 6 mg/kg x 3) were provided intraperitoneally to the mice at Day 24, 31, 38 and 45 (shown by arrows) post inoculation, and the animals were evaluated for tumor volume for up to 62 days.

[0063] **Figure 17** shows the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** against Calu-6 non-small cell lung carcinoma cells. **hmAb-C-DUBA** was introduced into groups of mice (n=7) that had been subcutaneously inoculated with Calu-6 non-small cell lung carcinoma cells. A dose of **hmAb-C-DUBA** or Ctrl-DUBA (3 mg/kg or 10 mg/kg) was provided to the mice at Day 20 (shown by arrow) post inoculation, and the animals were evaluated for tumor volume for up to 55 days.

[0064] **Figure 18** shows the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** against PA-1 ovarian carcinoma cells. **hmAb-C-DUBA** or Ctrl-DUBA was introduced into groups of mice (n=6) that had been subcutaneously inoculated with PA-1 ovarian carcinoma cells. A dose of **hmAb-C-DUBA** or Ctrl-DUBA (3 mg/kg, 6 mg/kg or 10 mg/kg) was provided to the mice at Day 25 (shown by arrow) post inoculation, and the animals were evaluated for tumor volume for up to 60 days.

[0065] **Figure 19** shows the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** against A375.S2 melanoma cells. **hmAb-C-DUBA** or Ctrl-DUBA was introduced into groups of mice (n=7) that had been subcutaneously inoculated with A375.S2 melanoma cells. A dose of **hmAb-C-DUBA** or Ctrl-DUBA (1 mg/kg or 3 mg/kg) was provided to the mice at Day 25 (shown by arrow) post inoculation, and the animals were evaluated for tumor volume for up to 60 days.

[0066] **Figures 20A-20D** show the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** against fat pad xenographs of MDA-MB468 breast carcinoma cells. **hmAb-C-DUBA** or Ctrl-DUBA was administered intraperitoneally into groups of mice at days 70, 74 and 78 after being inoculated with MDA-MB468 breast carcinoma cells in the mammary fat pad. A dose of **hmAb-C-DUBA** or Ctrl-DUBA (either a single dose of 3 mg/kg or 6 mg/kg) at Day 70 or three doses of 3 mg/kg at days 70, 74 and 78 (shown by arrows) was provided post inoculation, and the animals were evaluated for tumor volume for up to 110 days. **Figure 20A** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 6 mg/kg (single dose). **Figure 20B** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 3 mg/kg (single dose). **Figure 20C** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 3 mg/kg (three doses). **Figure 20D** shows all of the results on a single graph.

[0067] **Figures 21A-21D** show the results of an *in vivo* study of the efficacy of **hmAb-C-DUBA** against subcutaneously implanted xenographs of PA-1 ovarian carcinoma cells. **hmAb-C-DUBA** or Ctrl-DUBA was administered intraperitoneally (either a single dose of 3 mg/kg, 6 mg/kg or 10 mg/kg) at day 24 post-inoculation, or two doses of 10 mg/kg **hmAb-C-DUBA** or Ctrl-DUBA (at days 24 and 28 post-inoculation) or four doses of 6 mg/kg **hmAb-C-DUBA** or Ctrl-DUBA (at days 24, 28, 31 and 35 post-inoculation). The animals were evaluated for tumor volume for up to 70 days. **Figure 21A** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 10 mg/kg (single or double dose). **Figure 21B** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 6 mg/kg (single or quadruple dose).

Figure 21C shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 3 mg/kg (single doses). **Figure 21D** shows all of the results on a single graph.

[0068] **Figure 22** shows the pharmacokinetics of **chmAb-C-DUBA** administration in mice. The figure shows total human IgG and intact ADC of **chmAb-C-DUBA** at 3 mg/kg (n=3).

[0069] **Figures 23A-23B** show the pharmacokinetics of **hmAb-C-DUBA** administration in cynomolgus monkeys. The figures show total human IgG (**Figure 23A**) and intact ADC (**Figure 23B**) of **hmAb-C-DUBA** at 1 mg/kg (1 male; 1 female), 3 mg/kg (1 male; 1 female), 10 mg/kg (1 male; 1 female) or 27 mg/kg (2 males; 2 females)).

DETAILED DESCRIPTION OF THE INVENTION

[0070] The present invention is directed to novel B7-H3-binding molecules capable of binding to human and non-human B7-H3, and in particular to such molecules that are cross-reactive with B7-H3 of a non-human primate (*e.g.*, a cynomolgus monkey). The invention additionally pertains to B7-H3-binding molecules that comprise Variable Light Chain and/or Variable Heavy Chain (VH) Domains that have been humanized and/or deimmunized so as to exhibit a reduced immunogenicity upon administration to recipient subjects. The invention particularly pertains to bispecific, trispecific or multispecific B7-H3-binding molecules, including bispecific diabodies, BiTEs, bispecific antibodies, trivalent binding molecules, *etc.* that comprise: (i) such B7-H3-binding Variable Domains and (ii) a domain capable of binding to an epitope of a molecule present on the surface of an effector cell. The invention is also directed to pharmaceutical compositions that contain any of such B7-H3-binding molecules, and to methods involving the use of any of such B7-H3-binding molecules in the treatment of cancer and other diseases and conditions. The invention also particularly pertains to a molecule that comprises the human B7-H3 binding domain of a humanized anti-human B7-H3 antibody conjugated to at least one drug moiety (a "**B7-H3-ADC**"). The invention is also directed to pharmaceutical compositions that contain such **B7-H3-ADC**s, and to methods involving the use of any of such **B7-H3-ADC**s in the treatment of cancer and other diseases and conditions.

[0071] The present invention is also directed to a molecule that comprises the human B7-H3 binding domain of a humanized anti-human B7-H3 antibody conjugated to at least one drug moiety (a "**B7-H3-ADC**"). The invention is also directed to pharmaceutical

compositions that contain such **B7-H3-ADCs**, and to methods involving the use of any of such **B7-H3-ADCs** in the treatment of cancer and other diseases and conditions.

[0072] The **B7-H3-ADC** molecules of the present invention comprise the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n},$$

wherein:

Ab is an antibody that binds to B7-H3 that comprises a humanized Variable Heavy Chain (VH) Domain and a humanized Variable Light Chain (VL) Domain, or is a B7-H3-binding fragment thereof, and;

D is a cytotoxic drug moiety;

LM is a bond or a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

I. Antibodies and Their Binding Domains

[0073] The antibodies of the present invention are immunoglobulin molecules 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 Domain of the immunoglobulin molecule. The **B7-H3-ADC** molecules of the present invention thus comprise an antibody that binds to B7-H3 or a B7-H3-binding fragment thereof. As used herein, the terms "**antibody**" and "**antibodies**" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and epitope-binding fragments of any of the above. In particular, the term "antibody" includes immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an epitope-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass. Antibodies are capable of "**immunospecifically binding**" to a polypeptide or protein or a non-protein molecule (or of binding to such molecule in an "**immunospecific manner**") due to the presence on such

molecule of a particular domain or moiety or conformation (an "**epitope**"). An epitope-containing molecule may have immunogenic activity, such that it elicits an antibody production response in an animal; such molecules are termed "**antigens**". The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) "*The Use Of Antibodies In The Treatment Of Infectious Diseases*," Singapore Med. J. 50(7):663-666). Over 200 antibody-based drugs have been approved for use or are under development.

[0074] As used herein, an antibody, diabody or other epitope-binding molecule is said to "**immunospecifically**" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and/or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "immunospecific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "immunospecific" binding. Two molecules are said to be capable of binding to one another in a "**physiospecific**" manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0075] The term "**monoclonal antibody**" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring or non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv, *etc.*), single-chain (scFv) binding molecules, mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability

to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of "antibody." Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) "*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*," Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) "*Review of Selected Adjuvants Used in Antibody Production*," ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the monospecific or multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized, a chimeric antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0076] Natural antibodies (such as IgG antibodies) are composed of two "**Light Chains**" complexed with two "**Heavy Chains**." Each Light Chain contains a Variable Domain ("**VL**") and a Constant Domain ("**CL**"). Each Heavy Chain contains a Variable Domain ("**VH**"), three Constant Domains ("**CH1**," "**CH2**" and "**CH3**"), and a "**Hinge**" Region ("**H**") located between the **CH1** and **CH2** Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal ("**N-terminal**") portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal ("**C-terminal**") portion of each chain defines a constant region, with light chains having a single Constant Domain and heavy chains usually having three Constant Domains and a Hinge Domain. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is **n-VH-CH1-H-CH2-CH3-c** (where n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide).

A. Characteristics of Antibody Variable Domains

[0077] The Variable Domains of an IgG molecule consist of the complementarity determining regions ("**CDR**"), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments ("**FR**"), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the VL and VH Domains have the structure **n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c**. Polypeptides that are (or may serve as) the first, second and third CDR of the Light Chain of an antibody are herein respectively designated as: **CDR_L1 Domain**, **CDR_L2 Domain**, and **CDR_L3 Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of the Heavy Chain of an antibody are herein respectively designated as: **CDR_H1 Domain**, **CDR_H2 Domain**, and **CDR_H3 Domain**. Thus, the terms CDR_L1 Domain, CDR_L2 Domain, CDR_L3 Domain, CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to a specific epitope regardless of whether such protein is an antibody having light and heavy chains or is a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein. Accordingly, as used herein, the term "**epitope-binding fragment**" denotes a fragment of a molecule capable of immunospecifically binding to an epitope. An epitope-binding fragment may contain any 1, 2, 3, 4, or 5 the CDR Domains of an antibody,

or may contain all 6 of the CDR Domains of an antibody and, although capable of immunospecifically binding to such epitope, may exhibit an immunospecificity, affinity or selectivity toward such epitope that differs from that of such antibody. Preferably, however, an epitope-binding fragment will contain all 6 of the CDR Domains of such antibody. An epitope-binding fragment of an antibody may be a single polypeptide chain (*e.g.*, an scFv), or may comprise two or more polypeptide chains, each having an amino terminus and a carboxy terminus (*e.g.*, a diabody, a Fab fragment, an Fab_2 fragment, *etc.*). Unless specifically noted, the order of domains of the protein molecules described herein is in the "**N-terminal to C-Terminal**" direction.

[0078] The invention particularly encompasses single-chain Variable Domain fragments ("**scFv**") comprising a humanized anti-B7-H3-VL and/or VH Domain of this invention and multispecific binding molecules comprising the same. Single-chain Variable Domain fragments comprise VL and VH Domains that are linked together using a short "Linker" peptide. Such Linkers can be modified to provide additional functions, such as to permit the attachment of a drug or to permit attachment to a solid support. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0079] The invention also particularly encompasses the CDR_H1, CDR_H2, CDR_H3, CDR_L1, CDR_L2, CDR_L3, or the VL Domain and/or the VH Domain of humanized variants of the B7-H3 antibodies of the invention, as well as multispecific-binding molecules comprising the same. The term "**humanized**" antibody refers to a chimeric molecule, generally prepared using recombinant techniques, having an epitope-binding site of an immunoglobulin from a non-human species and a remaining immunoglobulin structure of the molecule that is based upon the structure and /or sequence of a human immunoglobulin. The anti-B7-H3 antibodies of the present invention particularly include humanized, chimeric or caninized variants of antibodies **mAb-A**, **mAb-B**, **mAb-C** or **mAb-D**. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic

manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the epitope-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0080] The epitope-binding site may comprise either a complete Variable Domain fused onto Constant Domains or only the complementarity determining regions (CDRs) of such Variable Domain grafted to appropriate framework regions. Epitope-binding domains may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable domain remains (LoBuglio, A.F. *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable domains as well so as to reshape them as closely as possible to human form. It is known that the variable domains of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable domains can be "reshaped" or "humanized" by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop*

Conformation," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

[0081] A number of humanized antibody molecules comprising an epitope-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent Variable Domain and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Winter *et al.* (1991) "*Man-made Antibodies*," Nature 349:293-299; Lobuglio *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989), Shaw *et al.* (1987) "*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*," J. Immunol. 138:4534-4538, and Brown *et al.* (1987) "*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*," Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody Constant Domain (see, for example, Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; and Jones *et al.* (1986) "*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*," Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example,

European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response towards rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) "*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*," Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

B. Characteristics of Antibody Constant Domains

1. Constant Domains of the Light Chain

[0082] As indicated above, each Light Chain of an antibody contains a Variable Domain ("**VL**") and a Constant Domain ("**CL**").

[0083] A preferred CL Domain is a human IgG CL Kappa Domain. The amino acid sequence of an exemplary human CL Kappa Domain is (**SEQ ID NO:1**):

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

[0084] Alternatively, an exemplary CL Domain is a human IgG CL Lambda Domain. The amino acid sequence of an exemplary human CL Lambda Domain is (**SEQ ID NO:2**):

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

2. Constant Domains of the Heavy Chain

[0085] As indicated above, the heavy chains of an antibody may comprise CH1, Hinge Domain, CH2 and CH3 constant domains. The CH1 Domains of the two heavy chains of an antibody complex with the antibody's Light Chain's "CL" constant region, and are attached to the heavy chains CH2 Domains via an intervening Hinge Domain.

[0086] An exemplary CH1 Domain is a human IgG1 CH1 Domain. The amino acid sequence of an exemplary human IgG1 CH1 Domain is (**SEQ ID NO:3**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV

[0087] An exemplary CH1 Domain is a human IgG2 CH1 Domain. The amino acid sequence of an exemplary human IgG2 CH1 Domain is (**SEQ ID NO:4**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTV

[0088] An exemplary CH1 Domain is a human IgG3 CH1 Domain. The amino acid sequence of an exemplary human IgG3 CH1 Domain is (**SEQ ID NO:5**):

ASTKGPSVFP LAPCSRSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YTCNVNHKPS NTKVDKRV

[0089] An exemplary CH1 Domain is a human IgG4 CH1 Domain. The amino acid sequence of an exemplary human IgG4 CH1 Domain is (**SEQ ID NO:6**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[0090] An exemplary Hinge Domain is a human IgG1 Hinge Domain. The amino acid sequence of an exemplary human IgG1 Hinge Domain is (**SEQ ID NO:7**): EPKSCDKTHTCPPCP

[0091] Another exemplary Hinge Domain is a human IgG2 Hinge Domain. The amino acid sequence of an exemplary human IgG2 Hinge Domain is (**SEQ ID NO:8**): ERKCCVECPPCP

[0092] Another exemplary Hinge Domain is a human IgG3 Hinge Domain. The amino acid sequence of an exemplary human IgG3 Hinge Domain is (**SEQ ID NO:9**):

ELKTPLGDTT HTCPRCPEPK SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK
SCDTPPPCPR CP

[0093] Another exemplary Hinge Domain is a human IgG4 Hinge Domain. The amino acid sequence of an exemplary human IgG4 Hinge Domain is (**SEQ ID NO:10**): ESKYGPPCPSCP. As described herein, an IgG4 Hinge Domain may comprise a stabilizing mutation such as the S228P substitution. The amino acid sequence of an exemplary S228P-stabilized human IgG4 Hinge Domain is (**SEQ ID NO:11**): ESKYGPPCPPCP.

[0094] The CH2 and CH3 Domains of the two heavy chains of an antibody interact to form an "**Fc Domain**," which is a domain that is recognized by cellular **Fc Receptors**, including but not limited to Fc gamma Receptors **(FcγRs)**. As used herein, the term "Fc Domain" is used to define a C-terminal region of an IgG heavy chain. An Fc Domain is

said to be of a particular IgG isotype, class or subclass if its amino acid sequence is most homologous to that isotype relative to other IgG isotypes. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents.

[0095] Throughout the present specification, the numbering of the residues in the constant region of an IgG heavy chain is that of the EU index as in Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NH1, MD (1991) ("**Kabat**"), expressly incorporated herein by reference. The term "**EU index as in Kabat**" refers to the numbering of the constant domains of human IgG1 EU antibody. Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid, and the CDRs are identified as defined by Kabat (it will be understood that CDR_H1 as defined by Chothia, C. & Lesk, A. M. ((1987) "*Canonical structures for the hypervariable regions of immunoglobulins*," J. Mol. Biol. 196:901-917) begins five residues earlier). Kabat's numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[0096] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:12**):

```
231       240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

          340         350         360         370         380
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
```

```
          440      447
ALHNHYTQKS LSLSPGX
```

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

[0097] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (**SEQ ID NO:13**):

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

          290         300         310         320         330
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA

          340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

          390         400         410         420         430
WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

          440      447
ALHNHYTQKS LSLSPGX
```

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

[0098] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (**SEQ ID NO:14**):

```
231       240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

          340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESSGQPENN YNTTPPMLDS DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE

          440      447
ALHNRFTQKS LSLSPGX
```

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

[0099] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (**SEQ ID NO:15**):

```
231       240         250         260         270         280
APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS

          340         350         360         370         380
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE

          440     447
ALHNHYTQKS LSLSLGX
```

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

[00100] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, "*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation.*" Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the antibodies of the present invention may incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue in the B7-H3-binding molecules (including **B7-H3-ADC** molecules) of the invention. Specifically encompassed by the instant invention are B7-H3-binding molecules (including **B7-H3-ADC** molecules) lacking the C-terminal residue of the CH3 Domain. Also specifically

encompassed by the instant invention are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

[00101] 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, and particularly to receptors (singularly referred to as an "**Fc gamma receptor**" "**FcγR**," and collectively as "**FcγRs**") found on the surfaces of multiple types of immune system cells (*e.g.*, B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). Such receptors have an "**extracellular**" portion (which is thus capable of ligating to an Fc Domain), a "**transmembrane**" portion (which extends through the cellular membrane, and a "**cytoplasmic**" portion (positioned inside the cell).

[00102] The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: **FcγRI (CD64)**, **CD32A (FcγRIIA)**, **FcγRIIB (CD32B)**, **CD16A (FcγRIIIA)** and **CD16B (FcγRIIIB)**. FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating receptors such that their ligation to an Fc Domain activates the immune system or enhances the immune response. In contrast, FcγRIIB (CD32B) is an inhibiting receptor; ligation to an Fc Domain inhibits an immune response or dampens an existing immune response. In addition, interaction of an Fc Domain with with the neonatal Fc Receptor (FcRn) mediates the recycling of IgG molecules from the endosome to the cell surface and release into the blood. The amino acid sequence of exemplary wild-type Fc Domains of IgG1 (**SEQ ID NO:12**), IgG2 (**SEQ ID NO:13**), IgG3 (**SEQ ID NO:14**), and IgG4 (**SEQ ID NO:15**) are presented above.

[00103] **CD16** is a generic name for the activating Fc receptors, **FcγRIIIA (CD16A)** and **FcγRIIIB (CD16B)**. CD16 is expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that bind aggregated but not monomeric human IgG (Peltz, G.A. *et al.* (1989) "*Human Fc Gamma RIII: Cloning, Expression, And Identification Of The Chromosomal Locus Of Two Fc Receptors For IgG*," Proc. Natl. Acad. Sci. (U.S.A.)

86(3):1013-1017; Bachanova, V. *et al.* (2014) "*NK Cells In Therapy Of Cancer*," Crit. Rev. Oncog. 19(1-2):133-141; Miller, J.S. (2013) "*Therapeutic Applications: Natural Killer Cells In The Clinic*," Hematology Am. Soc. Hematol. Educ. Program. 2013:247-253; Youinou, P. *et al.* (2002) "*Pathogenic Effects Of Anti-Fc Gamma Receptor IIIB (CD16) On Polymorphonuclear Neutrophils In Non-Organ-Specific Autoimmune Diseases*," Autoimmun Rev. 1(1-2):13-19; Peipp, M. *et al.* (2002) "*Bispecific Antibodies Targeting Cancer Cells*," Biochem. Soc. Trans. 30(4):507-511). These receptors bind to the Fc portion of IgG antibodies, thereby triggering the release of cytokines. If such antibodies are bound to the antigen of foreign cells (*e.g.*, tumor cells), then such release mediates the killing of the tumor cell. Since such killing is antibody-dependent, it is termed **antibody-dependent cell-mediated cytotoxicity** (**ADCC**).

[00104] **CD32A** (**FcγRIIA**) (Brandsma, A.M. (2015) "Fc Receptor Inside-Out Signaling And Possible Impact On Antibody Therapy," Immunol Rev. 268(1):74-87; van Sorge, N.M. et al. (2003) "Fcgammar Polymorphisms: Implications For Function, Disease Susceptibility And Immunotherapy," Tissue Antigens 61(3):189-202; Selvaraj, P. et al. (2004) "Functional Regulation Of Human Neutrophil Fc Gamma Receptors," Immunol. Res. 29(1-3):219-230) and **CD64** (**FcγRI**) (Lu, S. et al. (2015) "Structural Mechanism Of High Affinity FcγRI recognition Of Immunoglobulin G," Immunol. Rev. 268(1):192-200; Swisher, J.F. et al. (2015) "The Many Faces Of FcγRI: Implications For Therapeutic Antibody Function," Immunol. Rev. 268(1):160-174; Thepen, T. et al. (2009) "Fcgamma Receptor 1 (CD64), A Target Beyond Cancer," Curr. Pharm. Des. 15(23):2712-2718; Rouard, H. et al. (1997) "Fc Receptors As Targets For Immunotherapy," Int. Rev. Immunol. 16(1-2):147-185) are activating Fc receptors that are expressed on macrophages, neutrophils, eosinophils and dendritic cells (and for **CD32A**, also on platelets and Langerhan cells). In contrast, **CD32B** (**FcγRIIB**) is an inhibiting Fc receptor on B lymphocytes (macrophages, neutrophils, and eosinophils) (Stopforth, R.J. et al. (2016) "Regulation of Monoclonal Antibody Immunotherapy by FcγRIIB," J. Clin. Immunol. [2016 Feb 27 Epub], pp. 1-7; Bruhns, P. et al. (2009) "Specificity And Affinity Of Human Fcgamma Receptors And Their Polymorphic Variants For Human IgG Subclasses," Blood. 113(16):3716-3725; White, A.L. et al. (2014) "FcγRIIB As A Key Determinant Of Agonistic Antibody Efficacy," Curr. Top. Microbiol. Immunol. 382:355-372; Selvaraj, P. et al. (2004) "Functional Regulation Of Human Neutrophil Fc Gamma Receptors," Immunol. Res. 29(1-3):219-230).

[00105] The ability of the different FcγRs to mediate diametrically opposing functions reflects their structural differences, and in particular whether the FcγR possesses an immunoreceptor tyrosine-based activation motif ("**ITAM**") or an immunoreceptor tyrosine-based inhibitory motif ("**ITIM**"). The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγRs include FcγRI, FcγRIIA, FcγRIIIA, and activate the immune system when bound to Fc Domains (*e.g.*, aggregated Fc Domains present in an immune complex). FcγRIIB is the only currently known natural ITIM-containing FcγR; it acts to dampen or inhibit the immune system when bound to aggregated Fc Domains. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, the activation of which results in the activation of downstream substrates (*e.g.*, PI_3K). Cellular activation leads to release of pro-inflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR- mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus, cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness and aborts B-cell activation, B-cell proliferation and antibody secretion is thus aborted.

II. Bispecific Antibodies, Multispecific Diabodies and DART® Diabodies

[00106] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody's VL and VH Domains. Interaction of an antibody's Light Chain and Heavy Chain and, in particular, interaction of its VL and VH Domains forms one of the two epitope-binding sites of a natural antibody, such as an IgG. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bivalency or multivalency).

[00107] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (i.e., more than two binding sites) for the same epitope and/or antigen.

[00108] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple epitope-binding fragments (*e.g.*, two Fab fragments or scFvs). Alternative formats use linker peptides to fuse an epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). PCT Publications Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. PCT Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. PCT Publications Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Domains have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publication Nos. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. PCT Publications Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional binding domains or functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another).

[00109] The art has additionally noted the capability to produce diabodies that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bivalency or multivalency) (see, *e.g.*, Holliger *et al.* (1993) "*'Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 / WO 02/02781 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) "*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*," Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) "*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[00110] The design of a diabody is based on the antibody derivative known as a single-chain Variable Domain fragment (**scFv**). Such molecules are made by linking Light and/ or Heavy Chain Variable Domains by using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations

such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[00111] The provision of bispecific binding molecules (*e.g.*, non-monospecific diabodies) provides a significant advantage over antibodies, including but not limited to, a "trans" binding capability sufficient to co-ligate and/or co-localize different cells that express different epitopes and/or a "cis" binding capability sufficient to co-ligate and/or co-localize different molecules expressed by the same cell. Bispecific binding molecules (*e.g.*, non-monospecific diabodies) thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221-1225).

[00112] The ability to produce bispecific diabodies has led to their use (in "**trans**") to co-ligate two cells together, for example, by co-ligating receptors that are present on the surface of different cells (*e.g.*, cross-linking cytotoxic T-cells to tumor cells) (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631, and Holliger *et al.* (1996) *"Specific Killing Of* Lymphoma *Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; Marvin *et al.* (2005) "*Recombinant Approaches To IgG-Like Bispecific Antibodies*," Acta Pharmacol. Sin. 26:649-658). Alternatively (or additionally), bispecific (or tri- or multispecific) diabodies can be used (in "**cis**") to co-ligate molecules, such as receptors, *etc.*, that are present on the surface of the same cell. Co-ligation of different cells and/or receptors is useful to modulate effector functions and/or immune cell signaling. Multispecific molecules (*e.g.*, bispecific diabodies) comprising epitope-binding sites may be directed to a surface determinant of any immune cell such as CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*, which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-Presenting Cells or other mononuclear cells. In particular, epitope-binding sites directed to a cell surface

receptor that is present on immune effector cells, are useful in the generation of multispecific binding molecules capable of mediating redirected cell killing.

[00113] However, the above advantages come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain,*" Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[00114] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human* Recombinant *IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[00115] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART®** diabodies; see, *e.g.*, United States Patent Publication Nos. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Sloan, D.D. *et al.* (2015) "*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells*," PLoS Pathog. 11(11):e1005233. doi: 10.1371/journal.ppat.1005233; Al Hussaini, M. *et al.* (2015) "*Targeting CD123 In AML Using A T-Cell Directed Dual-Affinity Re-Targeting (DART®) Platform*," Blood pii: blood-2014-05-575704; Chichili, G.R. *et al.* (2015) "*A CD3xCD123 Bispecific DART For Redirecting Host T Cells To Myelogenous Leukemia: Preclinical Activity And Safety In Nonhuman Primates*," Sci. Transl. Med. 7(289):289ra82; Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond one or more pairs of such polypeptide chains to one another. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the involved polypeptide chains, stabilizing the resulting diabody without interfering with the diabody's binding characteristics.

[00116] Many variations of such molecules have been described (see, *e.g.*, United States Patent Publication Nos. 2015/0175697; 2014/0255407; 2014/0099318; 2013/0295121; 2010/0174053; 2009/0060910; 2007-0004909; European Patent Publication Nos. EP 2714079; EP 2601216; EP 2376109; EP 2158221; EP 1868650; and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538; WO 2006/113665), and are provided herein.

[00117] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required including, but not limited to, tetravalent tandem antibodies, also referred to as "**TandAbs**" (see, *e.g.* United States Patent Publications Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publications Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical polypeptide chains, each possessing a VH1, VL2, VH2, and VL2 Domain.

[00118] Recently, trivalent structures incorporating two diabody-type binding domains and one non-diabody-type domain and an Fc Domain have been described (see, *e.g.*, PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such trivalent binding molecules may be utilized to generate monospecific, bispecific or trispecific molecules. The ability to bind three different epitopes provides enhanced capabilities.

III. Human B7-H3

[00119] Human B7-H3 exists as a "4Ig" form and as a "2Ig" form. The amino acid sequence of a representative "4Ig" form of human B7-H3 (including a 29 amino acid residue signal sequence, shown underlined) is provided by NCBI Sequence NP_001019907 (**SEQ ID NO:16**, the 29 amino acid residue signal sequence, shown underlined):

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

[00120] The amino acid sequence of the "2Ig" form of human B7-H3 is completely embraced within the "4Ig" form of human B7-H3. The amino acid sequence of a representative "2Ig" form of human B7-H3 (including a 29 amino acid residue signal sequence, shown underlined) is provided by NCBI Sequence NP_079516 (**SEQ ID NO:17**):

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

[00121] In certain embodiments, B7-H3-binding molecules (*e.g.*, scFvs, antibodies, bispecific diabodies, *etc.*) of the invention are characterized by any one, two, three, four, five, six, seven, eight or nine of the following criteria:

(1) the ability to immunospecifically bind human B7-H3 as endogenously expressed on the surface of a cancer cell;

(2) specifically binds non-human primate B7-H3 (*e.g.*, B7-H3 of cynomolgus monkey);

(3) specifically binds human B7-H3 with an equilibrium binding constant (K_D) of 1 nM or less;

(4) specifically binds non-human primate B7-H3 with an equilibrium binding constant (K_D) of 1 nM or less;

(5) specifically binds human B7-H3 with an on rate (ka) of 1×10^6 $M^{-1}min^{-1}$ or more;

(6) specifically binds non-human primate B7-H3 with an on rate (ka) of 1×10^6 $M^{-1}min^{-1}$ or more;

(7) specifically binds human B7-H3 with an off rate (kd) of 15×10^{-4} min^{-1} or less;

(8) specifically binds non-human primate B7-H3 with an off rate (kd) of 15×10^{-4} min^{-1} or less;

(9) ability to mediate redirected cell killing *(e.g.*, killing of cancer cells expressing B7-H3).

[00122] As described elsewhere herein, the binding constants of a B7-H3-binding molecule may be determined using surface plasmon resonance *e.g.*, via a BIACORE® analysis. Surface plasmon resonance data may be fitted to a 1:1 Langmuir binding model (simultaneous ka kd) and an equilibrium binding constant K_D calculated from the ratio of rate constants kd/ka. Such binding constants may be determined for a monovalent B7-H3-binding molecule (*i.e.*, a molecule comprising a single B7-H3 epitope-binding site), a bivalent B7-H3-binding molecule (*i.e.*, a molecule comprising two B7-H3 epitope-binding

sites), or B7-H3-binding molecules having higher valency (*e.g.*, a molecule comprising three, four, or more B7-H3 epitope-binding sites).

[00123] As used herein the term "**redirected cell killing**" refers to the ability of a molecule to mediate the killing of a target cell (*e.g.*, cancer cell) by localizing an immune effector cell (*e.g.*, T-cell, NK cell, *etc.*) to the location of the target cell by binding epitopes present on the surfaces of such effector and target cells, resulting in the killing of the target cell. The ability of a B7-H3-binding molecule (*e.g.*, a bispecific B7-H3 x CD3-binding molecule) to mediate redirected cell killing activity may be determined using a cytotoxic T lymphocyte (CTL) assay. Such assays are well known in the art and preferred assays are described below.

[00124] The present invention particularly encompasses B7-H3-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) comprising anti-B7-H3 Variable Domains (*i.e.*, VL and/or VH Domains) that immunospecifically bind to an epitope of a human B7-H3 polypeptide. Unless otherwise stated, all such B7-H3-binding molecules are capable of immunospecifically binding to human B7-H3. As used herein such B7-H3 Variable Domains are referred to as "**anti-B7-H3-VL**" and "**anti-B7-H3-VH**," respectively.

IV. Murine Anti-Human B7-H3 Antibodies and Their Humanized Derivatives

[00125] Four exemplary anti-B7-H3 antibodies, designated "**mAb-A**," "**mAb-B**," "**mAb-C**" and "**mAb-D**," were isolated from hybridoma cells that had been produced through immunization with cells expressing human B7-H3, with a B7-H3 polypeptide or a peptide epitope thereof. Antibodies "**mAb-B**," "**mAb-C**" and "**mAb-D**," were humanized.

[00126] Antibodies "**mAb-C**," and "**mAb-D**" were found to be cross-reactive with B7-H3 of cynomolgus monkeys. The amino acid sequences of the VL and VH Domains of **mAb-C** and **mAb-D** are provided below. In one embodiment, the preferred anti-human B7-H3-binding molecules of the present invention possess 1, 2 or all 3 of the CDR_{HS} of the VH Domain and/or 1, 2 or all 3 of the CDR_{LS} of the VL Domain the VH and/or VL Domains of the murine anti-B7-H3 monoclonal antibody **mAb-D**, of chimeric monoclonal antibody **mAb-D** ("**chmAb-D**") or of humanized monoclonal antibody **mAb-C** or **mAb-D** ("**hmAb-C**" or "**hmAb-D**"). Such preferred anti-human B7-H3-binding molecules include bispecific (or multispecific) antibodies, chimeric or humanized antibodies, BiTes, diabodies, *etc*, and such binding molecules having variant Fc Domains. The invention encompasses the use of

any of **mAb-A**, **mAb-B**, **mAb-C** or **mAb-D** to form B7-H3 binding molecules, and in particular, **B7-H3-ADC**s.

A. Murine Anti-Human B7-H3 Antibody mAb-A

[00127] The amino acid sequence of the VL Domain of the murine anti-B7-H3 antibody designated "**mAb-A**" (**SEQ ID NO:95**) is shown below (CDR_L residues are shown underlined):

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

[00128] The amino acid sequence of the VH Domain of **mAb-A** (**SEQ ID NO:96**) is shown below (CDR_H residues are shown underlined):

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

B. Murine Anti-Human B7-H3 Antibody mAb-B

[00129] The amino acid sequence of the VL Domain of the murine anti-B7-H3 antibody designated "**mAb-B**" (**SEQ ID NO:97**) is shown below (CDR_L residues are shown underlined):

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

[00130] The amino acid sequence of the VH Domain of **mAb-B** (**SEQ ID NO:98**) is shown below (CDR_H residues are shown underlined).

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

C. Humanized Anti-Human B7-H3 Antibody hmAb-B

[00131] The amino acid sequence of the VL Domain of **hmAb-B** (**SEQ ID NO:99**) is shown below (CDR_L residues are shown underlined):

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

[00132] In some embodiments, the amino acid sequence of CDR_L1 of **hmAb-B** (RASQ***D***IS***N***YLN) **(SEQ ID NO:100)** may be replaced with an alternative CDR_L1 having the amino acid sequence RASQ***S***IS***S***YLN **(SEQ ID NO:101)**. Likewise, the amino acid sequence of CDR_L2 of **hmAb-B** (YTSRL***H***S) **(SEQ ID NO:102)** may be replaced with an alternative CDR_L2 having the amino acid sequence YTSRL***Q***S **(SEQ ID NO:103)**.

[00133] The amino acid sequence of the VH Domain of **hmAb-B (SEQ ID NO:104)** is shown below (CDR_H residues are shown underlined):

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

[00134] In some embodiments, the amino acid sequence of CDR_H2 of **hmAb-B** (TIYPG***D***GDTRYTQKF***K***G) **(SEQ ID NO:105)** may be replaced with an alternative CDR_H2 having the amino acid sequence: TIYPG***G***GDTRYTQKF***Q***G **(SEQ ID NO:106)**

D. Murine Anti-Human B7-H3 Antibody mAb-C

[00135] The amino acid sequence of the VL Domain of the murine anti-B7-H3 antibody designated "**mAb-C**" **(SEQ ID NO:18)** is shown below (CDR_L residues are shown underlined):

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

[00136] The amino acid sequence of the VH Domain of **mAb-C (SEQ ID NO:19)** is shown below (CDR_H residues are shown underlined).

EVQQVESGGD LVKPGGSLKL SCAASGFTFS **SYGMS**WVRQT PDKRLEWVA**T**
INSGGSNTYY PDSLKGRFTI SRDNAKNTLY LQMRSLKSED TAMYYCAR**HD**
GGAMDYWGQG TSVTVSS

E. Humanized Anti-Human B7-H3 Antibody hmAb-C

[00137] The Variable Domains of the anti-B7-H3 antibody **mAb-C** were humanized. In in some instances alternative humanized Variable Domains were generated to optimize binding activity and/or to remove antigenic epitopes and/or to remove potentially labile amino acid residues.

[00138] The amino acid sequence of the VL Domain of **hmAb-C** (**SEQ ID NO:20**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**RASESIY SYLA**WYQQKP GKAPKLLVY**N**
TKTLPEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYC**QH HYGTPPWT**FG
QGTRLEIK

[00139] The amino acid sequence of the VH Domain of **hmAb-C** (**SEQ ID NO:21**) is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYGMS**WVRQA PGKGLEWVA**T**
INSGGSNTYY PDSLKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR**HD**
GGAMDYWGQG TTVTVSS

F. Murine Anti-Human B7-H3 Antibody mAb-D

[00140] The amino acid sequence of the VL Domain of the murine anti-B7-H3 antibody designated "**mAb-D**" (**SEQ ID NO:22**) is shown below (CDR_L residues are shown underlined):

DIVMTQSQKF MSTSVGDRVS VTC**KASQNVD TNVA**WYQQKQ GHSPEALIY**S**
ASYRYSGVPA RFTGSGSGTD FTLTISNVQS EDLAEYFC**QQ YNNYPFT**FGG
GTKLEIK

[00141] The amino acid sequence of the **CDR_L1 Domain** of **mAb-D** is (**SEQ ID NO:23**): KASQNVDTNVA.

[00142] The amino acid sequence of the **CDR_L2 Domain** of **mAb-D** is (**SEQ ID NO:24**): SASYRYS.

[00143] The amino acid sequence of the **CDR_L3 Domain** of **mAb-D** is (**SEQ ID NO:25**): QQYNNYPFT.

[00144] The amino acid sequence of the VH Domain of **mAb-D** (**SEQ ID NO:26**) is shown below (CDR_H residues are shown underlined):

DVQLAESGGG LVQPGGSRKL SCAASGFTFS **SFGMH**WVRQA PEKGLEWVA**Y**
ISSGSGTIYY ADTVKGRFTI SRDNPKNSLF LQMTSLRSED TAMYYCAR**HG**
YRYEGFDYWG QGTTLTVSS

[00145] The amino acid sequence of the **CDR_H1 Domain** of **mAb-D** is (**SEQ ID NO:27**): SFGMH.

[00146] The amino acid sequence of the **CDR_H2 Domain** of **mAb-D** is (**SEQ ID NO:28**): YISSGSGTIYYADTVKG.

[00147] The amino acid sequence of the **CDR_H3 Domain** of **mAb-D** is (**SEQ ID NO:29**): HGYRYEGFDY.

G. Humanized Anti-Human B7-H3 Antibody mAb-D

[00148] The Variable Domains of the anti-B7-H3 antibody **mAb-D** were humanized. In in some instances alternative humanized Variable Domains were generated to optimize binding activity and/or to remove antigenic epitopes and/or to remove potentially labile amino acid residues.

[00149] The amino acid sequence of the VL Domain of **hmAb-D** (**SEQ ID NO:30**) is shown below (CDR_L residues are shown underlined).

DIQMTQSPSF LSASVGDRVT ITC**KASQNVD TNVA**WYQQKP GKAPKALIY**S**
ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYFC**QQ YNNYPFT**FGQ
GTKLEIK

[00150] The amino acid sequence of the VH Domain of **hmAb-D** (**SEQ ID NO:31**) is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **SFGMH**WVRQA PGKGLEWVA**Y**
ISSGSGTIYY ADTVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR**HG**
YRYEGFDYWG QGTTVTVSS

V. Chimeric Antigen Receptors

[00151] The B7-H3-binding molecules of the present invention may be monospecific single-chain molecules such as single-chain variable fragments ("**anti-B7-H3-scFvs**") or Chimeric Antigen Receptors ("**anti-B7-H3-CARs**"). As discussed above, scFvs are made by linking Light and Heavy Chain Variable Domains together via a short linking peptide. First-generation CARs typically had the intracellular domain from the CD3 ζ- chain, which is the primary transmitter of signals from endogenous TCRs. Second-generation CARs possessed additional intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 41BB, ICOS, *etc.*) to the cytoplasmic tail of the CAR in order to provide additional signals to the T-cell. Third-generation CARs combine multiple signaling domains, such as CD3z-CD28-41BB or CD3z-CD28-OX40, in order to further augment potency (Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*,"

Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

[00152] The anti-B7-H3-CARs of the present invention comprise an anti-B7-H3-scFv fused to an intracellular domain of a receptor. The Variable Light Chain and Variable Heavy Chain Domains of the anti-B7-H3-scFv are preferably **hmAb-C VL (SEQ ID NO:20)** and h**mAb-C VH (SEQ ID NO:21)** or are preferably **hmAb-D VL (SEQ ID NO:30)** and **hmAb-D VH (SEQ ID NO:31)**.

[00153] The intracellular domain of the anti-B7-H3-CARs of the present invention is preferably selected from the intracellular domain of any of: 41BB-CD3ζ, b2c-CD3ζ, CD28, CD28-4-1BB-CD3ζ, CD28-CD3ζ, CD28-FcεRIγ, CD28mut-CD3ζ, CD28-OX40-CD3ζ, CD28-OX40-CD3ζ, CD3ζ, CD4-CD3ζ, CD4-FcεRIγ, CD8-CD3ζ, FcεRIγ, FcεRIγCAIX, Heregulin-CD3ζ, IL-13-CD3ζ, or Ly49H-CD3ζ (Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*," Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

VI. Multispecific B7-H3-Binding Molecules

[00154] The present invention is also directed to multispecific (*e.g.*, bispecific, trispecific, *etc.*) B7-H3-binding molecules comprising an epitope-binding site (preferably comprising 1, 2 or all 3 of the CDR_{HS} of an anti-B7-H3-VH Domain of the invention and/or 1, 2 or all 3 of the CDR_{LS} of an anti-B7-H3-VL Domain of the invention, or such anti-B7-

H3-VH Domain and/or such anti-B7-H3-VL Domain) and further comprising a second epitope-binding site that immunospecifically binds to a second epitope, where such second epitope is (i) a different epitope of B7-H3, or (ii) an epitope of a molecule that is not B7-H3. Such multispecific B7-H3-binding molecules preferably comprise a combination of epitope-binding sites that recognize a set of antigens unique to target cells or tissue type. In particular, the present invention relates to multispecific B7-H3-binding molecules that are capable of binding to an epitope of B7-H3 and an epitope of a molecule present on the surface of an effector cell, especially a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. For example, such B7-H3-binding molecules of the present invention may be constructed to comprise an epitope-binding site that immunospecifically binds CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), or NKG2D.

[00155] One embodiment of the present invention relates to bispecific B7-H3-binding molecules that are capable of binding to a "**first epitope**" and a "**second epitope**," such epitopes not being identical to one another. Such bispecific molecules comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope, and "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope. The notation "**VL1**" and "**VH1**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "first" epitope of such bispecific molecules. Similarly, the notation "**VL2**" and "**VH2**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind the "second" epitope of such bispecific molecules. It is irrelevant whether a particular epitope is designated as the first vs. the second epitope; such notation having relevance only with respect to the presence and orientation of domains of the polypeptide chains of the binding molecules of the present invention. In one embodiment, one of such epitopes is an epitope of human B7-H3 and the other is a different epitope of B7-H3, or is an epitope of a molecule that is not B7-H3. In particular embodiments, one of such epitopes is an epitope of human B7-H3 and the other is an epitope of a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, a bispecific molecule comprises more than two epitope-binding sites. Such bispecific molecules will bind at least one epitope of B7-H3 and at least one epitope of a molecule that is not B7-H3, and may further bind additional epitopes of B7-H3 and/or additional epitopes of a molecule that is not B7-H3.

[00156] The present invention particularly relates to bispecific, trispecific and multispecific B7-H3-binding molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) that possess epitope-binding fragments of antibodies (*e.g.*, VL and VH Domains) that enable them to be able to coordinately bind to at least one epitope of B7-H3 and at least one epitope of a second molecule that is not B7-H3. Selection of the VL and VH Domains of the polypeptide domains of such molecules is coordinated so that the polypeptides chains that make up such multispecific B7-H3-binding molecules assemble to form at least one functional epitope-binding site that is specific for at least one epitope of B7-H3 and at least one functional epitope-binding site that is specific for at least one epitope of a molecule that is not B7-H3. Preferably, the multispecific B7-H3-binding molecules comprise 1, 2 or all 3 of the CDR_{H}s of an anti-B7-H3-VH Domain of the invention and/or 1, 2 or all 3 of the CDR_{L}s of an anti-B7-H3-VL Domain of the invention, or such anti-B7-H3-VH Domain and/or such anti-B7-H3-VL Domain, as provided herein.

A. Bispecific Antibodies

[00157] The instant invention encompasses bispecific antibodies capable of simultaneously binding to an epitope of B7-H3 and an epitope of a molecule that is not B7-H3. In some embodiments, the bispecific antibody capable of simultaneously binding to B7-H3 and a second molecule that is not B7-H3 is produced using any of the methods described in PCT Publication Nos. WO 1998/002463, WO 2005/070966, WO 2006/107786 WO 2007/024715, WO 2007/075270, WO 2006/107617, WO 2007/046893, WO 2007/146968, WO 2008/003103, WO 2008/003116, WO 2008/027236, WO 2008/024188, WO 2009/132876, WO 2009/018386, WO 2010/028797, WO2010028796, WO 2010/028795, WO 2010/108127, WO 2010/136172, WO 2011/086091, WO 2011/133886, WO 2012/009544, WO 2013/003652, WO 2013/070565, WO 2012/162583, WO 2012/156430, WO 2013/174873, and WO 2014/022540, each of which is hereby incorporated herein by reference in its entirety.

B. Bispecific Diabodies Lacking Fc Domains

[00158] One embodiment of the present invention relates to bispecific diabodies that are capable of binding to a first epitope and a second epitope, wherein the first epitope is an epitope of human B7-H3 and the second is an epitope of a molecule that is not B7-H3, preferably a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell

or other mononuclear cell. Such diabodies comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated diabody that is capable of simultaneously binding to an epitope of B7-H3 and the second epitope.

[00159] The first polypeptide chain of such an embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, the VL Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either $VL_{anti\text{-}B7\text{-}H3\text{-}VL}$ or $VL_{Epitope\ 2}$), a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such first polypeptide chain contains $VL_{anti\text{-}B7\text{-}H3\text{-}VL}$) or B7-H3 (if such first polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figure 1**).

[00160] The second polypeptide chain of this embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either $VL_{anti\text{-}B7\text{-}H3\text{-}VL}$ or $VL_{Epitope\ 2}$, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such second polypeptide chain contains $VL_{anti\text{-}B7\text{-}H3\text{-}VL}$) or to B7-H3 (if such second polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain, and a C-terminus (**Figure 1**).

[00161] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional epitope-binding site that is specific for a first antigen (*i.e.*, either B7-H3 or a molecule that contains the second epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional epitope-binding site that is specific for a second antigen (*i.e.*, either the molecule that comprises the second epitope or B7-H3). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to both an epitope of B7-H3 and to the second epitope (*i.e.*, they collectively comprise $VL_{anti\text{-}B7\text{-}H3\text{-}VL}/VH_{anti\text{-}B7\text{-}H3\text{-}VH}$ and $VL_{Epitope\ 2}/VH_{Epitope\ 2}$).

[00162] Most preferably, the length of the intervening spacer peptide (*i.e.*, "**Linker 1**," which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another (for example consisting of from 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 intervening linker amino acid residues). Thus, the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH Domains of the second polypeptide chain are substantially or completely incapable of binding to one another. A preferred intervening spacer peptide (Linker 1) has the sequence (**SEQ ID NO:32**): GGGSGGGG.

[00163] The length and composition of the second intervening spacer peptide ("**Linker 2**") is selected based on the choice of one or more polypeptide domains that promote such dimerization (i.e., a "**Heterodimer-Promoting Domain**"). Typically, the second intervening spacer peptide (Linker 2) will comprise 3-20 amino acid residues. In particular, where the employed Heterodimer-Promoting Domain(s) do/does not comprise a cysteine residue a cysteine-containing second intervening spacer peptide (Linker 2) is utilized. A cysteine-containing second intervening spacer peptide (Linker 2) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence GGCGGG (**SEQ ID NO:33**). Alternatively, Linker 2 does not comprise a cysteine (*e.g.*, GGG, GGGS (**SEQ ID NO:34**), LGGGSG (**SEQ ID NO:35**), GGGSGGGSGGG (**SEQ ID NO:36**), ASTKG (**SEQ ID NO:37**), LEPKSS (**SEQ ID NO:38**), APSSS (**SEQ ID NO:39**), *etc.*) and a Cysteine-Containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00164] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:40**) or VEPKSC (**SEQ ID NO:41**) or AEPKSC (**SEQ ID NO:42**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:43**) or FNRGEC (**SEQ ID NO:44**) on the other polypeptide chain (US2007/0004909).

[00165] In a preferred embodiment, the Heterodimer-Promoting Domains will comprise tandemly repeated coil domains of opposing charge for example, "E-coil" helical domains (**SEQ ID NO:45**: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, and "K-coil" domains (**SEQ ID**

NO:46: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Heterodimer-Promoting Domains that comprise modifications of the above-described E-coil and K-coil sequences so as to include one or more cysteine residues may be utilized. The presence of such cysteine residues permits the coil present on one polypeptide chain to become covalently bonded to a complementary coil present on another polypeptide chain, thereby covalently bonding the polypeptide chains to one another and increasing the stability of the diabody. Examples of such particularly preferred are Heterodimer-Promoting Domains include a Modified E-Coil having the amino acid sequence EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:47**), and a modified K-coil having the amino acid sequence KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:48**).

[00166] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of a polypeptide chain of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) "*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Module*s," J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:49**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00167] As disclosed in WO 2012/162068 (herein incorporated by reference), "**deimmunized**" variants of **SEQ ID NO:49** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized

ABD: 66D/70S +71A; 66S/70S +71A; 66S/70S +79A; 64A/65A/71A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**D**$_{66}$NAK**S**$_{70}$ **A**$_{71}$EGVKALIDE ILAALP **(SEQ ID NO:50)**,

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKN**A**$_{64}$**A**$_{65}$NNAKT VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:51)**,

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**S**$_{66}$NAK**S**$_{70}$ VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:52)**,

are particularly preferred as such deimmunized ABD exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an ABD contains a third linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the ABD (which is preferably a deimmunized ABD). A preferred sequence for such Linker 3 is **SEQ ID NO:34**: GGGS.

C. Multispecific Diabodies Containing Fc Domains

[00168] One embodiment of the present invention relates to multispecific diabodies capable of simultaneously binding to an epitope of B7-H3 and a second epitope (*i.e.*, a different epitope of B7-H3 or an epitope of a molecule that is not B7-H3) that comprise an Fc Domain. The addition of an IgG CH2-CH3 Domain to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Domain, increases the biological half-life and/or alters the valency of the diabody. Such diabodies comprise, two or more polypeptide chains whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated diabody that is capable of simultaneously binding to an epitope of B7-H3 and the second epitope. Incorporating an IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain bispecific Fc-Region-containing diabody to form (**Figure 2**).

[00169] Alternatively, incorporating an IgG CH2-CH3 Domains onto only one of the diabody polypeptides will permit a more complex four-chain bispecific Fc Domain-containing diabody to form (**Figures 3A-3C**). **Figure 3C** shows a representative four-chain diabody possessing the Constant Light (CL) Domain and the Constant Heavy CH1 Domain, however fragments of such domains as well as other polypeptides may alternatively be employed (see, *e.g.*, **Figures 3A and 3B**, United States Patent Publication Nos. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538). Thus, for example, in lieu of the CH1 Domain, one may employ a peptide having the amino acid sequence GVEPKSC (**SEQ ID NO:40**), VEPKSC (**SEQ ID NO:41**), or AEPKSC (**SEQ ID NO:42**), derived from the Hinge Domain of a human IgG, and in lieu of the CL Domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (**SEQ ID NO:43**) or FNRGEC (**SEQ ID NO:44**). A representative peptide containing four-chain diabody is shown in **Figure 3A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the "E-coil" helical domains (**SEQ ID NO:45**: EVAALEK-EVAALEK-EVAALEK-EVAALEK or **SEQ ID NO:47:** EVAACEK-EVAALEK-EVAALEK-EVAALEK-EVAALEK); and the "K-coil" domains (**SEQ ID NO:46**: KVAALKE-KVAALKE-KVAALKE-KVAALKE or **SEQ ID NO:48:** KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00170] The Fc Domain-containing molecules of the present invention may include additional intervening spacer peptides (Linkers), generally such Linkers will be incorporated between a Heterodimer-Promoting Domain (*e.g.*, an E-coil or K-coil) and a CH2-CH3 Domain and/or between a CH2-CH3 Domain and a Variable Domain (*i.e.*, VH or VL). Typically, the additional Linkers will comprise 3-20 amino acid residues and may optionally contain all or a portion of an IgG Hinge Domain (preferably a cysteine-containing portion of an IgG Hinge Domain). Linkers that may be employed in the bispecific Fc Domain-containing diabody molecules of the present invention include: GGGS (**SEQ ID NO:34**), LGGGSG (**SEQ ID NO:35**), GGGSGGGSGGG (**SEQ ID NO:36**), ASTKG (**SEQ ID NO:37**), LEPKSS (**SEQ ID NO:38**), APSSS (**SEQ ID NO:39**), APSSSPME (**SEQ ID NO:53**), VEPKSADKTHTCPPCP (**SEQ ID NO:54**), LEPKSADKTHTCPPC (**SEQ ID NO:55**), DKTHTCPPCP (**SEQ ID NO:56**), GGC, and GGG. LEPKSS (**SEQ ID NO:38**)

may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or LEPKSS (**SEQ ID NO:38**) may be immediately followed by DKTHTCPPCP (**SEQ ID NO:56**) to form the alternate linkers: GGGDKTHTCPPCP (**SEQ ID NO:57**); and LEPKSSDKTHTCPPCP (**SEQ ID NO:58**). Bispecific Fc Domain-containing molecules of the present invention may incorporate an IgG Hinge Domain in addition to or in place of a linker. Exemplary Hinge Domains include: EPKSCDKTHTCPPCP (**SEQ ID NO:7**) from IgG1, ERKCCVECPPCP (**SEQ ID NO:8**) from IgG2, ESKYGPPCPSCP (**SEQ ID NO:10**) from IgG4, and ESKYGPPCP**P**CP (**SEQ ID NO:11**) an IgG4 hinge variant comprising a stabilizing S228P substitution (as numbered by the EU index as set forth in Kabat) to reduce strand exchange.

[00171] As provided in **Figure 3A-3C**, Fc Domain-containing diabodies of the invention may comprise four chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide chains contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third polypeptide chains with the second/fourth polypeptide chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. The notation "**VL3**" and "**VH3**" denote respectively, the Light Chain Variable Domain and Variable Heavy Chain Domain that bind a "third" epitope of such diabody. Similarly, the notation "**VL4**" and "**VH4**" denote respectively, the Light Chain Variable Domain and Variable Heavy Chain Domain that bind a "fourth" epitope of such diabody. The general structure of the polypeptide chains of a representative four-chain bispecific Fc Domain-containing diabodies of invention is provided in **Table 1**:

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

HPD = Heterodimer-Promoting Domain

[00172] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies that are composed of four total polypeptide chains (**Figures 3A-3C**). The bispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope-binding sites immunospecific for B7-H3 (which may be capable of binding to the same epitope of B7-H3 or to different epitopes of B7-H3), and two epitope-binding sites immunospecific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to different epitopes of the second molecule). Preferably, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell.

[00173] In a further embodiment, the Fc Domain-containing diabodies of the present invention may comprise three polypeptide chains. The first polypeptide of such a diabody contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such a diabody contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such a diabody comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such a diabody associate together to form a VL1/VH1 epitope-binding site that is capable of binding to a first antigen (*i.e.*, either B7-H3 or a molecule that comprises a second epitope), as well as a VL2/VH2 epitope-binding site that is capable of binding to a second antigen (*i.e.*, either the molecule that contains the second epitope or B7-H3). The first and second polypeptides are bonded to one another through a

disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain that is stabilized via a disulfide bond. Such bispecific diabodies have enhanced potency. **Figures 4A** and **4B** illustrate the structures of such diabodies. Such Fc-Region-containing diabodies may have either of two orientations (**Table 2**):

Table 2

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

HPD = Heterodimer-Promoting Domain

[00174] In a specific embodiment, diabodies of the present invention are bispecific, bivalent (*i.e.*, possess two epitope-binding sites), Fc-containing diabodies that are composed of three total polypeptide chains (**Figures 4A-4B**). The bispecific, bivalent Fc-containing diabodies of the invention comprise one epitope-binding site immunospecific for B7-H3, and one epitope-binding site immunospecific for a second molecule. Preferably, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell.

[00175] In a further embodiment, the Fc Domain-containing diabodies may comprise a total of five polypeptide chains. In a particular embodiment, two of said five polypeptide chains have the same amino acid sequence. The first polypeptide chain of such a diabody contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, and (iii) a Domain containing a CH2-CH3 sequence. The first polypeptide chain may be the heavy chain of an antibody that contains a VH1 and a heavy chain constant region. The second and fifth polypeptide chains of such a diabody contain: (i) a VL1-containing domain, and (ii) a CL-containing domain. The second and/or fifth polypeptide chains of such a diabody may be light chains of an antibody that contains a VL1 complementary to the VH1 of the first/third polypeptide chain. The first, second and/or fifth polypeptide chains may be isolated from a naturally occurring antibody. Alternatively, they may be constructed recombinantly. The

third polypeptide chain of such a diabody contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, (iii) a Domain containing a CH2-CH3 sequence, (iv) a VL2-containing Domain, (v) a VH3-containing Domain and (vi) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the third chain with the fourth chain. The fourth polypeptide of such diabodies contains: (i) a VL3-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's third polypeptide chain.

[00176] Thus, the first and second, and the third and fifth, polypeptide chains of such diabodies associate together to form two VL1/VH1 epitope-binding sites capable of binding a first epitope. The third and fourth polypeptide chains of such diabodies associate together to form a VL2/VH2 epitope-binding site that is capable of binding to a second epitope, as well as a VL3/VH3 binding site that is capable of binding to a third epitope. The first and third polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective constant regions. Notably, the first and third polypeptide chains complex with one another to form an Fc Domain. Such multispecific diabodies have enhanced potency. **Figure 5** illustrates the structure of such diabodies. It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific. As provided herein, these domains are preferably selected so as to bind an epitope of B7-H3, an epitope of second molecule and optionally an epitope of a third molecule.

[00177] The VL and VH Domains of the polypeptide chains are selected so as to form VL/VH binding sites specific for a desired epitope. The VL/VH binding sites formed by the association of the polypeptide chains may be the same or different so as to permit tetravalent binding that is monospecific, bispecific, trispecific or tetraspecific. In particular, the VL and VH Domains maybe selected such that a multivalent diabody may comprise two binding sites for a first epitope and two binding sites for a second epitope, or three binding sites for a first epitope and one binding site for a second epitope, or two binding sites for a first epitope, one binding site for a second epitope and one binding site for a third epitope (as depicted in **Figure 5**). The general structure of the polypeptide chains of representative five-chain Fc Domain-containing diabodies of invention is provided in **Table 3**:

Table 3		
Bispecific (2x2)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH2-HPD-COOH
Bispecific (3x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL1-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH1-HPD-COOH
Trispecific (2x1x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH3-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL3-VH2-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00178] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies that are composed of five total polypeptide chains having two epitope-binding sites immunospecific for B7-H3 (which may be capable of binding to the same epitope of B7-H3 or to different epitopes of B7-H3), and two epitope-binding sites specific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to different epitopes of the second molecule). In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise three epitope-binding sites immunospecific for B7-H3 (which may be capable of binding to the same epitope of B7-H3 or to two or three different epitopes of B7-H3), and one epitope-binding site specific for a second molecule. In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise one epitope-binding site immunospecific for B7-H3, and three epitope-binding sites specific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to two or three different epitopes of the second molecule). As provided above, the VL and VH domains may be selected to permit trispecific binding. Accordingly, the invention also encompasses trispecific, tetravalent, Fc-

containing diabodies. The trispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope-binding sites immunospecific for B7-H3, one epitope-binding site immunospecific for a second molecule, and one epitope-binding site immunospecific for a third molecule. In certain embodiments, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the second molecule is CD3 and the third molecule is CD8.

D. Trivalent Binding Molecules Containing Fc Domains

[00179] A further embodiment of the present invention relates to trivalent binding molecules comprising an Fc Domain capable of simultaneously binding a first epitope, a second epitope and a third epitope, wherein at least one of such epitopes is not identical to another. Such trivalent binding molecules comprise three epitope-binding sites, two of which are Diabody-Type Binding Domains, which provide binding Site A and binding Site B, and one of which is a Fab-Type Binding Domain, or an scFv-Type Binding Domain, which provides binding Site C (see, *e.g.*, **Figures 6A-6F**, and PCT Application No: PCT/US15/33081; and PCT/US15/33076). Such trivalent binding molecules thus comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope and "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope and "**VL3**" and "**VH3**" domains that are capable of binding to the "third" epitope of such trivalent binding molecule. A "Diabody-Type Binding Domain" is the type of epitope-binding site present in a diabody, and especially, a DART® diabody, as described above. Each of a "Fab-Type Binding Domain" and an "scFv-Type Binding Domain" are epitope-binding sites that are formed by the interaction of the VL Domain of an immunoglobulin light chain and a complementing VH Domain of an immunoglobulin heavy chain. Fab-Type Binding Domains differ from Diabody-Type Binding Domains in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single epitope-binding site, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two epitope-binding sites. Similarly, scFv-Type Binding Domains also differ from Diabody-Type Binding Domains in that they comprise only a single epitope-binding site. Thus, as used herein Fab-Type, and scFv-Type Binding Domains are distinct from Diabody-Type Binding Domains.

[00180] Typically, the trivalent binding molecules of the present invention will comprise four different polypeptide chains (see **Figures 6A-6B**), however, the molecules

may comprise fewer or greater numbers of polypeptide chains, for example by fusing such polypeptide chains to one another (*e.g.*, via a peptide bond) or by dividing such polypeptide chains to form additional polypeptide chains, or by associating fewer or additional polypeptide chains via disulfide bonds. **Figures 6C-6F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 6A-6F**, the trivalent binding molecules of the present invention may have alternative orientations in which the Diabody-Type Binding Domains are N-terminal (**Figures 6A**, **6C** and **6D**) or C-terminal (**Figures 6B**, **6E** and **6F**) to an Fc Domain.

[00181] In certain embodiments, the first polypeptide chain of such trivalent binding molecules of the present invention contains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The VL1 and VL2 Domains are located N-terminal or C-terminal to the CH2-CH3-containing domain as presented in **Table 3** (also see, **Figures 6A and 6B**). The second polypeptide chain of such embodiments contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain. The third polypeptide chain of such embodiments contains: (i) a VH3-containing Domain, (ii) a CH1-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The third polypeptide chain may be the heavy chain of an antibody that contains a VH3 and a heavy chain constant region, or a polypeptide that contains such domains. The fourth polypeptide of such embodiments contains: (i) a VL3-containing Domain and (ii) a CL-containing Domain. The fourth polypeptide chains may be a light chain of an antibody that contains a VL3 complementary to the VH3 of the third polypeptide chain, or a polypeptide that contains such domains. The third or fourth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly, synthetically or by other means.

[00182] The Light Chain Variable Domain of the first and second polypeptide chains are separated from the Heavy Chain Variable Domains of such polypeptide chains by an intervening spacer peptide having a length that is too short to permit their VL1/VH2 (or their VL2/VH1) domains to associate together to form epitope-binding site capable of binding to either the first or second epitope. A preferred intervening spacer peptide (Linker 1) for this purpose has the sequence (**SEQ ID NO:32**): GGGSGGGG. Other Domains of the trivalent binding molecules may be separated by one or more intervening spacer peptides

(Linkers), optionally comprising a cysteine residue. In particular, as provided above, such Linkers will typically be incorporated between Variable Domains (*i.e.*, VH or VL) and peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and between such peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and CH2-CH3 Domains. Exemplary linkers useful for the generation of trivalent binding molecules are provided above and are also provided in PCT Application Nos: PCT/US15/33081; and PCT/US15/33076. Thus, the first and second polypeptide chains of such trivalent binding molecules associate together to form a VL1/VH1 binding site capable of binding a first epitope, as well as a VL2/VH2 binding site that is capable of binding to a second epitope. The third and fourth polypeptide chains of such trivalent binding molecules associate together to form a VL3/VH3 binding site that is capable of binding to a third epitope.

[00183] As described above, the trivalent binding molecules of the present invention may comprise three polypeptides. Trivalent binding molecules comprising three polypeptide chains may be obtained by linking the domains of the fourth polypeptide N-terminal to the VH3-containing Domain of the third polypeptide (*e.g.*, using an intervening spacer peptide (**Linker 4**)). Alternatively, a third polypeptide chain of a trivalent binding molecule of the invention containing the following domains is utilized: (i) a VL3-containing Domain, (ii) a VH3-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence, wherein the VL3 and VH3 are spaced apart from one another by an intervening spacer peptide that is sufficiently long (at least 9 or more amino acid residues) so as to allow the association of these domains to form an epitope-binding site. One preferred intervening spacer peptide for this purpose has the sequence: GGGGSGGGGSGGGGS (**SEQ ID NO:59**).

[00184] It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains of such trivalent binding molecules may be different so as to permit binding that is monospecific, bispecific or trispecific. In particular, the VL and VH Domains may be selected such that a trivalent binding molecule comprises two binding sites for a first epitope and one binding sites for a second epitope, or one binding site for a first epitope and two binding sites for a second epitope, or one binding site for a first epitope, one binding site for a second epitope and one binding site for a third epitope.

[00185] However, as provided herein, these domains are preferably selected so as to bind an epitope of B7-H3, an epitope of second molecule, and an epitope of a third molecule. In certain embodiments, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16,

T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the third molecule is CD8.

[00186] The general structure of the polypeptide chains of representative trivalent binding molecules of invention is provided in **Figures 6A-6F** and in **Table 4**:

Table 4

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

HPD = Heterodimer-Promoting Domain

[00187] One embodiment of the present invention relates to trivalent binding molecules that comprise two epitope-binding sites for B7-H3 and one epitope-binding site for a second molecule. The two epitope-binding sites for B7-H3 may bind the same epitope or different epitopes. Another embodiment of the present invention relates to trivalent binding molecules that comprise, one epitope-binding site for B7-H3 and two epitope-binding sites for a second molecule. The two epitope-binding sites for the second molecule may bind the same epitope or different epitopes of the second molecule. A further embodiment of the present invention relates to trispecific trivalent binding molecules that comprise, one epitope-binding site for B7-H3, one epitope-binding site for a second molecule, and one epitope-binding site for a third molecule. In certain embodiments, the second molecule is a

molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the second molecule is CD3 and the third molecule is CD8. As provided above, such trivalent binding molecules may comprise three, four, five, or more polypeptide chains.

VII. Modification of the Fc Domain

[00188] The Fc Domain of the Fc Domain-containing molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) of the present invention may be either a complete Fc Domain (*e.g.*, a complete IgG Fc Domain) or only a fragment of an Fc Domain. Optionally, the Fc Domain of the Fc Domain-containing molecules of the present invention lacks the C-terminal lysine amino acid residue.

[00189] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Domain of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. In addition, interaction with the neonatal Fc Receptor (FcRn) mediates the recycling of IgG molecules from the endosome to the cell surface and release into the blood. The amino acid sequence of exemplary wild-type IgG1 (**SEQ ID NO:12**), IgG2 (**SEQ ID NO:13**), IgG3 (**SEQ ID NO:14**), and IgG4 (**SEQ ID NO:15**) are presented above.

[00190] Modification of the Fc Domain may lead to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may therefore be desirable to modify an Fc Domain-containing B7-H3-binding molecule of the present invention with respect to effector function, for example, so as to enhance the effectiveness of such molecule in treating cancer. Reduction or elimination of effector function is desirable in certain cases, for example in the case of

antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*e.g.*, non-Hodgkin's lymphoma, CLL, and Burkitt's lymphoma). Molecules of the invention possessing such conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection in which an enhanced efficacy of effector function activity is desired.

[00191] Accordingly, in certain embodiments, the Fc Domain of the Fc Domain-containing molecules of the present invention may be an engineered variant Fc Domain. Although the Fc Domain of the bispecific Fc Domain-containing molecules of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such variant Fc Domain have altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Domain), *e.g.*, will have enhanced binding to an activating receptor and/or will have substantially reduced or no ability to bind to inhibitory receptor(s). Thus, the Fc Domain of the Fc Domain-containing molecules of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Domain, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Domain). Such Fc Domains may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Domains, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00192] Fc Domain modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer Res. 57(18):8882-8890). **Table 5** lists exemplary single, double, triple, quadruple and quintuple substitutions (numbering and

substitutions are relative to the amino acid sequence of **SEQ ID NO:12**) of exemplary modification that increase binding to activating receptors and/or reduce binding to inhibitory receptors.

Table 5
Variations of Preferred Activating Fc Domains

Single-Site Variations			
F243L	R292G	D270E	R292P
Y300L	P396L		
Double-Site Variations			
F243L and R292P	F243L and Y300L	F243L and P396L	R292P and Y300L
D270E and P396L	R292P and V305I	P396L and Q419H	P247L and N421K
R292P and P396L	Y300L and P396L	R255L and P396L	R292P and P305I
K392T and P396L			
Triple-Site Variations			
F243L, P247L and N421K		P247L, D270E and N421K	
F243L, R292P and Y300L		R255L, D270E and P396L	
F243L, R292P and V305I		D270E, G316D and R416G	
F243L, R292P and P396L		D270E, K392T and P396L	
F243L, Y300L and P396L		D270E, P396L and Q419H	
V284M, R292L and K370N		R292P, Y300L and P396L	
Quadruple-Site Variations			
L234F, F243L, R292P and Y300L		F243L, P247L, D270E and N421K	
L234F, F243L, R292P and Y300L		F243L, R255L, D270E and P396L	
L235I, F243L, R292P and Y300L		F243L, D270E, G316D and R416G	
L235Q, F243L, R292P and Y300L		F243L, D270E, K392T and P396L	
P247L, D270E, Y300L and N421K		F243L, R292P, Y300L, and P396L	
R255L, D270E, R292G and P396L		F243L, R292P, V305I and P396L	
R255L, D270E, Y300L and P396L		F243L, D270E, P396L and Q419H	
D270E, G316D, P396L and R416G			
Quintuple-Site Variations			
L235V, F243L, R292P, Y300L and P396L		F243L, R292P, V305I, Y300L and P396L	
L235P, F243L, R292P, Y300L and P396L			

[00193] Exemplary variants of human IgG1 Fc Domains with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R292P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc Domain in any combination. In one embodiment, the variant human IgG1 Fc Domain contains a F243L, R292P and Y300L substitution. In another embodiment, the variant human IgG1 Fc Domain contains a F243L, R292P, Y300L, V305I and P296L substitution.

[00194] In certain embodiments, it is preferred for the Fc Domains of B7-H3-binding molecules of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB

(CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:12**). In a specific embodiment, the B7-H3-binding molecules of the present invention comprise an IgG Fc Domain that exhibits reduced ADCC effector function. In a preferred embodiment, the CH2-CH3 Domains of such B7-H3-binding molecules include any 1, 2, 3, or 4 of the substitutions: L234A, L235A, D265A, N297Q, and N297G. In another embodiment, the CH2-CH3 Domains contain an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 Domain of a naturally occurring Fc Domain that inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding and effector function exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:12**)) is utilized. In a specific embodiment, the B7-H3-binding molecules of the present invention comprise an IgG2 Fc Domain (**SEQ ID NO:13**) or an IgG4 Fc Domain (**SEQ ID:NO:4**). When an IgG4 Fc Domain is utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such as the Hinge Domain S228P substitution described above (see, *e.g.*, **SEQ ID NO:11**). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00195] A preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Domain-containing molecules of the present invention having reduced or abolished effector function will comprise the substitutions L234A/L235A (**SEQ ID NO:60**):

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

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

[00196] The serum half-life of proteins comprising Fc Domains may be increased by increasing the binding affinity of the Fc Domain for FcRn. The term "half-life" as used herein means a pharmacokinetic property of a molecule that is a measure of the mean survival time of the molecules following their administration. Half-life can be expressed as the time required to eliminate fifty percent (50%) of a known quantity of the molecule from a subject's body (*e.g.*, a human patient or other mammal) or a specific compartment thereof, for example, as measured in serum, *i.e.*, circulating half-life, or in other tissues. In general,

an increase in half-life results in an increase in mean residence time (MRT) in circulation for the molecule administered.

[00197] In some embodiments, the B7-H3-binding molecules of the present invention comprise a variant Fc Domain, wherein said variant Fc Domain comprises at least one amino acid modification relative to a wild-type Fc Domain, such that said molecule has an increased half-life (relative to a molecule comprising a wild-type Fc Domain). In some embodiments, the B7-H3-binding molecules of the present invention comprise a variant IgG Fc Domain, wherein said variant Fc Domain comprises a half-live extending amino acid substitution at one or more positions selected from the group consisting of 238, 250, 252, 254, 256, 257, 256, 265, 272, 286, 288, 303, 305, 307, 308, 309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428, 433, 434, 435, and 436. Numerous mutations capable of increasing the half-life of an Fc Domain-containing molecule are known in the art and include, for example M252Y, S254T, T256E, and combinations thereof. For example, see the mutations described in U.S. Patents No. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and PCT Publication Nos. WO 98/23289; WO 2009/058492; and WO 2010/033279, which are herein incorporated by reference in their entireties. B7-H3-binding molecules with enhanced half-life also include those possessing variant Fc Domains comprising substitutions at two or more of Fc Domain residues 250, 252, 254, 256, 257, 288, 307, 308, 309, 311, 378, 428, 433, 434, 435 and 436. In particular, two or more substitutions selected from: T250Q, M252Y, S254T, T256E, K288D, T307Q, V308P, A378V, M428L, N434A, H435K, and Y436I.

[00198] In a specific embodiment, a B7-H3-binding molecule of the present invention possesses a variant IgG Fc Domain comprising the substitutions:

(A) M252Y, S254T and T256E;

(B) M252Y and S254T;

(C) M252Y and T256E;

(D) T250Q and M428L;

(E) T307Q and N434A;

(F) A378V and N434A;

(G) N434A and Y436I;

(H) V308P and N434A; or

(I) K288D and H435K.

[00199] In a preferred embodiment, a B7-H3-binding molecule of the present invention possesses a variant IgG Fc Domain comprising any 1, 2, or 3 of the substitutions: M252Y, S254T and T256E. The invention further encompasses B7-H3-binding molecules possessing variant Fc Domains comprising:

(A) one or more mutations which alter effector function and/or FcγR; and

(B) one or more mutations which extend serum half-life.

[00200] For certain antibodies, diabodies and trivalent binding molecules whose Fc Domain-containing first and third polypeptide chains are not identical, it is desirable to reduce or prevent homodimerization from occurring between the CH2-CH3 Domains of two first polypeptide chains or between the CH2-CH3 Domains of two third polypeptide chains. The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a "knob", *e.g.,* tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, "the hole" (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising CH2-CH3 Domains that forms an Fc Domain to foster heterodimerization. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.,* Ridgway *et al.* (1996) *"'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,"* Protein Engr. 9:617-621, Atwell *et al.* (1997) *"Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,"* J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) *"A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,"* J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety).

[00201] A preferred knob is created by modifying an IgG Fc Domain to contain the modification T366W. A preferred hole is created by modifying an IgG Fc Domain to contain the modification T366S, L368A and Y407V. To aid in purifying the hole-bearing

third polypeptide chain homodimer from the final bispecific heterodimeric Fc Domain-containing molecule, the protein A binding site of the hole-bearing CH2 and CH3 Domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the hole-bearing third polypeptide chain homodimer will not bind to protein A, whereas the bispecific heterodimer will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain. In an alternative embodiment, the hole-bearing third polypeptide chain may incorporate amino acid substitutions at positions 434 and 435 (N434A/N435K).

[00202] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Domain-containing molecule of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:61**):

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

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

[00203] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Domain-containing molecule of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Domain-containing molecule having three, four, or five polypeptide chains) will have the "**hole-bearing**" sequence (**SEQ ID NO:62**):

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

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

[00204] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:61,** and **SEQ ID NO:62** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Domain exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Domain (**SEQ ID NO:12**). The invention also encompasses such CH2-CH3 Domains, which comprise the wild-type alanine residues, alternative and/or additional substitutions which modify effector function and/or FγR

binding activity of the Fc Domain. The invention also encompasses such CH2-CH3 Domains, which further comprise one or more half-live extending amino acid substitutions. In particular, the invention encompasses such hole-bearing and such knob-bearing CH2-CH3 Domains which further comprise the M252Y/S254T/T256E.

[00205] It is preferred that the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:61**. However, as will be recognized, a "hole-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:62** could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:61**) would be employed in the second polypeptide chain of an Fc Domain-containing molecule of the present invention having two polypeptide chains (or in the third polypeptide chain of an Fc Domain-containing molecule having three, four, or five polypeptide chains).

[00206] In other embodiments, the invention encompasses B7-H3-binding molecules comprising CH2 and/or CH3 Domains that have been engineered to favor heterodimerization over homodimerization using mutations known in the art, such as those disclosed in PCT Publication No. WO 2007/110205; WO 2011/143545; WO 2012/058768; WO 2013/06867, all of which are incorporated herein by reference in their entirety.

VIII. Effector Cell Binding Capabilities

[00207] As provided herein, the B7-H3-binding molecules of the invention, including **B7-H3-ADC** molecules, can be engineered to comprise a combination of epitope-binding sites that recognize a set of antigens unique to a target cell or tissue type. In particular, the present invention relates to multispecific B7-H3-binding molecules that are capable of binding to an epitope of B7-H3 and an epitope of a molecule present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. For example, the B7-H3-binding molecules of the present invention may be construction to comprise an epitope-binding site that immunospecifically binds CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), or NKG2D. The invention also relates to trispecific B7-H3-binding molecules that are capable of binding to an epitope of CD3 and an epitope of CD8 (see, *e.g.*, PCT Publication No. WO 2015/026894).

A. CD2 Binding Capabilities

[00208] In one embodiment, the bispecific, trispecific or multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of

CD2. CD2 is a cell adhesion molecule found on the surface of T-cells and natural killer (NK) cells. CD2 enhances NK cell cytotoxicity, possibly as a promoter of NK cell nanotube formation (Mace, E.M. *et al.* (2014) "*Cell Biological Steps and Checkpoints in Accessing NK Cell Cytotoxicity*," Immunol. Cell. Biol. 92(3):245-255; Comerci, C.J. *et al.* (2012) "*CD2 Promotes Human Natural Killer Cell Membrane Nanotube Formation*," PLoS One 7(10):e47664:1-12). Molecules that specifically bind CD2 include the anti-CD2 antibody "**Lo-CD2a**."

[00209] The amino acid sequence of the VH Domain of Lo-CD2a (ATCC Accession No: 11423); **SEQ ID NO:63**) is shown below (CDR_H residues are shown underlined):

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

[00210] The amino acid sequence of the VL Domain of Lo-CD2a (ATCC Accession No: 11423; **SEQ ID NO:64**) is shown below (CDR_L residues are shown underlined):

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

B. CD3 Binding Capabilities

[00211] In one embodiment, the bispecific, trispecific or multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of CD3. CD3 is a T-cell co-receptor composed of four distinct chains (Wucherpfennig, K.W. *et al.* (2010) "*Structural Biology Of The T-Cell* Receptor*: Insights Into Receptor Assembly, Ligand Recognition, And Initiation Of Signaling*," Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14). In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with a molecule known as the T-Cell Receptor (TCR) in order to generate an activation signal in T lymphocytes. In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) "*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*," Immunology 129(2):170–177). CD3 is found bound to the membranes of all mature T-cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In: IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE," 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) "*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The*

CD3ε:γ Heterodimer," Cell 105(7):913-923; Kuhns, M.S. *et al.* (2006) "*Deconstructing The Form And Function Of The TCR/CD3 Complex*," Immunity. 2006 Feb;24(2):133-139). Molecules that specifically binds CD3 include the anti-CD3 antibodies "**CD3 mAb-1**" and "**OKT3**." The anti-CD3 antibody **CD3 mAb-1** is capable of binding non-human primates (*e.g.*, cynomolgus monkey).

[00212] The amino acid sequence of the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:65**) is shown below (CDR_H residues are shown underlined):

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

[00213] The amino acid sequence of an alternative VH Domain of **CD3 mAb-1 VH(2)** (**SEQ ID NO:66**) is shown below (CDR_H residues are shown in single underline; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:65**) are shown in double underline).

EVQLVESGGG LVQPGGSLRL SCAASGFTF**N** **TYAMN**WVRQA PGKGLEWV**A**R
IRSKYNNYAT YYADSVKDRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS WFAYWGQGTL VTVSS

[00214] The amino acid sequence of the VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**) is shown below (CDR_L residues are shown underlined):

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

[00215] The VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:65**) may be used with the VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**) to form a functional CD3-binding molecule in accordance with the present invention. Likewise, the VH Domain of **CD3 mAb-1 VH(2)** (**SEQ ID NO:66**) may be used with the VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**) to form a functional CD3-binding molecule in accordance with the present invention.

[00216] As discussed below, in order to better illustrate the present invention, bispecific B7-H3 x CD3-binding molecules were produced. In some of the B7-H3 x CD3 constructs, a variant of **CD3 mAb-1** was employed. The variant "**CD3 mAb-1 (D65G)**," comprises the VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**) and a VH **CD3 mAb-1** Domain having a D65G substitution (Kabat position 65, corresponding to residue 68 of **SEQ**

ID NO:65). The amino acid sequence of the VH Domain of **CD3 mAb-1** (D65G) (**SEQ ID NO:68**) is shown below (CDR_H residues are shown underlined, the substituted position (D65G) is shown in double underline):

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

[00217] Alternatively, an affinity variant of **CD3 mAb-1** may be incorporated. Variants include a low affinity variant designated "**CD3 mAb-1 Low**" and a variant having a faster off rate designated "**CD3 mAb-1 Fast.**" The VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**) is common to **CD3 mAb-1 Low** and **CD3 mAb1 Fast** and is provided above. The amino acid sequences of the VH Domains of each of **CD3 mAb-1 Low** and **CD3 mAb-1 Fast** are provided below.

[00218] The amino acid sequence of the VH Domain of anti-human **CD3 mAb-1 Low** (**SEQ ID NO:69**) is shown below (CDR_H residues are shown underlined; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:65**) are shown in double underline):

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

[00219] The amino acid sequence of the VH Chain Domain of anti-human **CD3 mAb-1 Fast** (**SEQ ID NO:70**) is shown below (CDR_H residues are shown underlined; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:65**) are shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAAS**GFTFS TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT YYADSVKGRF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
H*K*NFGNSYV*T* WFAYWGQGTL VTVSS

[00220] Another anti-CD3 antibody, which may be utilized is antibody Muromonab-CD3 "**OKT3**" (Xu *et al.* (2000) *"In Vitro Characterization Of Five Humanized OKT3 Effector Function Variant Antibodies,"* Cell. Immunol. 200:16-26); Norman, D.J. (1995) "*Mechanisms Of Action And Overview Of OKT3*," Ther. Drug Monit. 17(6):615-620; Canafax, D.M. *et al.* (1987) "*Monoclonal Antilymphocyte Antibody (OKT3)* Treatment *Of Acute Renal Allograft Rejection*," Pharmacotherapy 7(4):121-124; Swinnen, L.J. *et al.* (1993) "*OKT3 Monoclonal Antibodies Induce Interleukin-6 And Interleukin-10: A Possible*

Cause Of Lymphoproliferative Disorders Associated With Transplantation," Curr. Opin. Nephrol. Hypertens. 2(4):670-678).

[00221] The amino acid sequence of the VH Domain of **OKT3** (**SEQ ID NO:71**) is shown below (CDR_H residues are shown underlined):

QVQLQQSGAE LARPGASVKM SCKASGYTFT **RYTMH**WVKQR PGQGLEWIG**Y**
INPSRGYTNY **NQKFKD**KATL TTDKSSSTAY MQLSSLTSED SAVYYCAR**YY**
DDHYCLDYWG QGTTLTVSS

[00222] The amino acid sequence of the VL Domain of **OKT3** (**SEQ ID NO:72**) is shown below (CDR_L residues are shown underlined):

QIVLTQSPAI MSASPGEKVT MTC**SASSSVS** **YMN**WYQQKSG TSPKRWIY**DT**
SKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYC**QQW** **SSNPFTF**GSG
TKLEINR

[00223] Additional anti-CD3 antibodies that may be utilized include but are not limited to those described in PCT Publication Nos. WO 2008/119566; and WO 2005/118635.

C. CD8 Binding Capabilities

[00224] In one embodiment, the bispecific, trispecific or multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of CD8. CD8 is a T-cell co-receptor composed of two distinct chains (Leahy, D.J., (1995) "*A Structural View of CD4 and CD8*," FASEB J., 9:17-25) that is expressed on Cytotoxic T-cells. The activation of $CD8^+$ T-cells has been found to be mediated through co-stimulatory interactions between an antigen:major histocompability class I (**MHC I**) molecule complex that is arrayed on the surface of a target cell and a complex of CD8 and the T-cell Receptor, that are arrayed on surface of the $CD8^+$ T-cell (Gao, G., and Jakobsen, B., (2000). "*Molecular interactions of coreceptor CD8 and MHC class I: the molecular basis for functional coordination with the T-Cell Receptor*". Immunol Today 21: 630–636). Unlike MHC II molecules, which are expressed by only certain immune system cells, MHC I molecules are very widely expressed. Thus, cytotoxic T-cells are capable of binding to a wide variety of cell types. Activated cytotoxic T-cells mediate cell killing through their release of the cytotoxins perforin, granzymes, and granulysin. Antibodies that specifically bind CD8 include the anti-CD8 antibodies "**OKT8**" and "**TRX2**."

[00225] The amino acid sequence of the VH Domain of OKT8 (**SEQ ID NO:73**) is shown below (CDR_H residues are shown underlined):

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

[00226] The amino acid sequence of the VL Domain of OKT8 (**SEQ ID NO:74**) is shown below (CDR_L residues are shown underlined):

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

[00227] The amino acid sequence of the VH Domain of TRX2 (**SEQ ID NO:75**) is shown below (CDR_H residues are shown underlined):

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

[00228] The amino acid sequence of the VL Domain of TRX2 (**SEQ ID NO:76**) is shown below (CDR_L residues are shown underlined):

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

D. CD16 Binding Capabilities

[00229] In one embodiment, multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of CD16. CD16 is the FcγRIIIA receptor. CD16 is expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that bind aggregated but not monomeric human IgG (Peltz, G.A. *et al.* (1989) "*Human Fc Gamma RIII: Cloning, Expression, And Identification Of The Chromosomal Locus Of Two Fc Receptors For IgG*," Proc. Natl. Acad. Sci. (U.S.A.) 86(3):1013-1017; Bachanova, V. *et al.* (2014) "*NK Cells In Therapy Of Cancer*," Crit. Rev. Oncog. 19(1-2):133-141; Miller, J.S. (2013) "*Therapeutic Applications: Natural Killer Cells In The Clinic*," Hematology Am. Soc. Hematol. Educ. Program. 2013:247-253; Youinou, P. *et al.* (2002) "*Pathogenic Effects Of Anti-Fc Gamma Receptor IIIB (CD16) On Polymorphonuclear Neutrophils In Non-Organ-Specific Autoimmune Diseases*," Autoimmun Rev. 1(1-2):13-19; Peipp, M. *et al.* (2002) "*Bispecific Antibodies Targeting Cancer Cells*," Biochem. Soc. Trans. 30(4):507-511). Molecules that specifically bind

CD16 include the anti-CD16 antibodies "**3G8**" and "**A9**." Humanized A9 antibodies are described in PCT Publication WO 03/101485.

[00230] The amino acid sequence of the VH Domain of 3G8 (**SEQ ID NO:77**) is shown below (CDR_H residues are shown underlined):

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

[00231] The amino acid sequence of the VL Domain of 3G8 (**SEQ ID NO:78**) is shown below (CDR_L residues are shown underlined):

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

[00232] The amino acid sequence of the VH Domain of A9 (**SEQ ID NO:79**) is shown below (CDR_H residues are shown underlined):

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

[00233] The amino acid sequence of the VL Domain of A9 (**SEQ ID NO:80**) is shown below (CDR_L residues are shown underlined):

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

[00234] Additional anti-CD19 antibodies that may be utilized include but are not limited to those described in PCT Publication Nos. WO 03/101485; and WO 2006/125668.

E. TCR Binding Capabilities

[00235] In one embodiment, the bispecific, trispecific or multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of the T Cell Receptor (TCR). The T Cell Receptor is natively expressed by CD4+ or CD8+ T cells, and permits such cells to recognize antigenic peptides that are bound and presented by class I or class II MHC proteins of antigen-presenting cells. Recognition of a pMHC (peptide–MHC) complex by a TCR initiates the propagation of a cellular immune response that leads to the production of cytokines and the lysis of the antigen-presenting cell (see, *e.g.*, Armstrong, K.M. *et al.* (2008) "*Conformational Changes And Flexibility In T-Cell*

Receptor Recognition Of Peptide–MHC Complexes," Biochem. J. 415(Pt 2):183–196; Willemsen, R. (2008) "*Selection Of Human Antibody Fragments Directed Against Tumor T-Cell Epitopes For Adoptive T-Cell Therapy*," Cytometry A. 73(11):1093-1099; Beier, K.C. *et al.* (2007) "*Master Switches Of T-Cell Activation And Differentiation*," Eur. Respir. J. 29:804-812; Mallone, R. *et al.* (2005) "*Targeting T Lymphocytes For Immune Monitoring And Intervention In Autoimmune Diabetes*," Am. J. Ther. 12(6):534–550). CD3 is the receptor that binds to the TCR (Thomas, S. *et al.* (2010) "*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*," Immunology 129(2):170-177; Guy, C.S. *et al.* (2009) "*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex*," Immunol. Rev. 232(1):7-21; St. Clair, E.W. (Epub 2009 Oct 12) "*Novel Targeted Therapies For Autoimmunity*," Curr. Opin. Immunol. 21(6):648-657; Baeuerle, P.A. *et al.* (Epub 2009 Jun 9) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944; Smith-Garvin, J.E. *et al.* (2009) "*T Cell Activation*," Annu. Rev. Immunol. 27:591-619; Renders, L. *et al.* (2003) "*Engineered CD3 Antibodies For Immunosuppression*," Clin. Exp. Immunol. 133(3):307-309).

[00236] Molecules that specifically bind to the T Cell Receptor include the anti-TCR antibody "**BMA 031**" (EP 0403156; Kurrle, R. *et al.* (1989) "*BMA 031 – A TCR-Specific Monoclonal Antibody For Clinical Application*," Transplant Proc. 21(1 Pt 1):1017-1019; Nashan, B. *et al.* (1987) "Fine Specificity Of A Panel Of Antibodies Against The TCR/CD3 Complex," Transplant Proc. 19(5):4270-4272; Shearman, C.W. *et al.* (1991) "Construction, Expression, And Biologic Activity Of Murine/Human Chimeric Antibodies With Specificity For The Human α/β T Cell," J. Immunol. 146(3):928-935; Shearman, C.W. *et al.* (1991) "*Construction, Expression And Characterization of Humanized Antibodies Directed Against The Human α/β T Cell Receptor*," J. Immunol. 147(12):4366-4373).

[00237] The amino acid sequence of a VH Domain of BMA 031 (**SEQ ID NO:81**) is shown below (CDR_H residues are shown underlined):

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

[00238] The amino acid sequence of the VL Domain of BMA 031 (**SEQ ID NO:82**) is shown below (CDR_L residues are shown underlined):

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

F. NKG2D Binding Capabilities

[00239] In one embodiment, multispecific B7-H3-binding molecules of the invention are capable of binding to an epitope of B7-H3 and an epitope of the NKG2D receptor. The NKG2D receptor is expressed on all human (and other mammalian) Natural Killer cells (Bauer, S. *et al.* (1999) "*Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA*," Science 285(5428):727-729; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29) as well as on all $CD8^+$ T cells (Groh, V. *et al.* (2001) "*Costimulation Of CD8αβ T Cells By NKG2D Via Engagement By MIC Induced On Virus-Infected Cells*," Nat. Immunol. 2(3):255-260; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29). Such binding ligands, and particularly those which are not expressed on normal cells, include the histocompatibility 60 (H60) molecule, the product of the retinoic acid early inducible gene-1 (RAE-1), and the murine UL16-binding proteinlike transcript 1 (MULT1) (Raulet D.H. (2003) "*Roles Of The NKG2D Immunoreceptor And Its Ligands*," Nature Rev. Immunol. 3:781-790; Coudert, J.D. *et al.* (2005) "*Altered NKG2D Function In NK Cells Induced By Chronic Exposure To Altered NKG2D Ligand-Expressing Tumor Cells*," Blood 106:1711-1717). Molecules that specifically bind to the NKG2D Receptor include the anti-NKG2D antibodies "**KYK-1.0**" and "**KYK-2.0**" (Kwong, KY *et al.* (2008) "*Generation, Affinity Maturation, And Characterization Of A Human Anti-Human NKG2D Monoclonal Antibody With Dual Antagonistic And Agonistic Activity*," J. Mol. Biol. 384:1143-1156; and PCT/US09/54911).

[00240] The amino acid sequence of the VH Domain of KYK-1.0 (**SEQ ID NO:83**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG VVQPGGSLRL SCAASGFTFS **SYGMH**WVRQA PGKGLEWVA**F IRYDGSNKYY ADSVKG**RFTI SRDNSKNTKY LQMNSLRAED TAVYYCAK**DR FGYYLDY**WGQ GTLVTVSS

[00241] The amino acid sequence of the VL Domain of KYK-1.0 (**SEQ ID NO:84**) is shown below (CDR_L residues are shown underlined):

QPVLTQPSSV SVAPGETARI PCGGDDIETK SVHWYQQKPG QAPVLVIYDD DDRPSGIPER FFGSNSGNTA TLSISRVEAG DEADYYCQVW DDNNDEWVFG GGTQLTVL

[00242] The amino acid sequence of a VH Domain of KYK-2.0 (**SEQ ID NO:85**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG LVKPGGSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAF IRYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDR GLGDGTYFDY WGQGTTVTVS S

[00243] The amino acid sequence of a VL Domain of KYK-2.0 (**SEQ ID NO:86**) is shown below (CDR_L residues are shown underlined):

QSALTQPASV SGSPGQSITI SCSGSSSNIG NNAVNWYQQL PGKAPKLLIY YDDLLPSGVS DRFSGSKSGT SAFLAISGLQ SEDEADYYCA AWDDSLNGPV FGGGTKLTVL

IX. Multispecific B7-H3-Binding Molecules

A. B7-H3 x CD3 Bispecific Two Chain Diabodies

[00244] The VL and VH Domains of the above-described B7-H3 binding molecules may be used to construct B7-H3 x CD3 bispecific diabodies composed of two covalently linked polypeptide chains. To illustrate this aspect of the present invention, the VL and VH Domains of the above-described anti-B7-H3 **mAb-D** antibody is used to construct B7-H3 x CD3 bispecific diabodies composed of two covalently linked polypeptide chains and comprising the above-discussed murine or humanized VL and VH Domains of **mAb-D**. The general structure and amino acid sequences of such B7-H3 x CD3 bispecific diabodies is provided below.

[00245] The first polypeptide chain of one exemplary B7-H3 x CD3 bispecific two chain diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti-B7-H3 antibody (*e.g.*, **mAb-D VL** (**SEQ ID NO:22**), or **hmAb-D VL** (**SEQ ID NO:30**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of an anti-CD3 antibody (*e.g.*, CD3 mAb 1 (D65G) (**SEQ ID NO:68**)); a cysteine-containing intervening spacer peptide (**Linker 2:** GGCGGG (**SEQ ID NO:33**)); a Heterodimer-Promoting (*e.g.*, an E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:45**)); and a C-terminus.

[00246] The second polypeptide chain of such an exemplary B7-H3 x CD3 bispecific two chain diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of a corresponding anti-CD3 antibody (*e.g.*, a VL domain that in association with the VH Domain of the first polypeptide chain forms a CD3-binding site, *e.g.*, the VL Domain of **CD3 mAb-1** (**SEQ ID NO:67**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of a corresponding anti-B7-H3 antibody (*e.g.*, a VH domain that in association with the VL Domain of the first polypeptide chain forms an B7-H3-binding site, *e.g.*, **mAb-D VH** (**SEQ ID NO:26**) or **hmAb-D VH** (**SEQ ID NO:31**); a cysteine-containing intervening spacer peptide (**Linker 2:** GGCGGG (**SEQ ID NO:33**)); a Heterodimer-Promoting (*e.g.*, K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:46**)); and a C-terminus.

[00247] As provided herein, alternative linkers and/or alternative Heterodimer-Promoting Domains may be utilized in the generation of such diabodies. For example, the first polypeptide chain of an alternative exemplary B7-H3 x CD3 bispecific two chain diabody may comprise, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti-B7-H3 antibody; the intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of the anti-CD3 antibody or of a corresponding anti-CD3 antibody; an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:37**)); a cysteine-containing Heterodimer-Promoting (*e.g.*, K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:46**)); and a C-terminus. The second polypeptide chain of such alternative exemplary diabody may comprise, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of a corresponding anti-CD3 antibody; an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of a corresponding anti-B7-H3 antibody (*e.g.*, **mAb-D VH** (**SEQ ID NO:26**) or **hmAb-D VH** (**SEQ ID NO:31**)); an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:37**)); a cysteine-containing Heterodimer-Promoting (*e.g.*, E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:47**)); and a C-terminus.

1. DART-D1

[00248] A representative B7-H3 x CD3 bispecific two chain diabody comprising the VH and VL Domains of **hmAb-C** ("**DART-D1**") is constructed.

[00249] The amino acid sequence of the first polypeptide chain of **DART-D1** (**SEQ ID NO:87**) is shown below (the sequence of the **hmAb-C VL** Domain (**SEQ ID NO:20**) is underlined):

DIQMTQSPSS LSASVGDRVT ITCRASESIY SYLAWYQQKP GKAPKLLVYN TKTLPEGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQH HYGTPPWTFG QGTRLEIKGG GSGGGGEVQL VESGGGLVQP GGSLRLSCAA SGFTFSTYAM NWVRQAPGKG LEWVGRIRSK YNNYATYYAD SVKGRFTISR DDSKNSLYLQ MNSLKTEDTA VYYCVRHGNF GNSYVSWFAY WGQGTLVTVS SGGCGGGEVA ALEKEVAALE KEVAALEKEV AALEK

[00250] The amino acid sequence of the second polypeptide chain of **DART-D1** (**SEQ ID NO:88**) is shown below (the sequence of the **hmAb-C VH** Domain (**SEQ ID NO:21**) is underlined):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGG**EV QLVESGGGLV KPGGSLRLSC AASGFTFSSY GMSWVRQAPG KGLEWVATIN SGGSNTYYPD SLKGRFTISR DNAKNSLYLQ MNSLRAEDTA VYYCARHDGG AMDYWGQGTT VTVSS**GGCGG GKVAALKEKV AALKEKVAAL KEKVAALKE

2. DART-D2

[00251] A representative B7-H3 x CD3 bispecific two chain diabody comprising the VH and VL Domains of **hmAb-D** ("**DART-D2**") is constructed.

[00252] The amino acid sequence of the first polypeptide chain of **DART-D2** (**SEQ ID NO:89**) is shown below (the sequence of the **hmAb-D VL** Domain (**SEQ ID NO:30**) is underlined):

DIQMTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYFCQQ YNNYPFTFGQ GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISRD DSKNSLYLQM NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLVTVSS ASTKGEVAAC EKEVAALEKE VAALEKEVAA LEK

[00253] The amino acid sequence of the second polypeptide chain of **DART-D2** (**SEQ ID NO:90**) is shown below (the sequence of the **hmAb-D VH** Domain (**SEQ ID NO:31**) is underlined):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF GGGTKLTVLG GGGSGGGG**EV QLVESGGGLV QPGGSLRLSC AASGFTFSSF**

GMHWVRQAPG KGLEWVAYIS SGSGTIYYAD TVKGRFTISR DNAKNSLYLQ MNSLRAEDTA VYYCARHGYR YEGFDYWGQG TTVTVSSAST KGKVAACKEK VAALKEKVAA LKEKVAALKE

[00254] It will be appreciated in view of the teachings provided herein that different domain orientations, VH Domains, VL Domains, linkers, and/or heterodimer promoting domains, could be utilized to generate alternative B7-H3 x CD3 bispecific two chain diabodies.

B. B7-H3 x CD3 Bispecific Three Chain Diabodies

[00255] A B7-H3 x CD3 diabody having three chains and possessing an Fc Domain is generated having one binding site specific for B7-H3 (comprising humanized VH and VL Domains of **hmAb-D**) and one binding site specific for CD3 (comprising the VL and VH Domains of CD3 mAb 1 (D65G)). The diabody is designated "**DART-D3**."

[00256] The first polypeptide chain of the exemplary B7-H3 x CD3 bispecific three chain **DART-D3** diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti- B7-H3 antibody (**hmAb-D VL** (**SEQ ID NO:30**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of CD3 mAb 1 (D65G) (**SEQ ID NO:68**); an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:37**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:47**)); an intervening spacer peptide (**Linker 3:** GGGDKTHTCPPCP (**SEQ ID NO:57**)); a knob-bearing IgG1 CH2-CH3 Domain (**SEQ ID NO:61**); and a C-terminus. Polynucleotides encoding this polypeptide chain may encode the C-terminal lysine residue of **SEQ ID NO:61** (*i.e.*, **X** of **SEQ ID NO:61**), however, as discussed above, this lysine residue may be post-translationally removed in some expression systems. Accordingly, the invention encompasses such a first polypeptide chain that contains such lysine residue (*i.e.*, **SEQ ID NO:61**, wherein **X** is lysine), as well as a first polypeptide chain that lacks such lysine residue (*i.e.*, **SEQ ID NO:61**, wherein **X** is absent). The amino acid sequences of such first polypeptide chain (**SEQ ID NO:91**) is provided below (the sequence of the **hmAb-D VL** Domain (**SEQ ID NO:30**) is underlined):

DIQMTQSPSF LSASVGDRVT ITCKASQNVD TNVAWYQQKP GKAPKALIYS ASYRYSGVPS RFSGSGSGTD FTLTISSLQP EDFAEYFCQQ YNNYPFTFGQ GTKLEIKGGG SGGGGEVQLV ESGGGLVQPG GSLRLSCAAS GFTFSTYAMN WVRQAPGKGL EWVGRIRSKY NNYATYYADS VKGRFTISRD DSKNSLYLQM

NSLKTEDTAV YYCVRHGNFG NSYVSWFAYW GQGTLVTVSS ASTKGEVAAC
EKEVAALEKE VAALEKEVAA LEKGGGDKTH TCPPCPAPEA AGGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LWCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PG**X**

wherein **X** is Lysine (K) or is absent.

[00257] The second polypeptide chain of the exemplary B7-H3 x CD3 bispecific three chain **DART-D3** diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of CD3 mAb-1 (**SEQ ID NO:67**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:32**)); the VH Domain of an anti-B7-H3 antibody (**hmAb-D VH** (**SEQ ID NO:31**); an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:37**)); a cysteine-containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:48**)); and a C-terminus. The amino acid sequence of such second polypeptide chain (**SEQ ID NO:92**) is provided below (the sequence of the **hmAb-D VH** Domain (**SEQ ID NO:31**) is underlined):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
GGGTKLTVLG GGGSGGGG**EV QLVESGGGLV QPGGSLRLSC AASGFTFSSF**
GMHWVRQAPG KGLEWVAYIS SGSGTIYYAD TVKGRFTISR DNAKNSLYLQ
MNSLRAEDTA VYYCARHGYR YEGFDYWGQG TTVTVSSAST KGKVAACKEK
VAALKEKVAA LKEKVAALKE

[00258] The third polypeptide chain of the exemplary B7-H3 x CD3 bispecific three chain **DART-D3** diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; a spacer peptide (DKTHTCPPCP (**SEQ ID NO:56**)); a hole-bearing IgG1 CH2-CH3 Domain (**SEQ ID NO:62**); and a C-terminus. Polynucleotides encoding this polypeptide chain may encode the C-terminal lysine residue of **SEQ ID NO:62** (*i.e.*, **X** of **SEQ ID NO:62**), however, as discussed above, this lysine residue may be post-translationally removed in some expression systems. Accordingly, the invention encompasses such a third polypeptide chain that contains such lysine residue (*i.e.*, **SEQ ID NO:62**, wherein **X** is lysine), as well as a third polypeptide chain that lacks such lysine residue (*i.e.*, **SEQ ID NO:62**, wherein **X** is absent). The amino acid sequence of such third polypeptide chain (**SEQ ID NO:93**) is provided below:

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK

CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL TVDKSRWQQG
NVFSCSVMHE ALHNRYTQKS LSLSPG**X**

wherein **X** is Lysine (K) or is absent.

[00259] It will be appreciated in view of the teachings provided herein that different domain orientations, VH Domains, VL Domains, linkers, and/or heterodimer promoting domains, could be utilized to generate alternative B7-H3 x CD3 bispecific three chain diabodies. In particular, the VH Domain and VL Domain of **hmAb-C (SEQ ID NOs:20-21)** may be utilized.

C. B7-H3 x CD3 x CD8 Trivalent Binding Molecules

[00260] Exemplary trivalent "**B7-H3 x CD3 x CD8**" binding molecules having one binding site specific for B7-H3 (comprising a parental and/or humanized anti-B7-H3-VL Domain and a corresponding anti-B7-H3-VH Domain, as described above), one binding site specific for CD3 (comprising, for example, the VL Domain of **CD3 mAb-1 (SEQ ID NO:67)** and the VH Domain of anti-CD3 antibody (*e.g.*, **CD3 mAb 1 (D65G) (SEQ ID NO:68)**), and one binding site specific for CD8 (comprising, for example, the VH and VL Domains of **TRX2 (SEQ ID NOs:75 and 76**, respectively). Such trivalent binding molecules may have two polypeptide chains (see, *e.g.*, **Figure 6E**, and **Figure 6F**), three polypeptide chains (see, *e.g.*, **Figure 6C** and **Figure 6D**), four polypeptide chains (see, *e.g.*, **Figure 6A** and **Figure 6B**), or five polypeptide chains (see, *e.g.*, **Figure 5**).

X. Methods of Production

[00261] The B7-H3-binding molecules of the present invention are most preferably produced through the recombinant expression of nucleic acid molecules that encode such polypeptides, as is well-known in the art.

[00262] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "S*olid Phase Synthesis*," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) "*Solid-Phase Synthesis In The Twenty-First Century*," Mini Rev. Med. Chem. 6(1):3-10).

[00263] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants {*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) "*Production Of Antibodies And Antibody Fragments In Plants*," Vaccine 19:2756; Lonberg, N. *et al.* (1995) "*Human Antibodies From Transgenic Mice*," Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art, and have been described above. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patents No. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) "*Making Antibodies By Phage Display Technology*," Annu. Rev. Immunol. 12.433-455).

[00264] Vectors containing polynucleotides of interest (*e.g.*, polynucleotides encoding the polypeptide chains of the B7-H3-binding molecules of the present invention) can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00265] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of expressing a polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells.

[00266] The invention includes polypeptides comprising an amino acid sequence of an B7-H3-binding molecule of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially

shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available.

[00267] The invention includes variants of B7-H3-binding molecules, including functionally equivalent polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the Variable Domain. Changes in the Variable Domain can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00268] The invention encompasses fusion proteins comprising one or more of the anti-B7-H3-VL and/or VH of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a Light Chain

Variable Domain and a Heavy Chain Variable Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to B7-H3 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

[00269] The present invention particularly encompasses B7-H3-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) conjugated to a diagnostic or therapeutic moiety. For diagnostic purposes, B7-H3-binding molecules of the invention may be coupled to a detectable substance. Such B7-H3-binding molecules are useful for monitoring and/or prognosing the development or progression of a disease as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Examples of detectable substances include various enzymes (*e.g.,* horseradish peroxidase, beta-galactosidase, *etc.*), prosthetic groups (*e.g.,* avidin/biotin), fluorescent materials (e.g., umbelliferone, fluorescein, or phycoerythrin), luminescent materials (*e.g.*, luminol), bioluminescent materials (*e.g.,* luciferase or aequorin), radioactive materials (*e.g.*, carbon-14, manganese-54, strontium-85 or zinc-65), positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the B7-H3-binding molecule or indirectly, through an intermediate (*e.g.*, a linker) using techniques known in the art.

[00270] For therapeutic purposes, B7-H3-binding molecules of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells such as, for example, Pseudomonas exotoxin, Diptheria toxin, a botulinum toxin A through F, ricin abrin, saporin, and cytotoxic fragments of such agents. A therapeutic agent includes any agent having a therapeutic effect to prophylactically or therapeutically treat a disorder. Such therapeutic agents may be may be chemical therapeutic agents, protein or polypeptide therapeutic agents, and include therapeutic agents that possess a desired biological activity and/or modify a given biological response. Examples of therapeutic agents include alkylating agents, angiogenesis inhibitors, anti-mitotic agents, hormone therapy agents, and antibodies useful for the treatment of cell proliferative disorders. The therapeutic moiety may be coupled or conjugated either directly

to the B7-H3-binding molecule or indirectly, through an intermediate (*e.g.*, a linker) using techniques known in the art.

XI. Antibody Drug Conjugates

[00271] The present invention relates to therapeutic anti-human B7-H3 antibodies (or the B7-H3 binding domains thereof), and particularly to any of the above-described anti-human B7-H3 antibodies or B7-H3 binding domains thereof, that are conjugated to a drug (a "**B7-H3-ADC**" molecule). Such **B7-H3-ADC**s enhance the cytotoxicity of anti-human B7-H3 therapy, particularly in the treatment of cancer. As indicated above, the **B7-H3-ADC** molecules of the present invention comprise the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n,}$$

wherein:

Ab is an antibody that binds to B7-H3 that comprises a humanized Variable Heavy Chain (VH) Domain and a humanized Variable Light Chain (VL) Domain, or is a B7-H3-binding fragment thereof, and;

D is a cytotoxic drug moiety;

LM is a bond or a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

[00272] In preferred embodiments, the **B7-H3-ADC** will bind to a tumor cell expressing B7-H3, and will then be internalized into such cell through receptor-mediated endocytosis. Once inside a lysosome, the **B7-H3-ADC** will preferably be degraded so as to thereby cause the release of the cytotoxic drug moiety inside the cell, resulting in cell death. As will be appreciated, the mechanism of action of cell death can vary based on the class of cytotoxic drug used (e.g., disruption of cytokinesis by tubulin polymerization inhibitors such as maytansines and auristatins, DNA damage by DNA interacting agents such as calcheamicins and duocarmycins), *etc.* Neighboring cancer cells may also be killed when free drug is released into the tumor environment by the dying cell in a process known as the bystander effect (Panowski, S. *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs 6(1):34-45; Kovtun, Y.V. *et al.* (2006) "*Antibody-Drug*

Conjugates Designed To Eradicate Tumors With Homogeneous And Heterogeneous Expression Of The Target Antigen," Cancer Res. 66:3214-3221).

[00273] The **B7-H3-ADC**s of the present invention may comprise an Fc Domain, which may be a naturally occurring Fc Domain, or may have a sequence that possesses one or more differences from a naturally occurring Fc Domain, and which may be a complete Fc Domain (*e.g.*, a complete IgG Fc Domain) or only a portion of a complete Fc Domain. Such Fc Domains may be of any isotype (e.g., IgG1, IgG2, IgG3, or IgG4). Such Fc Domain may comprise, or may lack, the C-terminal lysine residue of a CH3 Domain. The **B7-H3-ADC**s of the invention may further comprise a CH1 Domain and/or a Hinge Domain. When present, the CH1 Domain and/or Hinge Domain may be of any isotype (e.g., IgG1, IgG2, IgG3, or IgG4), and will preferably of the same isotype as the desired Fc Domain.

A. Exemplary Linker Molecules of the Invention

[00274] The invention thus particularly contemplates such **B7-H3-ADCs** wherein the Linker Molecule **LM** is absent (*i.e.*, **m** = 0), and **B7-H3-ADCs** that possess more than one Linker Molecule **LM** (*i.e.*, **m** is an integer from 2 through **n**, wherein n is an integer from 2 through 10), each of which Linker Molecule **LM** covalently links a cytotoxic drug moiety **D** to the **Ab** of such **B7-H3-ADCs**.

[00275] The invention further provides **B7-H3-ADCs** whose **Ab** are covalently linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are identical. The cytotoxic drug moieties **D** that are covalently linked to the **Ab** of such **B7-H3-ADCs** may all be identical or may include 2, 3, 4, or more independently different cytotoxic drug moieties **D**.

[00276] The invention further provides such **B7-H3-ADCs** whose **Ab** are covalently linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are not identical and may independently differ. The cytotoxic drug moieties **D** that are covalently linked to the **Ab** of such **B7-H3-ADCs** may all be identical or may include 2, 3, 4, or more independently different cytotoxic drug moieties **D**.

[00277] Exemplary humanized VH and VL Domains of antibodies that bind to human B7-H3, and exemplary human antibody Constant Domains that may be included in a **B7-H3-ADC** of the invention are provided above. As stated above, the **B7-H3-ADC** of the

invention additionally comprise at least one cytotoxic drug moiety, which is preferably covalently linked to an atom of a side chain of an amino acid residue of such VH Domain or VL Domain and/or Constant Domain, either directly, or via a Linker Molecule intercalated between the side chain atom and the drug moiety. The Linker Molecule may be a non-peptide molecule, or a molecule that comprises a non-peptide portion and a peptide portion, or it may be a molecule that is composed solely of amino acid residues. The amino acid residues of any such Linker Molecules may contain naturally occurring or non-naturally occurring amino acid residues, including D-versions of naturally occurring amino acid residues, *p*-acetylphenylalanine, selenocysteine, *etc*. Optionally, or additionally, particular residues having a desired side chain (*e.g.*, a -CH_2-SH side chain, a-CH_2-OH side chain, a -$CH(CH_2)$-SH side chain, a -CH_2-CH_2-S-CH_3 side chain; a -CH_2-C(O)-NH_2 side chain, a -CH_2-CH_2-C(O)-NH_2 side chain, a -CH_2-C(O)OH- side chain, a CH_2-CH_2-C(O)OH- side chain, a -CH_2-CH_2-CH_2-CH_2-NH_2 side chain, a -CH_2-CH_2-CH_2-NH-$C(NH_2)_2$ side chain, an imidazole side chain, a benzyl side chain, a phenol side chain, an indole side chain, *etc.*) may be engineered into a **B7-H3-ADC** of the invention.

[00278] The Linker Molecule may be non-cleavable under physiologic conditions, for example composed of a hydrolytically stable moiety, for example, a thioether linker or a hindered disulfide linker. Hydrolytically stable linkers are substantially stable in water and do not react with water at useful pH values, including but not limited to, under physiological conditions for an extended period of time. In contrast, hydrolytically unstable or degradable linkers are degradable in water or in aqueous solutions, including for example, blood.

[00279] Alternatively, the Linker Molecule may be cleavable, or may contain a cleavable portion. Examples of such a cleavable portion includes an acid labile linker (*e.g.*, a 4-(4'-acetylpheonxy)butanoic acid linker which forms a hydrazine bond), a cleavable disulfide linker (that is cleaved in the reducing intracellular environment), and a protease cleavable linker. Acid-labile linkers are designed to be stable at pH levels encountered in the blood, but become unstable and degrade when the low pH environment in lysosomes is encountered. Protease-cleavable linkers are also designed to be stable in blood/plasma, but rapidly release free drug inside lysosomes in cancer cells upon cleavage by lysosomal enzymes (Panowski, S. *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs 6(1):34-45). Alternatively, the Linker Molecule may be an enzyme-cleavable-substrate or contain an enzyme-cleavable-substrate, such as a cleavable peptide,

(*e.g.*, a cleavable dipeptide such as a valine-citrulline dipeptide para-aminobenzylalcohol linker (cAC10-mc-vc-PABA) which is selectively cleaved by lysosomal enzymes). Suitable cleavable linkers are known in the art, see, *e.g.*, de Groot, Franciscus M.H., *et al.* (2002) "*Design, Synthesis, and Biological Evaluation of a Dual Tumor-Specific Motive Containing Integrin-Targeted Plasmin-Cleavable Doxorubicin Prodrug*," Molecular Cancer Therapeutics, 1: 901-911; Dubowchik *et al.*, (2002) "Doxorubicin Immunoconjugates Containing Bivalent, Lysosomally-Cleavable Dipeptide Linkages." Bioorganic & Medicinal Chemistry Letters12:1529-1532; US Patent Nos. 5547667; 6,214,345; 7,585,491; 7,754,681; 8,080,250; 8,461,117; and WO 02/083180.

[00280] Enzymatically unstable or degradable linkers can be employed. Such linkers are degraded by one or more enzymes. By way of example only, PEG and related polymers can include a degradable Linker Molecule(s) in the polymer backbone or in the linker group between the polymer backbone and one or more of the terminal functional groups of the polymer molecule. Such degradable Linker Molecule(s) include, but are not limited to, ester linkages formed by the reaction of PEG carboxylic acids or activated PEG carboxylic acids with alcohol groups on a biologically active agent, wherein such ester groups generally hydrolyze under physiological conditions to release the biologically active agent. Other hydrolytically degradable Linker Molecules include but are not limited to carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; hydrazone linkages that are a reaction product of a hydrazide and an aldehyde; acetal linkages that are the reaction product of an aldehyde and an alcohol; orthoester linkages that are the reaction product of a formate and an alcohol; peptide linkages formed by an amine group, including but not limited to, at an end of a polymer such as PEG, and a carboxyl group of a peptide; and oligonucleotide linkages formed by a phosphoramidite group, including but not limited to, at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

[00281] In one embodiment, the Linker Molecule of the present invention may be, or may comprise, a cleavable Linker Molecule, **V-(W)$_k$-(X)$_1$-A**, as disclosed in PCT Publication WO 02/083180, having the formula:

$$\mathbf{Ab - [V\text{-}(W)_k\text{-}(X)_1\text{-}A] - D}$$

wherein:

V is an optional cleavable moiety,

(W)$_k$-(X)$_1$-A is an elongated, self-eliminating spacer system, that self-eliminates via a 1,(4+2n)-elimination,

W and **X** are each a 1,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula (**Y**)$_m$, wherein **Y** is a 1,(4+2n) electronic cascade spacer, or a group of formula **U**, being a cyclisation elimination spacer,

k, 1 and m are independently an integer of 0 (included) to 5 (included),

n is an integer of 0 (included) to 10 (included),

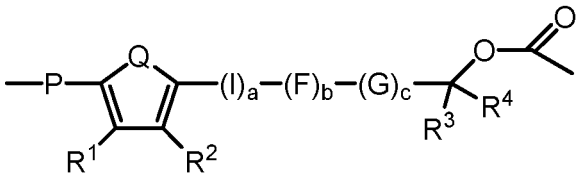
with the provisos that:

when **A** is (**Y**)$_m$: then k+1+m ≥ 1, and

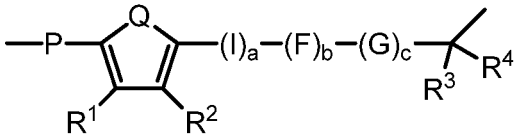
if k+1+m=1, then n>1;

when **A** is **U**: then k+1 ≥ 1.

W, **X**, and **Y** are independently selected from compounds having the formula:



or the formula:

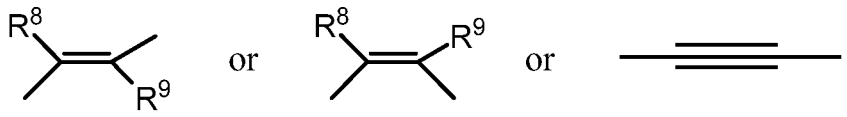


wherein: Q is -R^{5}C=CR^6-, S, O, NR^5, -R^{5}C=N-, or -N=CR^5-

P is NR^7, O or S

a, b, and c are independently an integer of 0 (included) to 5 (included);

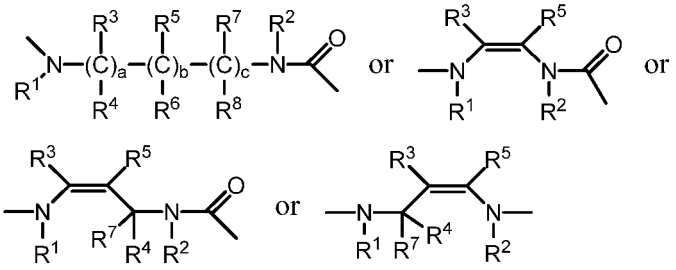
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C$_{1-6}$ alkyl, C$_{3-20}$ heterocyclyl, C$_{5-20}$ aryl, C$_{1-6}$ alkoxy, hydroxy

(OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are independently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures;

U is selected from compounds having the formula:



wherein:

a, b and c are independently selected to be an integer of 0 or 1; provided that a + b + c = 2 or 3;

R^1 and/or R^2 independently represent H, C1-6 alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_X), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_X$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)ORx), sulphinyl (S(=O)Rx), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7 and R^8 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, and two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 are optionally connected to one another to form one or more aliphatic or aromatic cyclic structures.

[00282] Exemplary molecules include:

p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

p-ammocinnamyloxycarbonyl;

p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl;

p-amino-benzyloxycarbonyl-p-aminocinnamyloxycarbonyl;

p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl;

p-aminophenylpentadienyloxycarbonyl;

p-aminophenylpentadienyloxycarbonyl-p-arninocinnamyloxycarbonyl;

p-aminophenylpentadienyloxycarbonyl-paminobenzyloxycarbonyl;

p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl;

p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl;

p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl;

p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl;

p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl;

p-aminobenzyloxycarbonyl-p-arninocinnamyloxycarbonyl(methylamino)ethyl(methylamino)-carbonyl;

p-aminocinnamyloxycarbonyl-p-
aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino)carbonyl;
p-aminobenzyloxycarbonyl-p-aminobenzyl;
p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyl;
p-aminocinnamyl;
p-aminocinnamyloxycarbonyl-p-aminobenzyl;
p-aminobenzyloxycarbonyl-p-aminocinnamyl;
p-amino-cinnamyloxycarbonyl-p-aminocinnamyl;
p-aminophenylpentadienyl;
p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl;
p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl;
and
p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

[00283] In some embodiments, a **B7-H3-ADC** of the invention comprises two, three, four, five, six, seven, eight, nine or ten cytotoxic drug moieties, which may be the same, or may independently be the same or different from another cytotoxic drug moiety of the **B7-H3-ADC**. In one embodiment, each such cytotoxic drug moiety is conjugated to the **Ab** of the **B7-H3-ADC** of the invention via a separate Linker Molecule. Alternatively, more than one cytotoxic drug moiety may be attached to the **Ab** of the **B7-H3-ADC** of the invention via the same Linker Molecule.

[00284] Cytotoxic drug moieties may be conjugated to the **Ab** of the **B7-H3-ADC** of the invention by means known in the art (see, *e.g.*, Yao, H. *et al.* (2016) "*Methods to Design and Synthesize Antibody-Drug Conjugates (ADC)*," Intl. J. Molec. Sci. 17(194):1-16); Behrens, C. R. *et al.* (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies*," mAbs 6(1):46-53; Bouchard, H. *et al.* (2014) "*Antibody-Drug Conjugates – A New Wave Of Cancer Drugs*," Bioorganic & Medicinal Chem. Lett 24:5357-5363). The thiol group of a cysteine, the amino side group of lysine, glutamine or arginine, or the carboxyl group of glutamate or aspartate can be employed to conjugate the Linker Molecule-cytotoxic drug moiety (**LM-D**) to the **Ab** of the **B7-H3-ADC** of the invention. Native antibodies contain numerous lysine conjugation sites, and thus are capable of linking multiple conjugated molecules per antibody. Indeed, peptide mapping has determined that conjugation occurs on both the heavy and light chain at approximately 20 different lysine residues (40 lysines

per mAb). Therefore, greater than one million different ADC species can be generated. Cysteine conjugation occurs after reduction of one to four inter-chain disulfide bonds, and the conjugation is thus limited in native VL and VH Domains to the eight exposed sulfhydryl groups. However, if desired, additional reactive (*e.g.*, lysine, cysteine, selenocysteine, *etc.*) residues may be engineered into an antibody (*e.g.*, within a VL Domain and/or a VH Domain and/or a Constant Domain). For example, one or more native amino acid residues may be substituted with a cysteine residue. An unnatural amino acid (e.g. *p*-acetylphenylalanine) may be genetically incorporated into an antibody using an amber stop codon suppressor tRNA/aaRS pair. (See, *e.g.*, Behrens CR, and Liu B. (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies*," mAbs 6(1):46-53. doi:10.4161/mabs.26632; Panowksi, S., *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs, 6(1), 34–45, doi:10.4161/mabs.27022; and WO 2008/070593). Alternatively, or additionally, enzymes (*e.g.*, a glycotransferase) may be used to conjugate the Linker Molecule-cytotoxic drug moiety (**LM-D**) to the **Ab** of the **B7-H3-ADC** of the invention. The glycotransferase platform attaches a sugar moiety to a glycosylation site on an antibody (for example, position N297 of the Fc Domain of a human IgG antibody), which can then serve as the Linker Molecule of the present invention and conjugate the cytotoxic drug moiety (**D**) to the **Ab** of the **B7-H3-ADC** of the invention. Alternatively, a transglutaminase may be used to catalyze the formation of a covalent bond between a free amine group and a glutamine side chain.

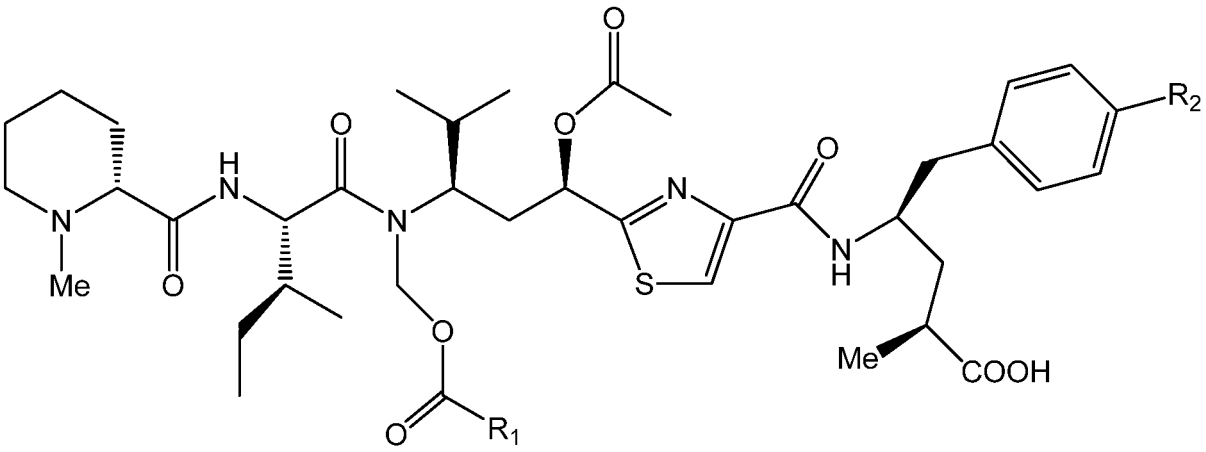
[00285] Preferred for such purpose is the commercially available transglutaminase from Streptoverticillium mobaraense (mTG) (Pasternack, R. *et al.* (1998) "*Bacterial Pro-Transglutaminase From Streptoverticillium mobaraense – Purification, Characterisation And Sequence Of The Zymogen*," Eur. J. Biochem. 257(3):570-576; Yokoyama, K. *et al.* (2004) "*Properties And Applications Of Microbial Transglutaminase*," Appl. Microbiol. Biotechnol. 64:447-454). This enzyme does not recognize any of the natural occurring glutamine residues in the Fc Domain of glycosylated antibodies, but does recognize the tetrapeptide LLQL (**SEQ ID NO:94**) (Jeger, S. et al. (2010) "*Site-Specific And Stoichiometric Modification Of Antibodies By Bacterial Transglutaminase*," Angew Chem. Int. Ed. Engl. 49:9995-9997) that may be engineered into a VL Domain and/or a VH Domain and/or a Constant Domain. Such considerations are reviewed by Panowski, S. *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs 6(1):34-45.

B. Exemplary Cytotoxic Drug Moieties of the Invention

[00286] In some embodiments, the cytotoxic drug moiety of the **B7-H3-ADC** of the invention comprises a cytotoxin, a radioisotope, an immunomodulator, a cytokine, a lymphokine, a chemokine, a growth factor, a tumor necrosis factor, a hormone, a hormone antagonist, an enzyme, an oligonucleotide, a DNA molecule, an RNA molecule, an siRNA molecule, an RNAi molecule, a microRNA molecule, a photoactive therapeutic agent, an anti-angiogenic agent, a pro-apoptotic agent, a peptide, a lipid, a carbohydrate, a chelating agent, or a combination thereof.

1. Tubulysin Cytotoxic Drug Moieties

[00287] The **B7-H3-ADC** of the invention may comprise a tubulysin cytotoxic drug moiety:



Tubulysin Derivative	R1	R2
Tubulysin A	$CH_2CH(CH_3)_2$	OH
Tubulysin B	$CH(CH_3)_2$	OH
Tubulysin C	CH_2CH_3	OH
Tubulysin D	$CH_2CH(CH_3)_2$	H

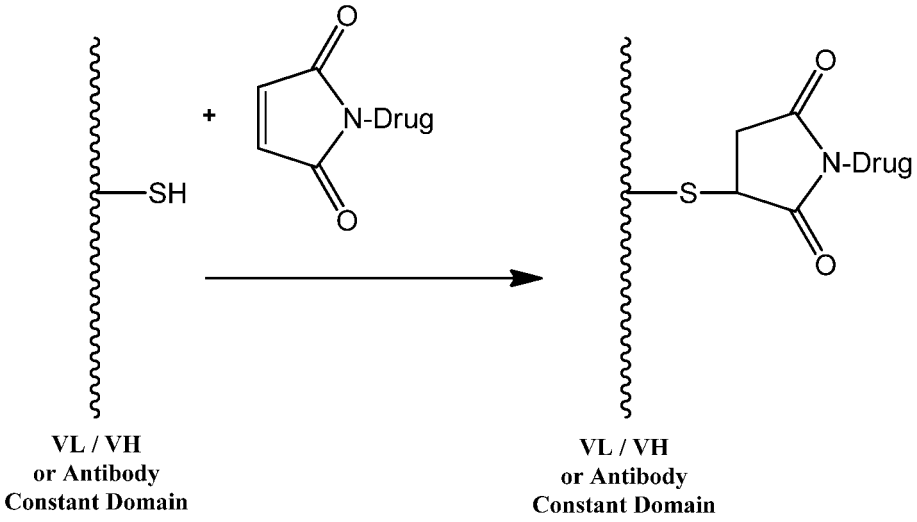
[00288] Tubulysins are members of a class of natural products isolated from myxobacterial species (Sasse *et al.* (2000) "*Tubulysins, New Cytostatic Peptides From Myxobacteria Acting On Microtubuli. Production, Isolation, Physico-Chemical And Biological Properties*," J. Antibiot. 53:879-885). As cytoskeleton interacting agents, tubulysins are mitotic poisons that inhibit tubulin polymerization and lead to cell cycle arrest and apoptosis (Steinmetz *et al.* (2004) "*Isolation, Crystal And Solution Structure*

Determination, And Biosynthesis Of Tubulysins--Powerful Inhibitors Of Tubulin Polymerization From Myxobacteria," Chem. Int. Ed. 43:4888-4892; Khalil *et al.* (2006) "*Mechanism Of Action Of Tubulysin, An Antimitotic Peptide From Myxobacteria*," ChemBioChem. 7:678-683; Kaur *et al.* (2006) "*Biological Evaluation Of Tubulysin A: A Potential Anticancer And Antiangiogenic Natural Product*," Biochem. J. 396: 235-242). Tubulysins are extremely potent cytotoxic molecules, exceeding the cell growth inhibition of any clinically relevant traditional chemotherapeutic, *e.g.*, epothilones, paclitaxel, and vinblastine. Furthermore, they are potent against multidrug resistant cell lines (Domling, A. *et al.* (2005) "*Myxobacterial Epothilones And Tubulysins As Promising Anticancer Agents*," Mol. Diversity 9:141-147). These compounds show high cytotoxicity tested against a panel of cancer cell lines with IC_{50} values in the low picomolar range; thus, they are of interest as anticancer therapeutics. See, *e.g.*, WO 2012/019123, WO 2015/157594. Tubulysin conjugates are disclosed, *e.g.*, in U.S. Patent No. 7,776,814. In some embodiments, the tubulysin molecule or derivative thereof is a prodrug.

2. Auristatin Cytotoxic Drug Moieties

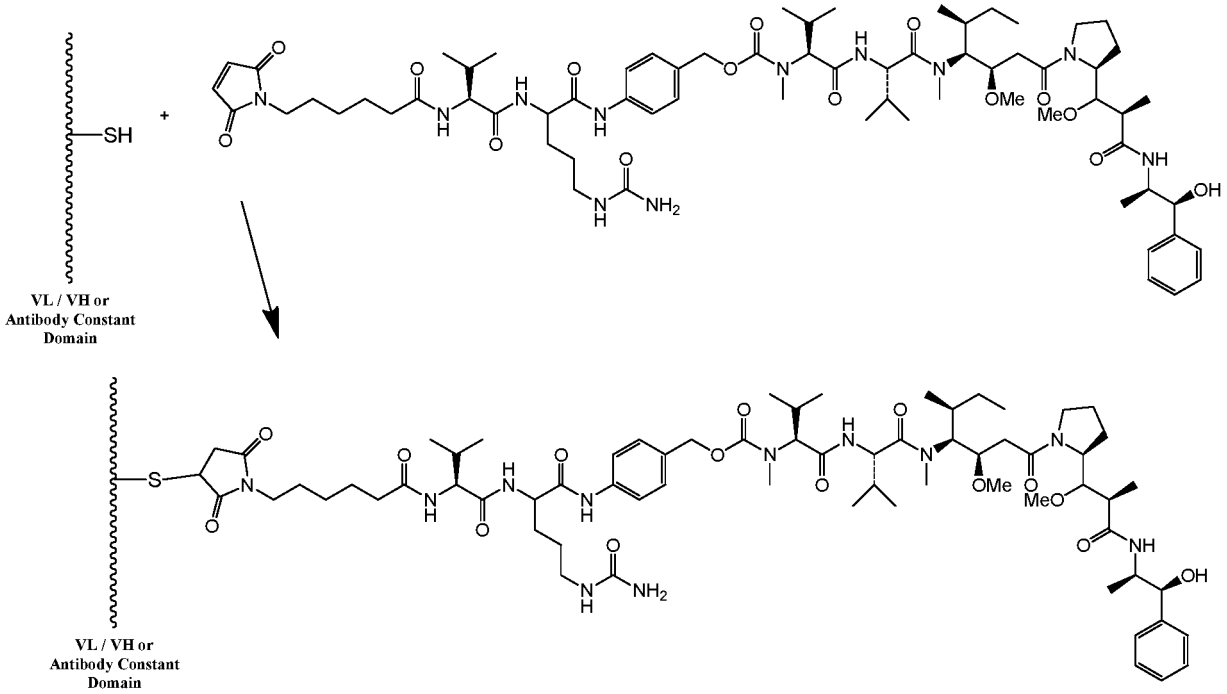
[00289] The **B7-H3-ADC** of the invention may alternatively or additionally comprise an auristatin cytotoxic drug moiety (*e.g.*, MMAE (N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine) and MMAF (N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine). Dolastatins were originally discovered as constituents of the sea hare Dolabella auricularia and have been modified to generate derivatives also known as auristatins (*e.g.*, monomethyl auristatin E and F). Dolastatins and auristatins interact with the Vinca alkaloid binding site on α-tubulin and block its polymerization. They have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke *et al.*, Antimicrob. Agents and Chemother. 45:3580-3584 (2001)) and have anticancer activity (U.S. Pat. Nos. 5,663,149, 6,884,869, 7,964,566). The auristatin drug moiety can be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (See, *e.g.*, WO 2002/088172). In some embodiments, the auristatine or dolastatine molecule, variant, or derivative thereof is a prodrug. MMAE may be conjugated to a protein via modification of native cysteine side chain thiols (Senter, P.D. *et al.* (2012) "*The Discovery And Development Of Brentuximab Vedotin For Use In Relapsed Hodgkin Lymphoma And Systemic Anaplastic Large Cell Lymphoma*," Nat. Biotechnol. 30:631-637; van de Donk, N.W. *et al.* (2012) "*Brentuximab vedotin*," MAbs 4:458-465). This method involves reduction of one or more solvent-exposed disulfide

bonds of cysteine residues with a reducing agent (*e.g.*, dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP)) followed by modification of the resulting thiols with a maleimide-containing drug (see, Behrens, C. R. *et al.* (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies*," mAbs 6(1):46-53).



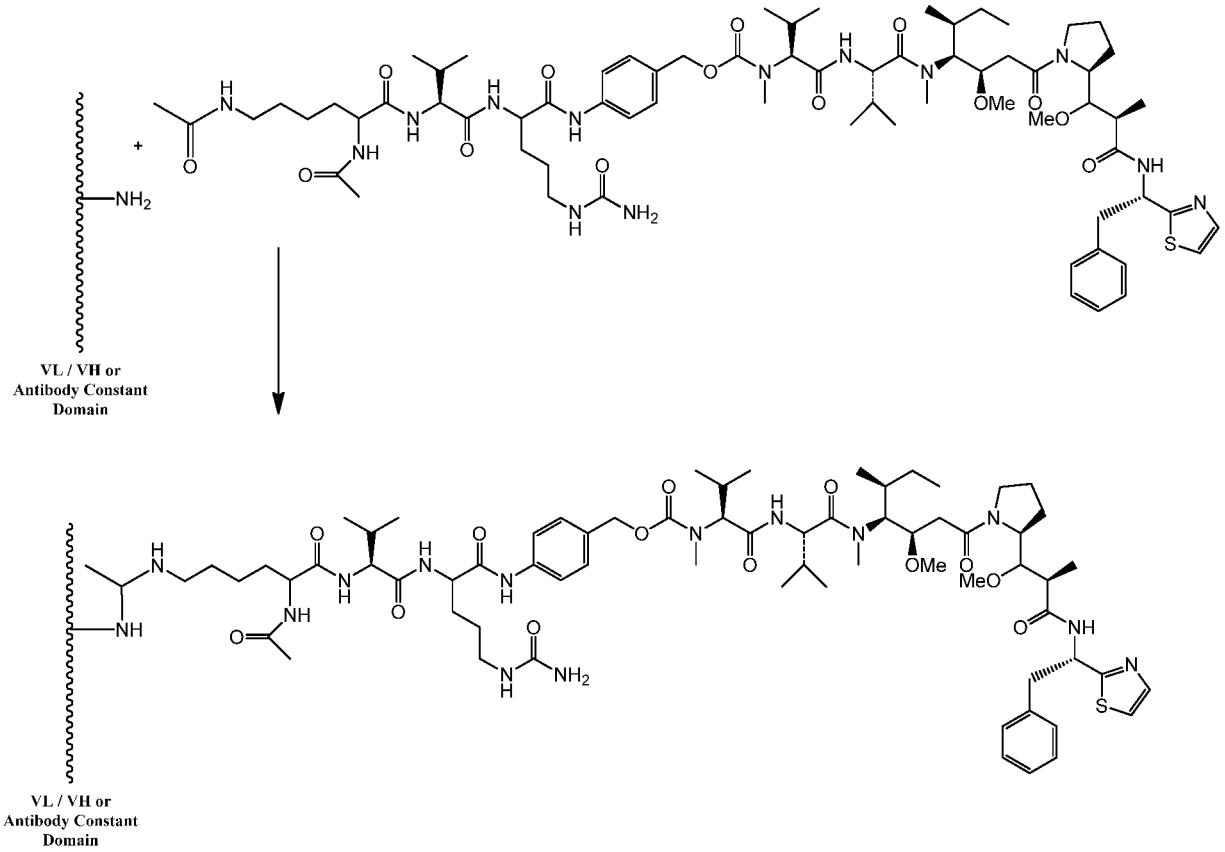
Scheme 1

[00290] An exemplary cytotoxic drug that may be conjugated in this manner incorporates a cathepsin B protease cleavage site25 (VC: valine, citrulline) and a self-immolative linker (PAB: para-aminobenzyloxycarbonyl) between the maleimide group (MC: maleimidocaproyl) and the cytotoxic drug (MMAE) (Doronina, S.O. *et al.* (2003) "*Development Of Potent Monoclonal Antibody Auristatin Conjugates For Cancer Therapy*," Nat. Biotechnol. 21:778-784).



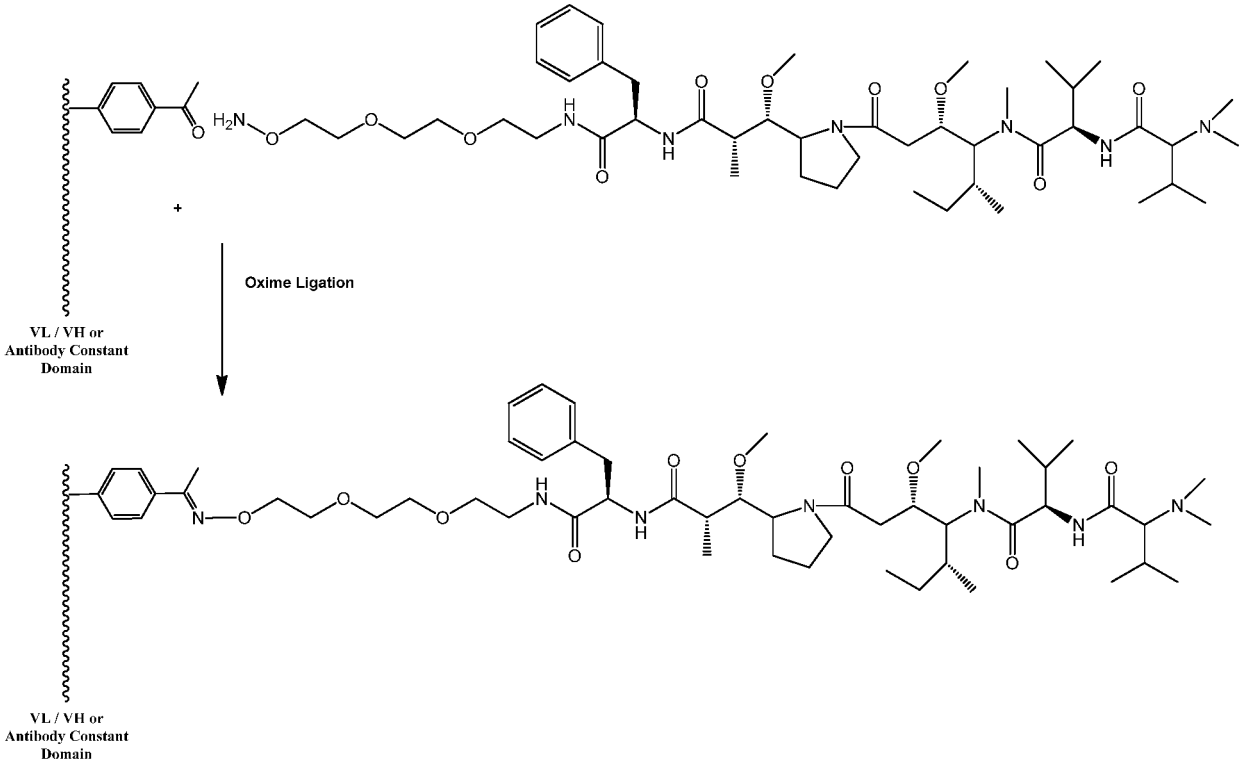
Scheme 2: Synthesis of MC-VC-PAB-MMAE

[00291] Alternatively, an auristatin cytotoxic drug moiety may be AcLys-VC-PAB-MMAD (acetyllysinevalinecitrulline-p-aminobenzyloxycarbonyl-monomethyldolastatin), which may be conjugated to an NH_2 side chain group of a glutamine residue of a VL Domain and/or a VH Domain and/or a Constant Domain of the **Ab** portion of the **B7-H3-ADC** of the invention using the enzyme microbial transglutaminase to catalyze the site-specific reaction between the side chain of the acetylated lysine residue and the glutamine side chains:



Scheme 3

[00292] Alternatively, p-acetylphenylalanine may be incorporated into a VL Domain and/or a VH Domain and/or a Constant Domain of the **Ab** portion of the **B7-H3-ADC** of the invention and then employed to conjugate auristatin F-oxyamine to such Domain via oxime ligation:



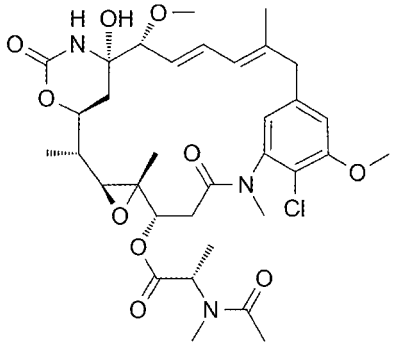
Scheme 4

3. Maytansinoid Cytotoxic Drug Moieties

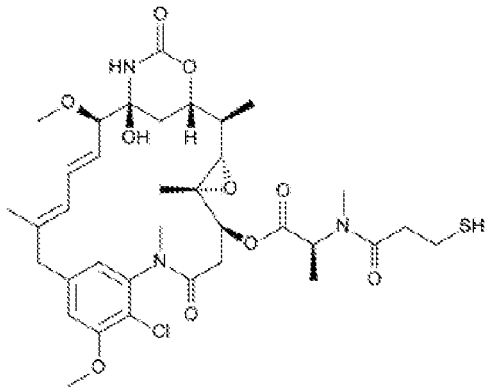
[00293] The **B7-H3-ADC** of the invention may alternatively or additionally comprise a maytansinoid cytotoxic drug moiety *e.g.*, an ansamycin antibiotic characterized by a 19-member ansamacrolide structure attached to a chlorinated benzene ring chromophore. Maytansinoids are mitotic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Patent No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Patent No. 4,151,042). Synthetic maytansinol and derivatives and analogues thereof are disclosed, for example, in U.S. Patents No. 4,137,230 and 4,248,870. Maytansinoid drug moieties are attractive drug moieties in antibody drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification, derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through the non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv)

effective against a variety of tumor cell lines. Immunoconjugates containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Patents No. 5,208,020 and 5,416,064 and European Patent EP 0425235B1; Liu, C. *et al.* (1996) "*Eradication Of Large Colon Tumor Xenografts By Targeted Delivery Of Maytansinoids*," Proc. Natl. Acad. Sci. (U.S.A.) 93:8618-8623 (described immunoconjugates comprising a maytansinoid designated DM1) and Chari, R.V. *et al.* (1992) "*Immunoconjugates Containing Novel Maytansinoids: Promising Anticancer Drugs*," Cancer Research 52:127-131.

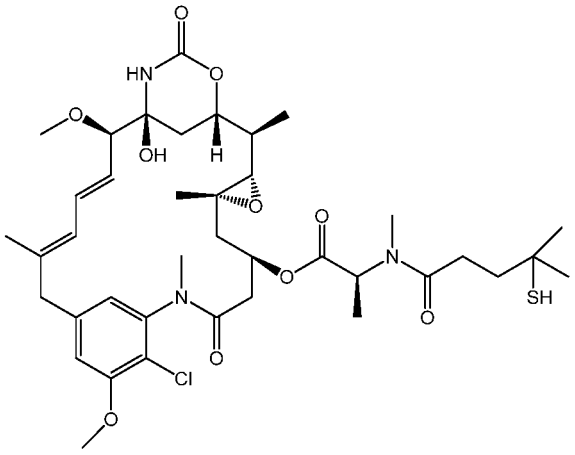
[00294] Maytansine, DM1 and DM4 are exemplary maytansinoid cytotoxic drug moieties:



Maytansine



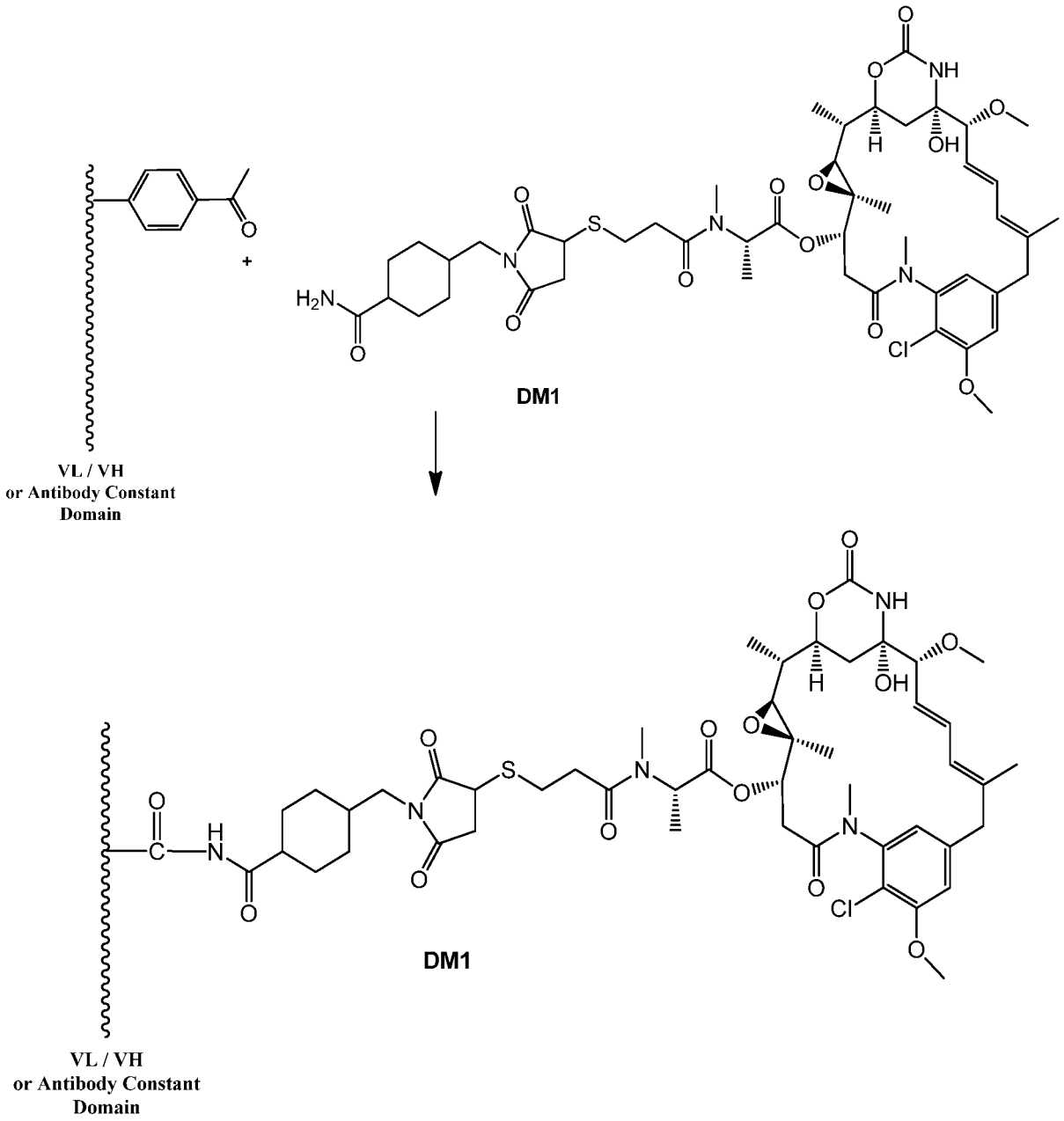
DM1



DM4

[00295] Maytansine may be conjugated to the **Ab** portion of the **B7-H3-ADC** of the invention by reaction with a lysine or glutamine side chain. DM1 and DM4 may be conjugated to a COOH side chain of a glutamate or aspartate residue of a VL Domain and/or a VH Domain and/or a Constant Domain of the **Ab** portion of the **B7-H3-ADC** of the

invention (see, Behrens, C. R. *et al.* (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies*," mAbs 6(1):46-53; Bouchard, H. *et al.* (2014) "*Antibody-Drug Conjugates – A New Wave Of Cancer Drugs*," Bioorganic & Medicinal Chem. Lett 24:5357-5363):



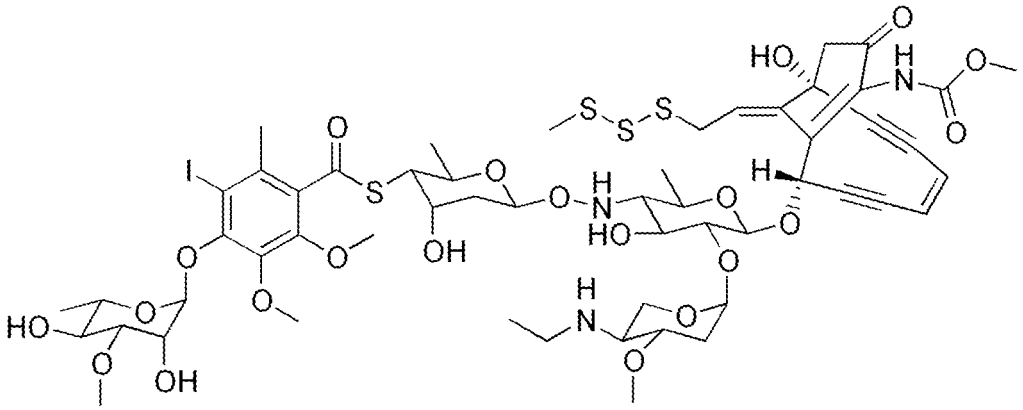
Scheme 5

[00296] Trastuzumab emtansine (ado-trastuzumab emtansine, T-DM1, trade name KADCYLA®) is an antibody-drug conjugate consisting of the monoclonal antibody trastuzumab (HERCEPTIN®) conjugated to the maytansinoid mertansine (DM1). See, *e.g.*, LoRusso *et al.* (2011) "*Trastuzumab Emtansine: A Unique Antibody-Drug Conjugate In Development For Human Epidermal Growth Factor Receptor 2-Positive Cancer*," Clin. Cancer Res. 20:6437-6447. An engineered thio-Trastuzumab-DM1 ADC has also been

described in Junutual *et al.* (2010) "*Engineered Thio-Trastuzumab-DM1 Conjugate With An Improved Therapeutic Index To Target Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer*," Clin, Cancer Res. 16:4769-4778. In some embodiments, the maytansinoid molecule, variant, or derivative thereof is a prodrug.

4. Calicheamicin Cytotoxic Drug Moieties

[00297] The **B7-H3-ADC** of the invention may alternatively or additionally comprise a calicheamicin cytotoxic drug moiety:



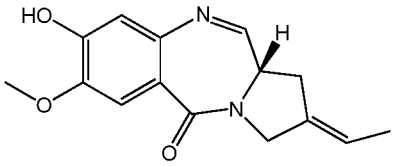
[00298] The described calicheamicin-based antibody conjugates are disulfide versions of the trisulfide parent compound. Two coupling strategies with N-acetyl-c-calicheamicin dimethyl hydrazide (CalichDMH) have been reported to-date: (i) hydrazide; and (ii) amide coupling (Bouchard, H. *et al.* (2014) "*Antibody-Drug Conjugates – A New Wave Of Cancer Drugs*," Bioorganic & Medicinal Chem. Lett 24:5357-5363).

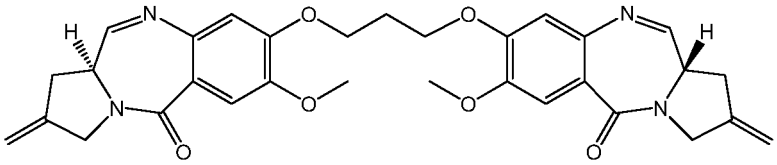
[00299] The calicheamicin family of enediyne antitumor antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. The calicheamicins are a class of enediyne antibiotics derived from the bacterium *Micromonospora echinospora*, with calicheamicin γ1 being the most notable. Other calicheamicins are β1Br, γ1Br, α2I, α3I, β1I, γ1I, and Δ1I (*see* Lee, M.D. *et al.* (1989) "*Calicheamicins, A Novel Family Of Antitumor Antibiotics. 3. Isolation, Purification And Characterization Of Calicheamicins Beta 1Br, Gamma 1Br, Alpha 2I, Alpha 3I, Beta 1I, Gamma 1I And Delta 1I.*," J. Antibiotics 42(7):1070-1087). For the preparation of conjugates of the calicheamicin family, see U.S. Patents No. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001 and 5,877,296. Structural analogues of calicheamicin which can be used include, but are not limited to, γ1I, α2I, α3I, N-acetyl-γ1I, PSAG and θ11 (Hinman *et al.* (1993) "*Preparation And Characterization Of*

Monoclonal Antibody Conjugates Of The Calicheamicins: A Novel And Potent Family Of Antitumor Antibiotics," Cancer Research 53:3336-3342 (1993), Lode *et al.* (1998) "*Targeted Therapy With A Novel Enediyene Antibiotic Calicheamicin Theta(I)1 Effectively Suppresses Growth And Dissemination Of Liver Metastases In A Syngeneic Model Of Murine Neuroblastoma*," Cancer Research 58:2925-2928 (1998). In some embodiments, the calicheamicin molecule, variant, or derivative thereof is a prodrug.

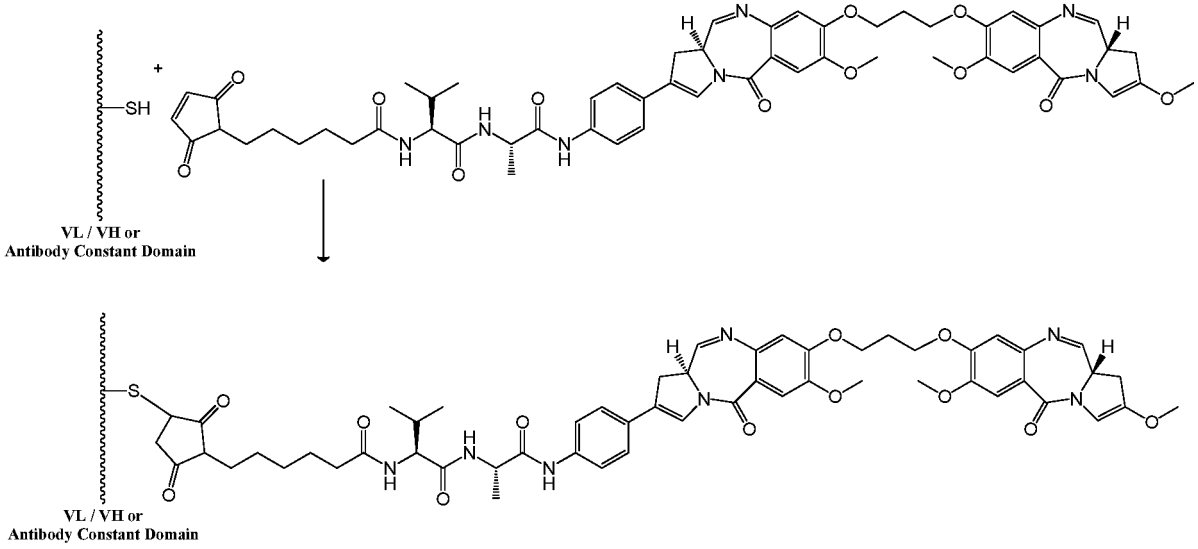
5. Pyrrolobenzodiazepine Cytotoxic Drug Moieties

[00300] The **B7-H3-ADC** of the invention may comprise alternatively or additionally a pyrrolobenzodiazepine drug moiety (*e.g.*, natural pyrrolobenzodiazepine and SJG-136, a derivative thereof):





[00301] A preferred pyrrolobenzodiazepine drug moiety is vadastuximab talirine (SGN-CD33A; Seattle Genetics):



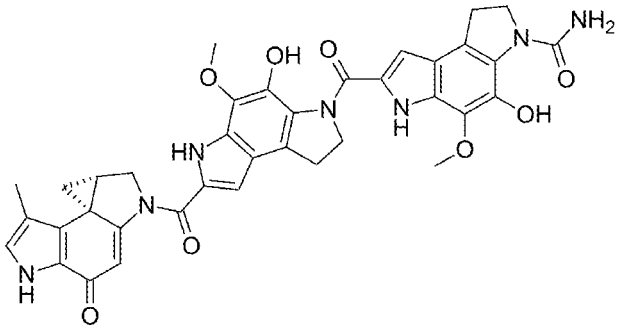
Scheme 6

[00302] Pyrrolobenzodiazepines (PBD) are a class of natural products with antibiotic or anti-tumor activity. They are naturally produced by actinomycetes. They are DNA alkylating compound and some are sequence-selective. A number of PBDs and derivatives thereof are known in the art, for example, PBD dimers (e.g., SJG-136 or SG2000), C2-unsaturated PBD dimers, pyrrolobenzodiazepine dimers bearing C2 aryl substitutions (e.g., SG2285), PBD dimer pro-drug that is activated by hydrolysis (e.g., SG2285), and polypyrrole-PBD (e.g., SG2274). PBDs are further described WO 2000/012507, WO 2007/039752, WO 2005/110423, WO 2005/085251, and WO 2005/040170, and WO 2014/057119. In some embodiments, the PBD molecule, variant, or derivative thereof is a prodrug.

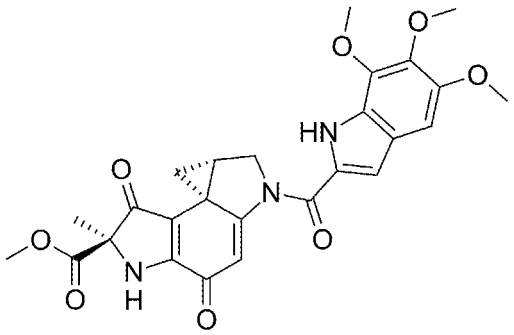
6. Duocarmycin Cytotoxic Drug Moieties

[00303] The **B7-H3-ADC** of the invention may alternatively or additionally comprise a duocarmycin drug moiety. Duocarmycins are members of a series of related natural products first isolated from Streptomyces bacteria and they are potent antitumor antibiotics (*see* Dokter, W. *et al.* (2014) "*Preclinical Profile of the HER2-Targeting ADC SYD983/SYD985: Introduction of a New Duocarmycin-Based Linker-Drug Platform*," Mol. Cancer Ther. 13(11):2618-2629; Boger, D.L. *et al.* (1991). "*Duocarmycins - A New Class Of Sequence Selective DNA Minor Groove Alkylating Agents*," Chemtracts: Organic Chemistry 4 (5): 329-349 (1991); Tercel *et al.* (2013) "*The Cytotoxicity Of Duocarmycin Analogues Is Mediated Through Alkylation Of DNA, Not Aldehyde Dehydrogenase 1: A Comment*," Chem. Int. Ed. Engl. 52(21):5442-5446; Boger, D.L. *et al.* (1995) "*CC-1065 And The Duocarmycins: Unraveling The Keys To A New Class Of Naturally Derived DNA Alkylating Agents*," Proc. Natl. Acad. Sci. (U.S.A.) 92(9):3642-3649; Cacciari, B. *et al.* (2000) "*CC-1065 And The Duocarmycins: Recent Developments*," Expert Opinion on Therapeutic Patents 10(12):1853-1871).

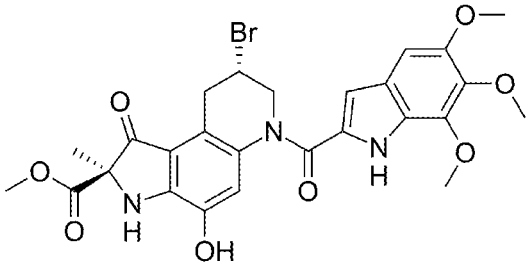
[00304] Natural duocarmycins include duocarmycin A, duocarmycin B1, doucarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, and CC-1065 (PCT Publication No. WO 2010/062171; Martin, D.G. *et al.* (1980) "*Structure Of CC-1065 (NSC 298223), A New Antitumor Antibiotic*," J. Antibiotics 33:902-903; Boger, D.L. *et al.* (1995) "*CC-1065 And The Duocarmycins: Unraveling The Keys To A New Class Of Naturally Derived DNA Alkylating Agents*," Proc. Natl. Acad. Sci. (U.S.A.) 92:3642-3649).



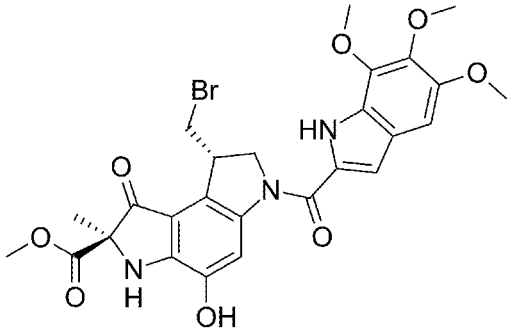
CC-1065



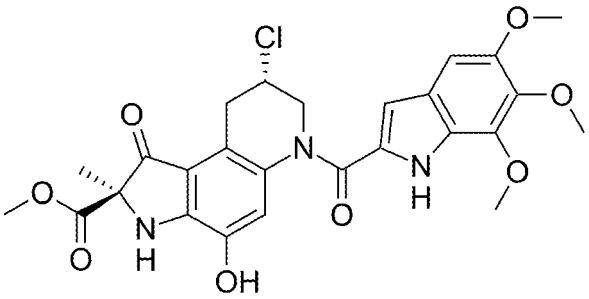
Duocarmycin A



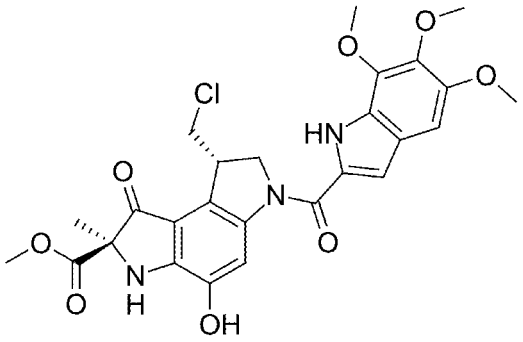
Duocarmycin B1



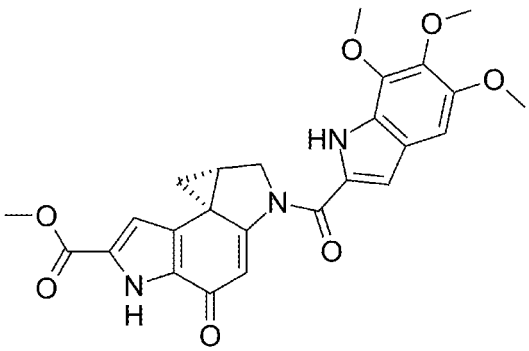
Duocarmycin B2



Duocarmycin C1

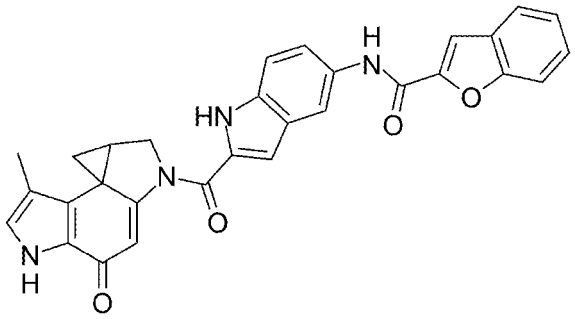


Duocarmycin C2

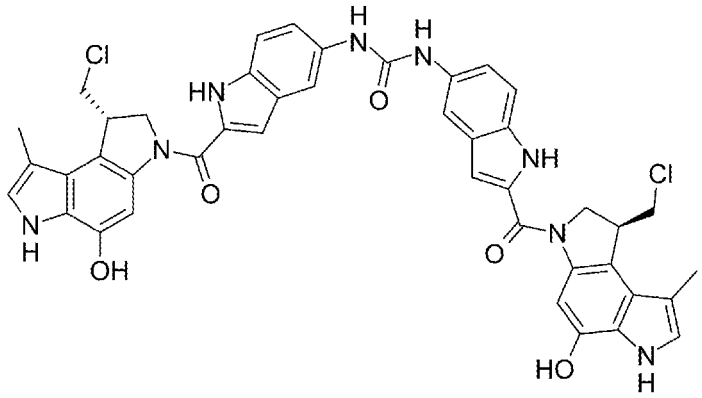


Duocarmycin SA

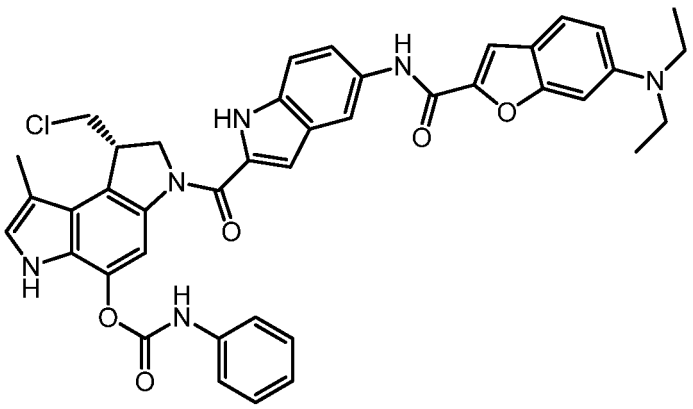
[00305] Suitable synthetic duocarmycin analogs include adozelesin, bizelesin, carzelesin (U-80244) and spiro-duocarmycin (DUBA) (Dokter, W. *et al.* (2014) "*Preclinical Profile of the HER2-Targeting ADC SYD983/SYD985: Introduction of a New Duocarmycin-Based Linker-Drug Platform*," Mol. Cancer Ther. 13(11):2618-2629; Elgersma, R.C. *et al.* (2014) "*Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody–Drug Conjugate SYD985*," Mol. Pharmaceut. 12:1813-1835):



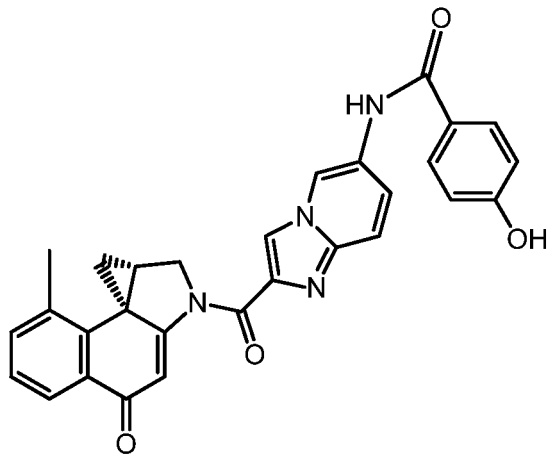
Adozelesin



Bizelesin

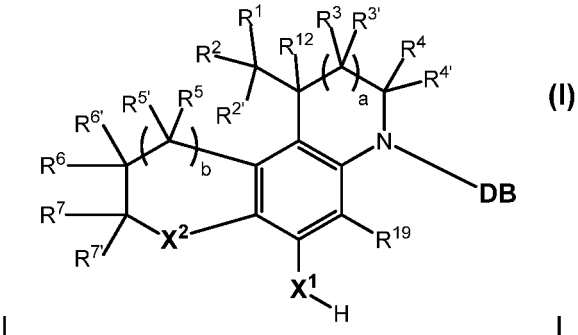


Carzelesin

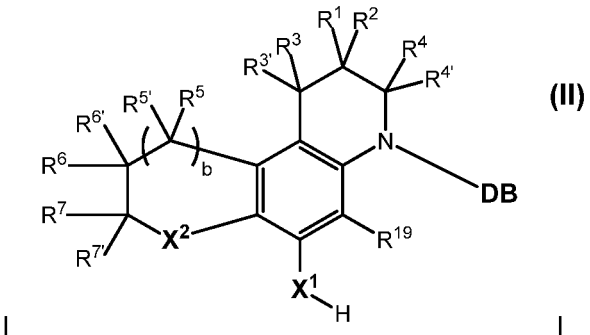


DUBA (*spiro*-duocarmycin)

[00306] Additional synthetic duocarmycin analogs include those disclosed in PCT Publication No. WO 2010/062171, and particularly such analogs that have the formula:



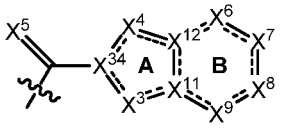
(I)



(II)

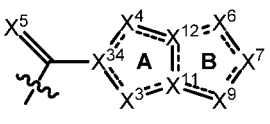
DA1 DA2

or a pharmaceutically acceptable salt, hydrate, or solvate thereof, wherein **DB** is a DNA-binding moiety and is selected from the group consisting of:



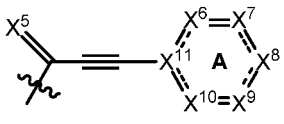
DB1

and

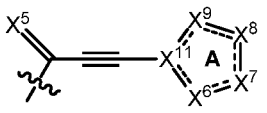


DB2

and

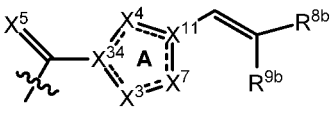


DB3



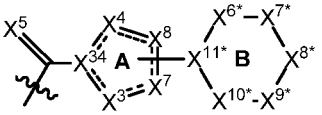
DB4

and

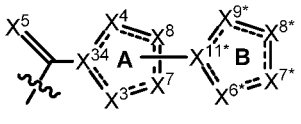


DB5

and

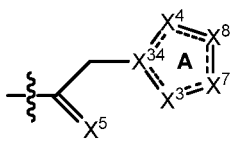


DB6



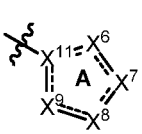
DB7

and



DB8

and



DB9

wherein:

R is a leaving group;

R^2, $R^{2'}$, R^3, $R^{3'}$, R^4, $R^{4'}$, R^{12}, and R^{19} are independently selected from H, OH, SH, NH_2, N_3, NO_2, NO, CF_3, CN, $C(O)NH_2$, C(O)H, C(O)OH, halogen, Ra, SR^a, $S(O)R^a$, $S(O)2R^a$, $S(O)OR^a$, $S(O)_2OR^a$, $OS(O)R^a$, $OS(O)_2R^a$, $OS(O)OR^a$, $OS(O)_2OR^a$, OR^a, NHR^a, $N(R^a)R^b$, $+N(R^a)(R^b)R^c$, $P(O)(OR^a)(OR^b)$, $OP(O)(OR^a)(OR^b)$, $SiR^aR^bR^c$, $C(O)R^a$, $C(O)OR^a$, $C(O)N(R^a)R^b$, $OC(O)R^a$, $OC(O)OR^a$, $OC(O)N(R^a)R^b$, $N(R^a)C(O)R^b$, $N(R^a)C(O)OR^b$, and $N(R^a)C(O)N(R^b)R^c$, wherein R^a, R^b, and R^c are independently selected from H and optionally substituted C_{1-3} alkyl or C_{1-3} heteroalkyl, or R^3 + $R^{3'}$ and/or R^4 + $R^{4'}$ are independently selected from =O, =S, $=NOR^{18}$, $=C(R^{18})R^{18'}$, and $=NR^{18}$, R^{18} and $R^{18'}$ being independently selected from H and optionally substituted C_{1-3} alkyl, two or more of R^2, $R^{2'}$, R^3, $R^{3'}$, R^4, $R^{4'}$ and R^{12} optionally being joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles;

X^2 is selected from O, $C(R^{14})(R^{14'})$, and $NR^{14'}$, wherein R^{14} and $R^{14'}$ have the same meaning as defined for R^7 and are independently selected, or $R^{14'}$ and $R^{7'}$ are absent resulting in a double bond between the atoms designated to bear $R^{7'}$ and $R^{14'}$;

R^5, $R^{5'}$, R^6, $R^{6'}$, R^7, and $R^{7'}$ are independently selected from H, OH, SH, NH_2, N_3, NO_2, NO, CF_3, CN, $C(O)NH_2$, C(O)H, C(O)OH, halogen, R^e, SR^e, $S(O)R^e$, $S(O)_2R^e$, $S(O)OR^e$, $S(O)_2OR^e$, $OS(O)R^e$, $OS(O)_2R^e$, $OS(O)OR^e$, $OS(O)_2OR^e$, OR^e, NHR^e, $N(R^e)R^f$, $^+N(R^e)(R^f)R^g$, $P(O)(OR^e)(OR^f)$, $OP(O)(OR^e)(OR^f)$, $SiR^eR^fR^g$, $C(O)R^e$, $C(O)OR^e$, $C(O)N(R^e)R^f$, $OC(O)R^e$, $OC(O)OR^e$, $OC(O)N(R^e)R^f$, $N(R^e)C(O)R^f$, $N(R^e)C(O)OR^f$, $N(R^e)C(O)N(R^f)R^g$, and a water-soluble group,

wherein

R^e, R^f, and R^g are independently selected from H and optionally substituted $(CH_2CH_2O)_{ee}CH_2CH_2\mathbf{X^{13}}R^{e1}$, C_{1-15} alkyl, C_{1-15} heteroalkyl, C_{3-15} cycloalkyl, C_{1-15} heterocycloalkyl, C_{5-15} aryl, or C_{1-15} heteroaryl, wherein ee is selected from 1 to 1000, $\mathbf{X^{13}}$ is selected from O, S, and NR^{f1}, and R^{f1} and R^{e1} are independently selected from H and C_{1-3} alkyl, one or more of the optional substituents in R^e, R^f, and/or R^g optionally being a water-soluble group, two or more of R^e, R^f, and R^g optionally being joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles,

or R^5 + $R^{5'}$ and/or R^6 + $R^{6'}$ and/or R^7 + $R^{7'}$ are independently selected from =O, =S, $=NOR^{e3}$, $=C(R^{e3})R^{e4}$, and $=NR^{e3}$, R^{e3} and R^{e4} being independently selected from H and optionally substituted C_{1-3} alkyl, or $R^{5'}$ + $R^{6'}$ and/or $R^{6'}$ + $R^{7'}$ and/or $R^{7'}$ + $R^{14'}$ are absent, resulting in a double bond between the atoms designated to bear $R^{5'}$ + $R^{6'}$ and/or $R^{6'}$ + $R^{7'}$ and/or $R^{7'}$ + $R^{14'}$ respectively, two or more of R^5, $R^{5'}$, R^6, $R^{6'}$, R^7, $R^{7'}$, R^{14} and $R^{14'}$ optionally being joined by

one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles;

$\mathbf{X^1}$ is selected from O, S, and NR, wherein R is selected from H and optionally substituted C_{1-8} alkyl or C_{1-8} heteroalkyl and not joined with any other substituent;

$\mathbf{X^3}$ is selected from O, S, $C(R^{15})R^{15'}$, $-C(R^{15})(R^{15'})-C(R^{15''})(R^{15'''})-$, $-N(R^{15})-N(R^{15'})-$, $-C(R^{15})(R^{15'})-N(R^{15''})-$, $-N(R^{15''})-C(R^{15})(R^{15'})-$, $-C(R^{15})(R^{15'})-O-$, $-O-C(R^{15})(R^{15'})-$, $-C(R^{15})(R^{15'})-S-$, $-S-C(R^{15})(R^{15'})-$, $-C(R^{15})=C(R^{15'})-$, $=C(R^{15})-C(R^{15'})=$, $-N=C(R^{15'})-$, $=N-C(R^{15'})=$, $-C(R^{15})=N-$, $=C(R^{15})-N=$, $-N=N-$, $=N-N=$, CR^{15}, N, NR^{15}, or in **DB1** and **DB2-X3-** represents $-\mathbf{X^{3a}}$ and $\mathbf{X^{3b}}-$, wherein $\mathbf{X^{3a}}$ is connected to $\mathbf{X^{34}}$, a double bond is present between $\mathbf{X^{34}}$ and $\mathbf{X^4}$, and $\mathbf{X^{3b}}$ is connected to $\mathbf{X^{11}}$, wherein $\mathbf{X^{3a}}$ is independently selected from H and optionally substituted $(CH_2CH_2O)_{ee}CH_2CH_2\mathbf{X^{13}}R^{e1}$, C_{1-8} alkyl, or C_{1-8} heteroalkyl and not joined with any other substituent;

$\mathbf{X^4}$ is selected from O, S, $C(R^{16})R^{16'}$, NR^{16}, N, and CR^{16};

$\mathbf{X^5}$ is selected from O, S, $C(R^{17})R^{17'}$, NOR^{17}, and NR^{17}, wherein R^{17} and $R^{17'}$ are independently selected from H and optionally substituted C_{1-8} alkyl or C_{1-8} heteroalkyl and not joined with any other substituent;

$\mathbf{X^6}$ is selected from CR^{11}, $CR^{11}(R^{11'})$, N, NR^{11}, O, and S;

$\mathbf{X^7}$ is selected from CR^{8}, $CR^{8}(R^{8'})$, N, NR^{8}, O, and S;

$\mathbf{X^8}$ is selected from CR^{9}, $CR^{9}(R^{9'})$, N, NR^{9}, O, and S;

$\mathbf{X^9}$ is selected from CR^{10}, $CR^{10}(R^{10'})$, N, NR^{10}, O, and S;

$\mathbf{X^{10}}$ is selected from CR^{20}, $CR^{20}(R^{20'})$, N, NR^{20}, O, and S;

$\mathbf{X^{11}}$ is selected from C, CR^{21}, and N, or $\mathbf{X^{11}}$-$\mathbf{X^{3b}}$ is selected from CR^{21}, $CR^{21}(R^{21'})$, N, NR^{21}, O, and S;

$\mathbf{X^{12}}$ is selected from C, CR^{22}, and N;

$\mathbf{X^{6*}}$, $\mathbf{X^{7*}}$, $\mathbf{X^{8*}}$, $\mathbf{X^{9*}}$, $\mathbf{X^{10*}}$, and $\mathbf{X^{11*}}$ have the same meaning as defined for $\mathbf{X^6}$, $\mathbf{X^7}$, $\mathbf{X^8}$, $\mathbf{X^9}$, $\mathbf{X^{10}}$, and $\mathbf{X^{11}}$, respectively, and are independently selected;

$\mathbf{X^{34}}$ is selected from C, CR^{23}, and N;

the ring **B** atom of $\mathbf{X^{11*}}$ in **DB6** and **DB7** is connected to a ring atom of ring **A** such that ring **A** and ring **B** in **DB6** and **DB7** are directly connected via a single bond;

a dashed double bond means that the indicated bond may be a single bond or a non-cumulated, optionally delocalized, double bond;

R^8, $R^{8'}$, R^9, $R^{9'}$, R^{10}, $R^{10'}$, R^{11}, $R^{11'}$, R^{15}, $R^{15'}$, $R^{15''}$, $R^{15''}$, R^{16}, $R^{16'}$, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{22}, and R^{23} are each independently selected from H, OH, SH, NH_2, N_3, NO_2, NO, CF_3, CN, $C(O)NH_2$, C(O)H, C(O)OH, halogen, R^h, SR^h, $S(O)R^h$, $S(O)_2R^h$, $S(O)OR^h$, $S(O)_2OR^h$,

$OS(O)R^h$, $OS(O)_2R^h$, $OS(O)OR^h$, $OS(O)_2OR^h$, OR^h, NHR^h, $N(R^h)R^i$, $^+N(R^h)(R^i)R^j$, $P(O)(OR^h)(OR^i)$, $OP(O)(OR^h)(OR^i)$, $SiR^hR^iR^j$, $C(O)R^h$, $C(O)OR^h$, $C(O)N(R^h)R^i$, $OC(O)R^h$, $OC(O)OR^h$, $OC(O)N(R^h)R^i$, $N(R^h)C(O)R^i$, $N(R^h)C(O)OR^i$, $N(R^h)C(O)N(R^i)R^j$, and a water-soluble group, wherein

R^h, R^i, and R^j are independently selected from H and optionally substituted $(CH_2CH_2O)_{ee}CH_2CH_2\mathbf{X^{13}}R^{e1}$, C_{1-15} alkyl, C_{1-15} heteroalkyl, C_{3-15} cycloalkyl, C_{1-15} heterocycloalkyl, C_{5-15} aryl, or C_{1-15} heteroaryl, one or more of the optional substituents in R^h, R^i, and/or R^j optionally being a water-soluble group, two or more of R^h, R^i, and R^j optionally being joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles,

or $R^8 + R^{8'}$ and/or $R^9 + R^{9'}$ and/or $R^{10} + R^{10'}$ and/or $R^{11} + R^{11'}$ and/or $R^{15} + R^{15'}$ and/or $R^{15''} + R^{15'''}$ and/or $R^{16} + R^{16'}$ and/or $R^{20} + R^{20'}$ and/or $R^{21} + R^{21'}$ are independently selected from =0, =S, $=NOR^{h1}$, $= C(R^{hl})R^{h2}$, and $=NR^{hl}$, R^{hl} and R^{h2} being independently selected from H and optionally substituted C_{1-3} alkyl, two or more of R^8, $R^{8'}$, R^9, $R^{9'}$, R^{10}, $R^{10'}$, R^{11}, $R^{11'}$, R^{15}, $R^{15'}$, $R^{15''}$, $R^{15'''}$, R^{16}, R^{20}, $R^{20'}$, R^{21}, $R^{21'}$, R^{22}, and R^{23} optionally being joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles;

R^{8b} and R^{9b} are independently selected and have the same meaning as R^8, except that they may not be joined with any other substituent;

one of R^4 and $R^{4'}$ and one of R^{16} and $R^{16'}$ may optionally be joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles;

one of R^4 and $R^{4'}$ and one of R^{16} and $R^{16'}$ may optionally be joined by

one of R^2, $R^{2'}$, R^3, and $R^{3'}$ and one of R^5 and $R^{5'}$ may optionally be joined by one or more bonds to form one or more optionally substituted carbocycles and/or heterocycles;

a and b are independently selected from 0 and 1;

the **DB** moiety does not comprise a **DA1**, **DA2**, **DA1'**, or **DA2'** moiety;

ring **B** in **DB1** is a heterocycle;

if $\mathbf{X^3}$ in **DB1** represents $-\mathbf{X^{3a}}$ and $\mathbf{X^{3b}}-$ and ring **B** is aromatic, then two vicinal substituents on said ring **B** are joined to form an optionally substituted carbocycle or heterocycle fused to said ring **B**;

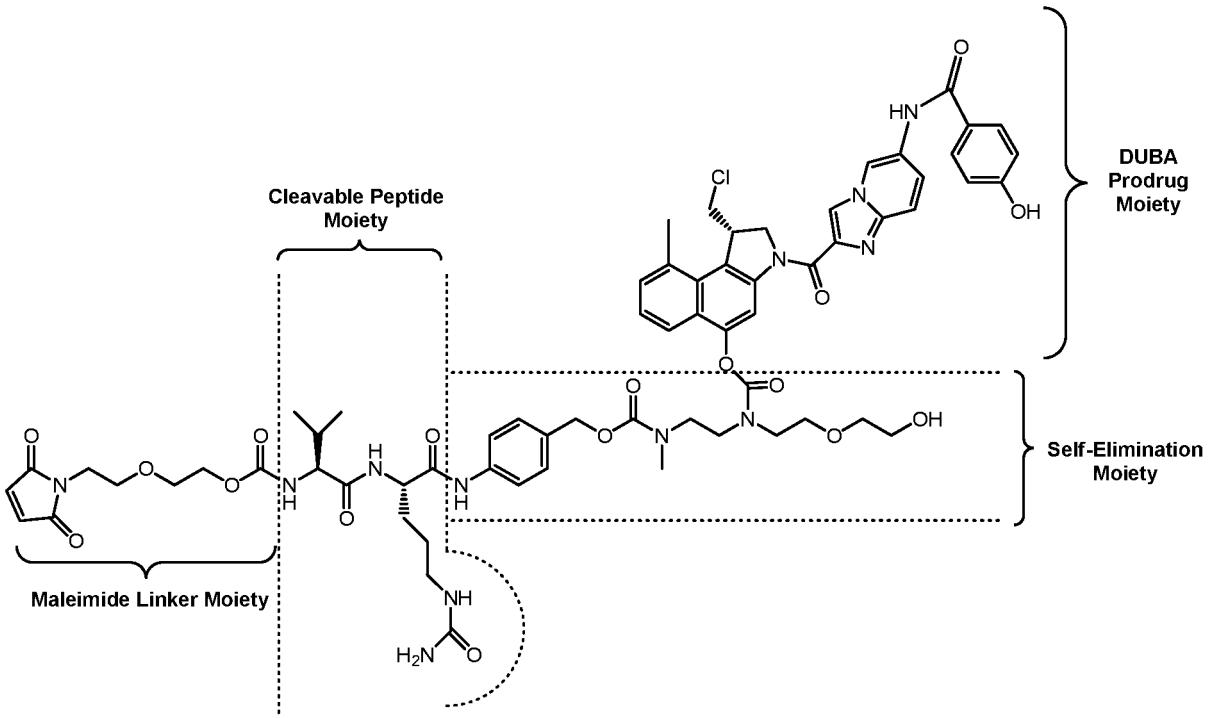
if $\mathbf{X^3}$ in **DB2** represents $-\mathbf{X^{3a}}$ and $\mathbf{X^{3b}}-$ and ring **B** is aromatic, then two vicinal substituents on said ring **B** are joined to form an optionally substituted heterocycle fused to said ring **B**, an optionally substituted non-aromatic carbocycle fused to said ring **B**, or a substituted aromatic carbocycle which is fused to said ring **B** and to which at least one substituent is attached that contains a hydroxy group, a primary amino group, or a secondary amino group,

the primary or secondary amine not being a ring atom in an aromatic ring system nor being part of an amide;

if ring **A** in **DB2** is a 6-membered aromatic ring, then substituents on ring **B** are not joined to form a ring fused to ring **B**;

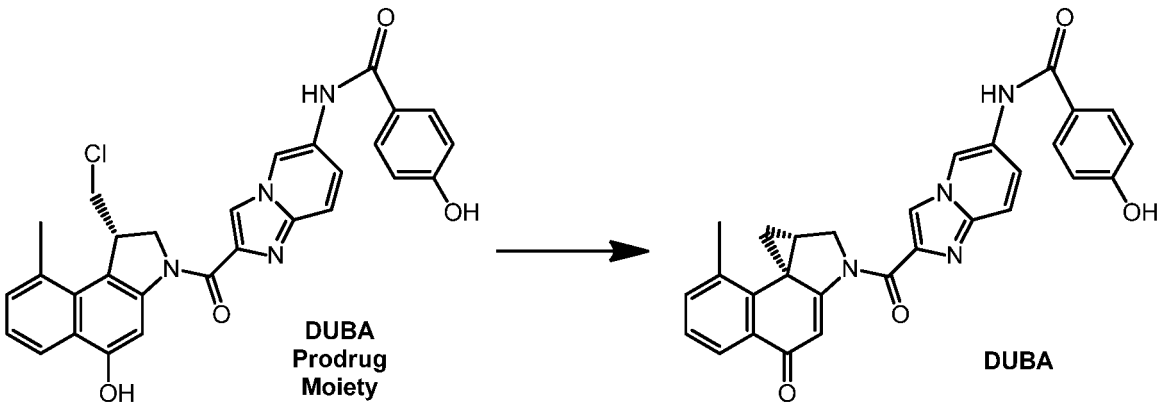
two vicinal substituents on ring **A** in **DB8** are joined to form an optionally substituted carbocycle or heterocycle fused to said ring **A** to form a bicyclic moiety to which no further rings are fused; and ring **A** in **DB9** together with any rings fused to said ring **A** contains at least two ring heteroatoms.

[00307] The above-described Linker Molecules can be conjugated to a cysteine thiol group using thiol-maleimide chemistry, as shown above. In some embodiments, the cytotoxic duocarmycin drug moiety is a prodrug. For example, the prodrug, vc-*seco*-DUBA can be conjugated to a self-elimination moiety linked to maleimide linker moiety via a cleavable peptide moiety:



Scheme 7

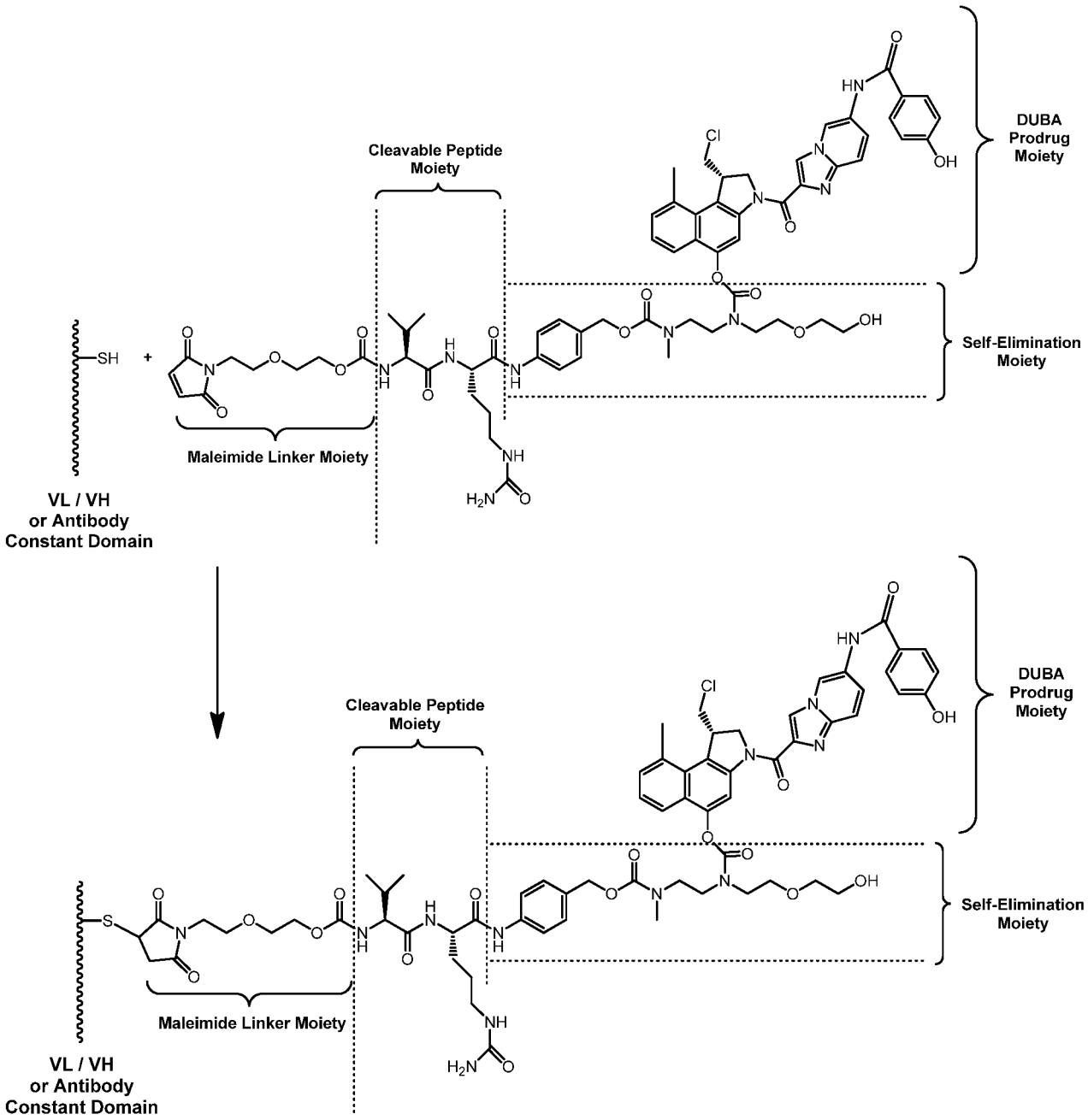
[00308] The maleimide linker moiety of the molecule can be conjugated to a thiol group of a cysteine residue of a VL Domain and/or a VH Domain and/or a Constant Domain of the **Ab** portion of the **B7-H3-ADC** of the invention. Subsequent proteolytic cleavage of the cleavable peptide moiety is followed by the spontaneous elimination of the self-elimination moiety, leading to the release of *seco*-DUBA, which spontaneously rearranges to form the active drug, DUBA:



Scheme 8

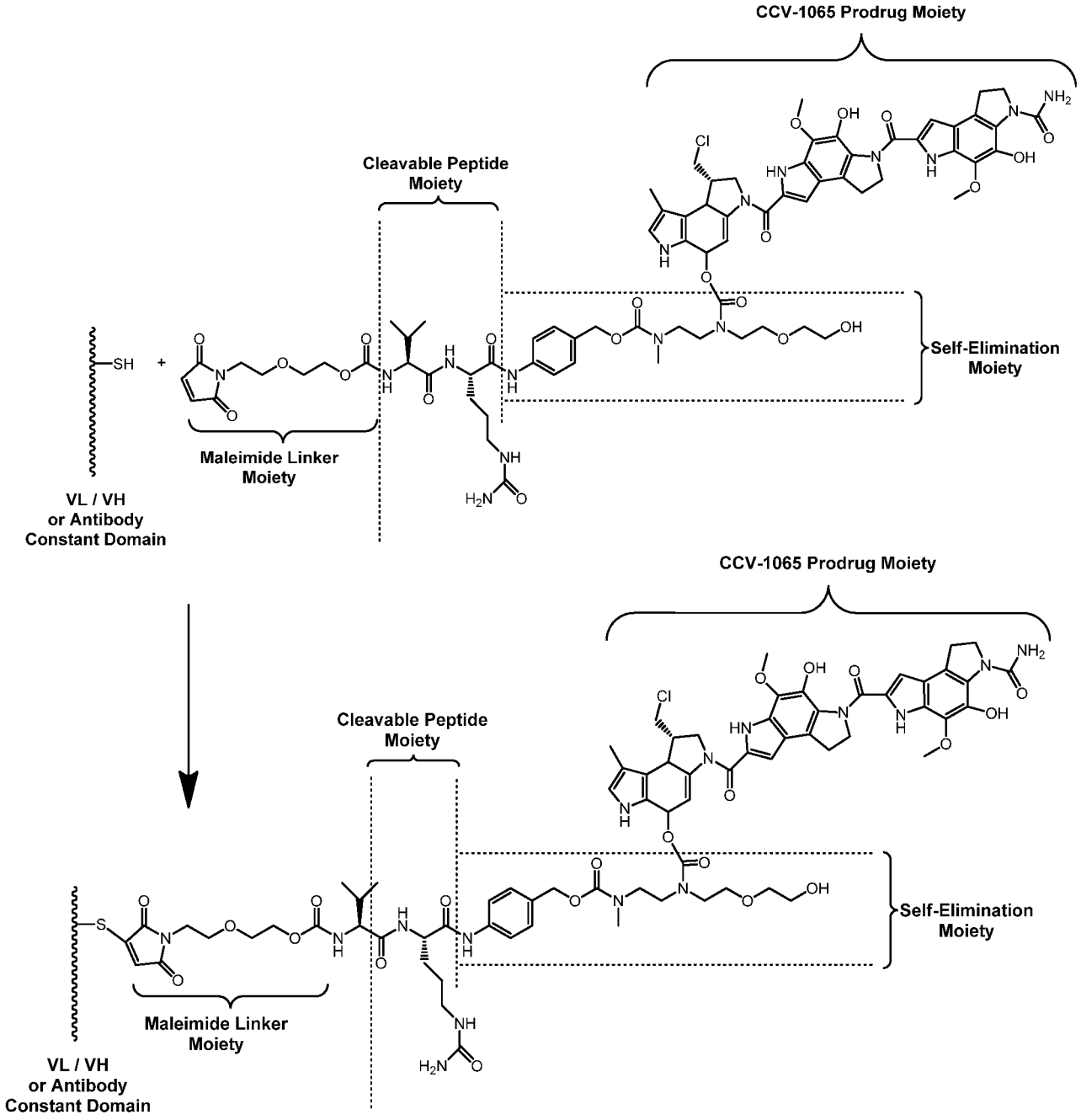
(see, Dokter, W. *et al.* (2014) "*Preclinical Profile of the HER2-Targeting ADC SYD983/SYD985: Introduction of a New Duocarmycin-Based Linker-Drug Platform*," Mol. Cancer Ther. 13(11):2618-2629).

[00309] In a preferred method for the production of B7-H3-duocarmycin drug moiety conjugates, the method of by Elgersma, R.C. *et al.* (2014) "*Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody–Drug Conjugate SYD985*," Mol. Pharmaceut. 12:1813-1835 or that of WO 2011/133039 will be employed. Thus, a thiol-containing group of the VL or VH chain of an anti-B7-H3 antibody or antibody fragment is conjugated to a *seco*-DUBA or other prodrug through a Maleimide Linker Moiety-Cleavable Peptide Moiety-Self-Elimination Moiety (**Scheme 9A**):


Cleavable Peptide Moiety
DUBA Prodrug Moiety
Self-Elimination Moiety
Maleimide Linker Moiety
VL / VH or Antibody Constant Domain
SH
Cleavable Peptide Moiety
DUBA Prodrug Moiety
Self-Elimination Moiety
Maleimide Linker Moiety
VL / VH or Antibody Constant Domain

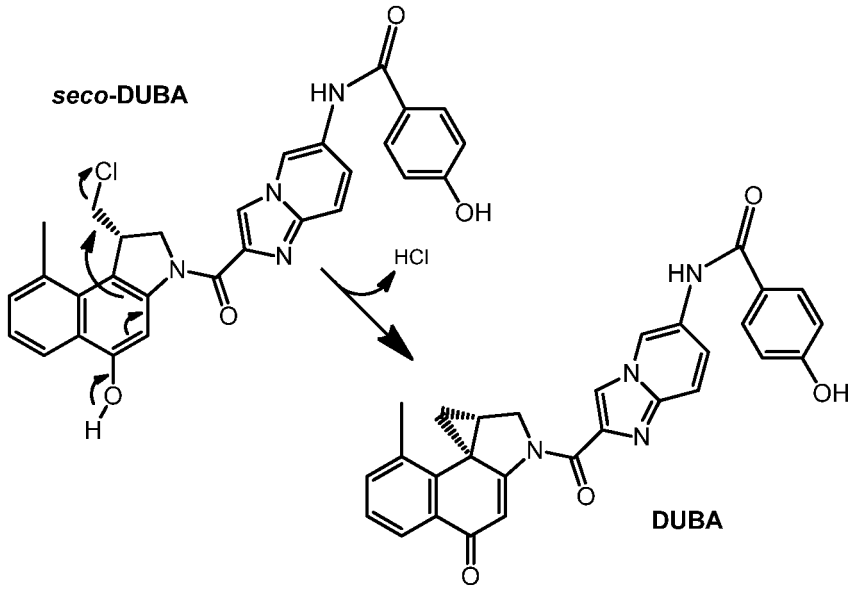
Scheme 9A

[00310] Although the invention is illustrated with regard to a DUBA prodrug, other prodrugs, *e.g.*, CC-1065, may be alternatively employed, as shown in **Scheme 9B**:



Scheme 9B

[00311] Upon cleavage of the Cleavable Peptide Moiety and elimination of the Self-Elimination Moiety, the Prodrug Moiety is believed to undergo a Winstein spirocyclization to yield the active drug (*e.g.*, DUBA from *seco*-DUBA as shown in **Scheme 9C**).

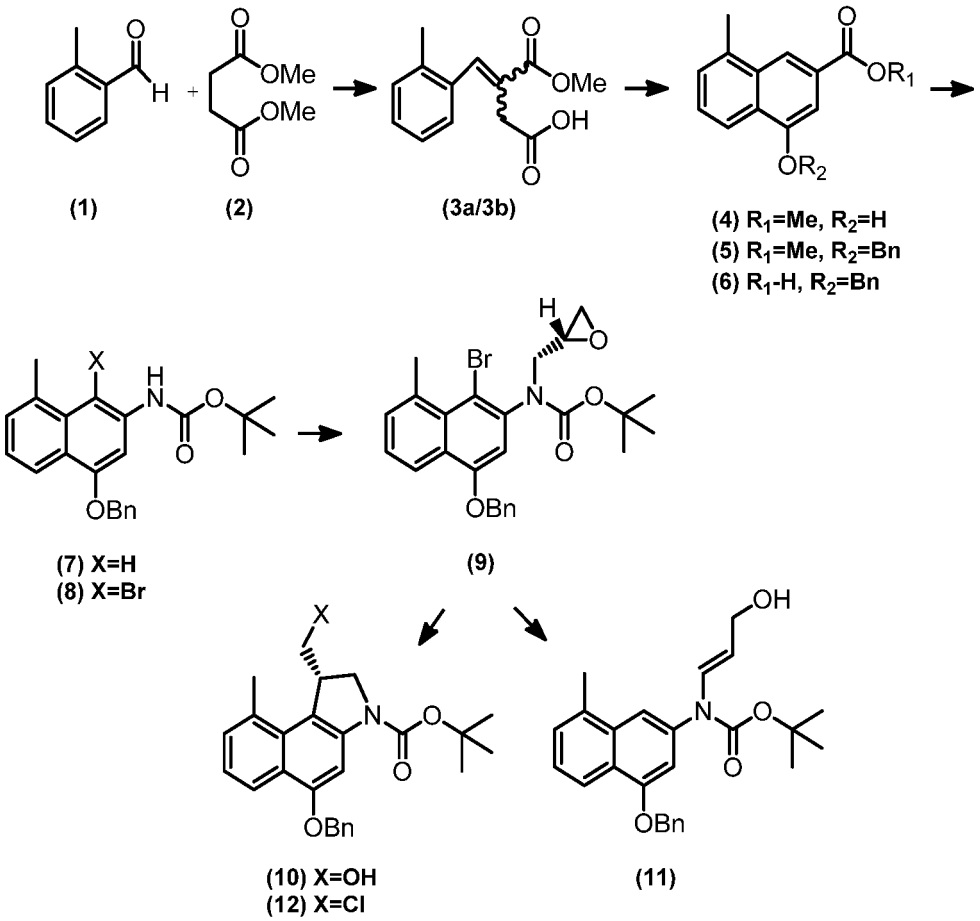


Scheme 9C

[00312] seco-DUBA is prepared from the corresponding DNA-alkylating and DNA-binding moieties (*e.g.*, a 1,2,9,9a-tetrahydrocyclopropa-[*c*]benzo[*e*]indole-4-one framework as described by Elgersma, R.C. *et al.* (2014) "*Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody–Drug Conjugate SYD985*," Mol. Pharmaceut. 12:1813-1835 (see, Boger, D.L. *et al.* (1989) "*Total Synthesis and Evaluation of (±)-N-(tert-Butoxycarbonyl)-CBI, (±)-CBI-CDPI1, and (±)-CBI-CDPI2: CC-1065 Functional Agents Incorporating the Equivalent 1,2,9,9a-Tetrahydrocyclopropa[1,2-c]benz[1,2-e]indol-4-one (CBI) Left-Hand Subunit*," J. Am. Chem. Soc. 111:6461-6463; Boger, D.L. *et al.* (1992) "*DNA Alkylation Properties of Enhanced Functional Analogs of CC-1065 Incorporating the 1,2,9,9a-Tetrahydrocyclopropa[1,2-c]benz[1,2-e]indol-4-one (CBI) Alkylation Subunit*," J. Am. Chem. Soc. 114:5487-5496).

[00313] **Scheme 9D** illustrates the invention by showing the synthesis of the DNA-alkylating moiety for DUBA. Thus, *o*-tolualdehyde **(1)** and dimethyl succinate **(2)** are reacted to produce a mixture of acids (3a/3b) through a Stobbe condensation. Ring closure of the mixture of acids may be accomplished with trifluoroacetic anhydride and gave alcohol **(4)**, which is then protected with benzyl chloride to afford benzyl ether **(5)**. The ensuing

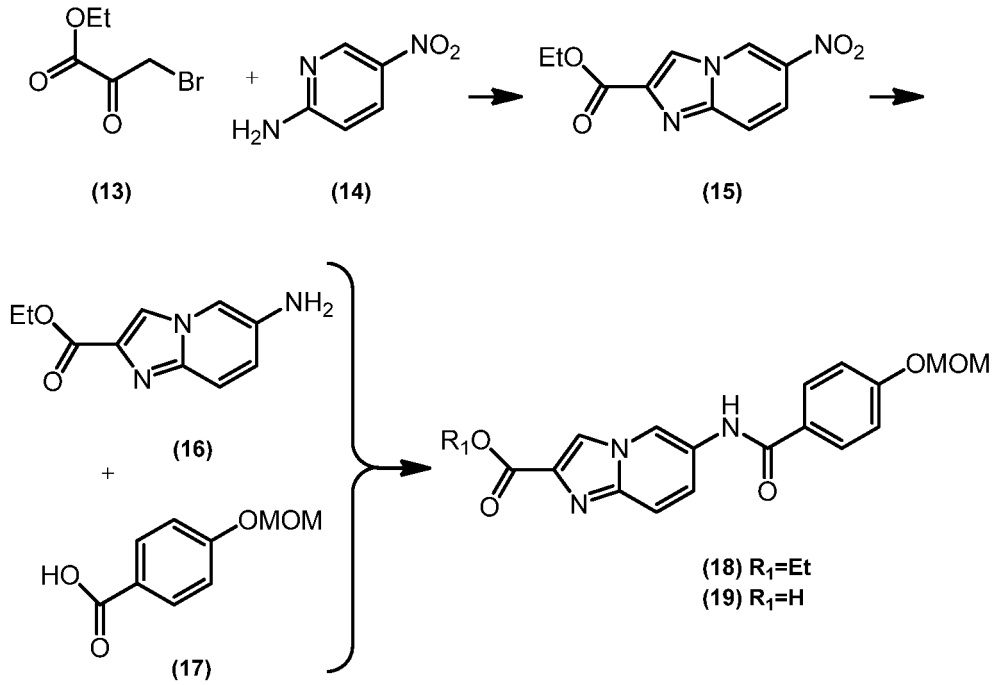
hydrolysis of the methyl ester group yields the carboxylic acid **(6)** which is followed by a Curtius rearrangement in a mixture of toluene and tert-butyl alcohol to provide the carbamate **(7)**. Bromination with N-bromosuccinimide give the bromide **(8)**. The bromide **(8)** is alkylated with (S)-glycidyl nosylate in the presence of potassium *tert*-butoxide to give epoxide **(9)**. Reaction with n-butyllithium provides a mixture of desired compound **(10)** and debrominated, rearranged derivative **(11)**. Yields for desired compound **(10)** are higher when tetrahydrofuran is used as the solvent and the reaction temperature is kept between -25 and -20 °C. Under these conditions, desired compound **(10)** and debrominated, rearranged derivative **(11)** are obtainable in an approximate 1:1 ratio. Workup with *p*-toluenesulfonic acid results in conversion of debrominated, rearranged derivative **(11)** to **(7)**, thereby aiding recovery of desired compound **(10)**. Mesylation of the hydroxyl group in **(10)** followed by chloride substitution using lithium chloride gives key intermediate **(12)**.



Scheme 9D

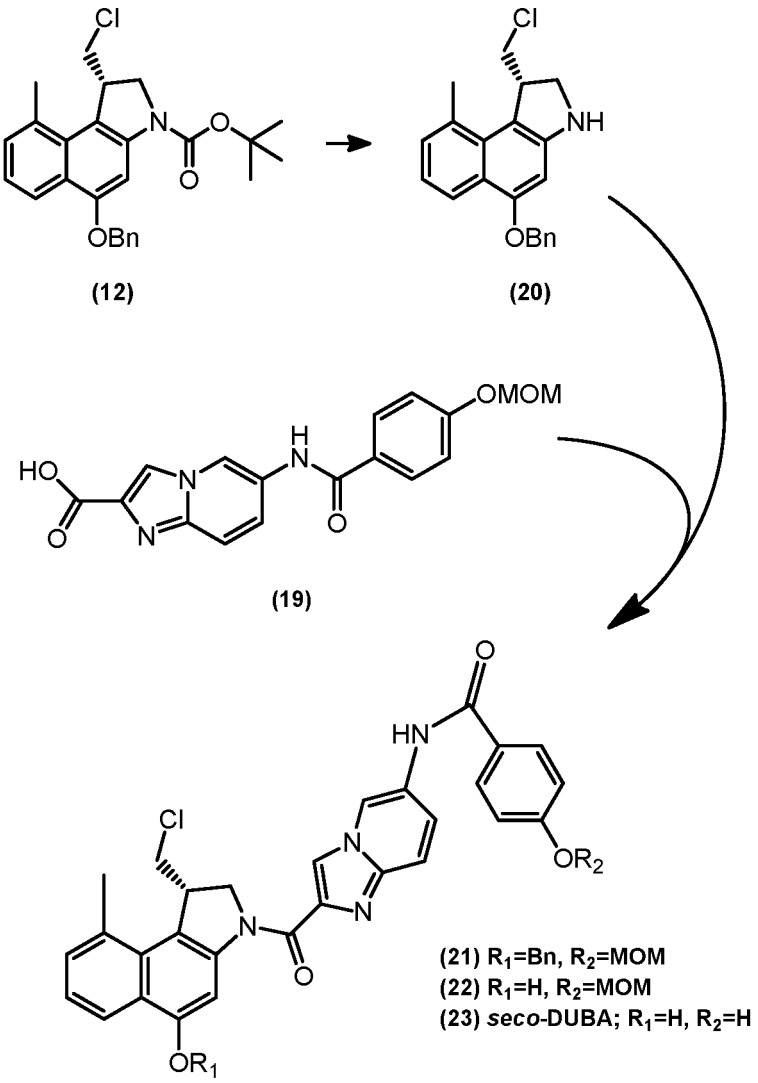
[00314] **Scheme 9E** illustrates the invention by showing the synthesis of the DNA-binding moiety for DUBA. Thus, a Chichibabin cyclization reaction is permitted to proceed between ethyl bromopyruvate **(13)** and 5-nitropyridin-2-amine **(14)**, thereby obtaining nitro

compound **(15)**. Reduction of the nitro group with zinc under acidic conditions gives amine **(16)**. Coupling with methoxymethyl (MOM)-protected 4-hydroxybenzoic acid **(17)**, prepared from methyl 4-hydroxybenzoate through reaction with chloromethyl methyl ether followed by ester hydrolysis (see, WO 2004/080979) gives the ethyl ester **(18)**, which may be hydrolyzed with sodium hydroxide in aqueous 1,4-dioxane to provide acid **(19)**.



Scheme 9E

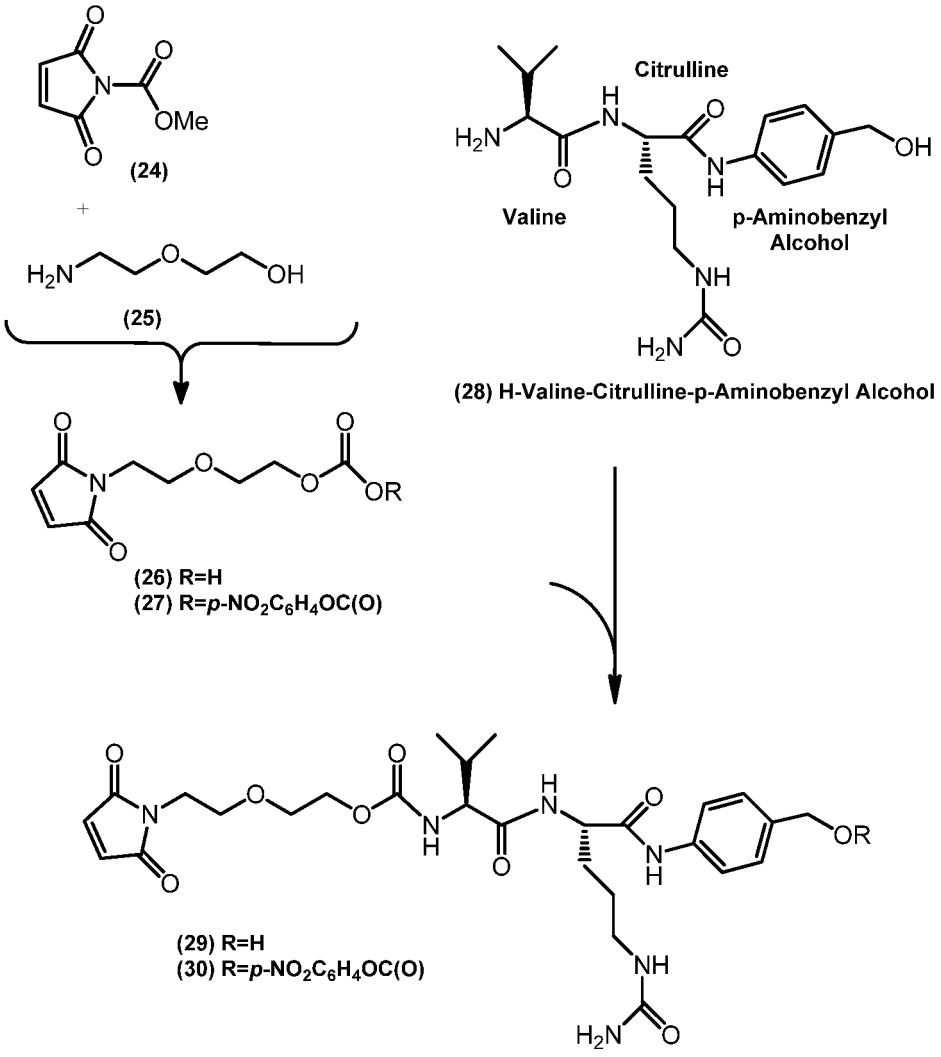
[00315] *seco*-DUBA is then synthesized from DNA-alkylating unit **(12)** and DNA-binding moiety **(19)**. The tert-butoxycarbonyl (Boc) protective group is removed from **(12)** under acidic conditions to form the amine **(20)**. EDC-mediated coupling of amine **(20)** and compound **(19)** yields protected compound **(21)**, which is then fully deprotected in two consecutive steps (with Pd/C, NH_4HCO_2, MeOH/THF, 3 hours, 90%, to yield **(22)** and then with HCl, 1,4-dioxane/water, 1 h, 95% to provide *seco*-DUBA **(23)** as its HCl salt (**Scheme 9F**).



Scheme 9F

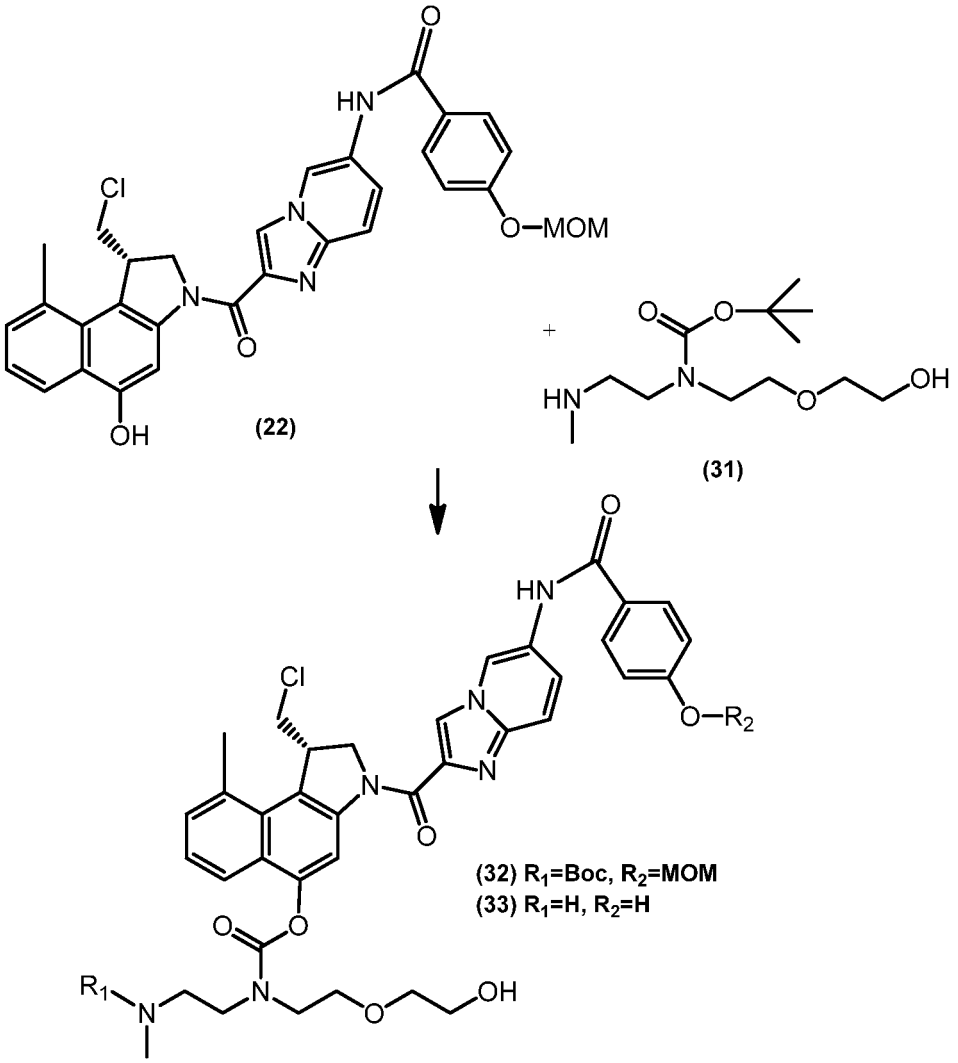
[00316] Prodrugs of other drugs, *e.g.*, CC-1065, may be synthesized as described for example in WO 2010/062171.

[00317] The Prodrug Moiety is preferably linked to the other moieties of the ADC according to **Scheme 9G**. The Maleimide Linker building block was synthesized by starting with a condensation reaction between **(24)** and 2-(2-aminoethoxy)ethanol **(25)** to give alcohol **(26)**, which was then converted to reactive carbonate **(27)** through reaction with 4-nitrophenyl chloroformate. Coupling of **(27)** to H-Valine-Citrulline-PABA **(28)**, prepared according to Dubowchik, G.M. *et al.* (2002) "*Cathepsin B-Labile Dipeptide Linkers For Lysosomal Release Of Doxorubicin From Internalizing Immunoconjugates: Model Studies Of Enzymatic Drug Release And Antigen-Specific In Vitro Anticancer Activity*," Bioconjugate Chem. 13:855-869) results in the formation of linker **(29)**, which was treated with bis(4-nitrophenyl) carbonate to give activated linker **(30)**.


(24)
(25)
(26) R=H
(27) R=p-NO2C6H4OC(O)
Citrulline
Valine
p-Aminobenzyl Alcohol
(28) H-Valine-Citrulline-p-Aminobenzyl Alcohol
(29) R=H
(30) R=p-NO2C6H4OC(O)

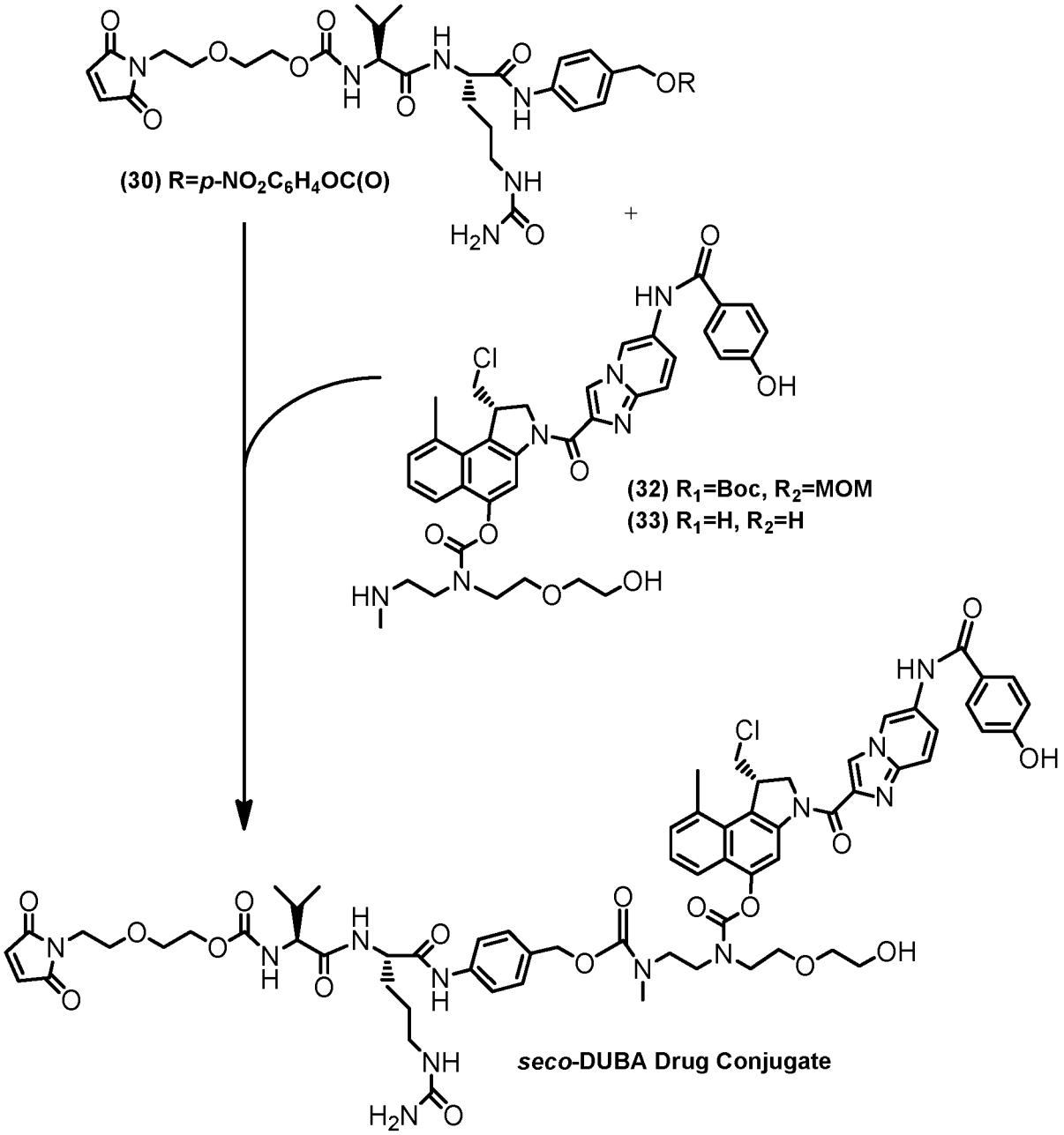
Scheme 9G

As shown in **Scheme 9H**, *seco*-DUBA-MOM **(22)** is modified for conjugation in two steps. Consecutive treatment of **(22)** with 4-nitrophenyl chloroformate and *tert*-butyl methyl(2-(methylamino)ethyl)carbamate **(31)** gives compound **(32)**. Removal of the Boc and MOM protective groups in **(32)** with trifluoroacetic acid (TFA) provided **(33)** as its TFA salt.



Scheme 9H

[00318] The ADC was synthesized through reaction of activated linker **(30)** with cyclization spacer-duocarmycin construct **(33)** under slightly basic conditions. Under these conditions, self-elimination of the cyclization spacer and resulting formation of 3a was suppressed (**Scheme 9I**).



Scheme 9I

[00319] The process generates on average two free thiol groups per mAb leading to a statistical distribution of **B7-H3-ADC** with an average drug-to-antibody-ratio (DAR) of about two, and low amounts of high-molecular weight species and residual unconjugated duocarmycin moiety.

[00320] The order of the steps of the synthesis may be varied as desired. Preferably, the method used will be that of **Schemes 9A-9I**, as described above.

XII. Uses of the B7-H3-Binding Molecules of the Present Invention

[00321] The present invention encompasses compositions, including pharmaceutical compositions, comprising the B7-H3-binding molecules of the present invention (*e.g.*, antibodies, bispecific antibodies, bispecific diabodies, trivalent binding molecules, **B7-H3-ADC**, *etc.*), polypeptides derived from such molecules, polynucleotides comprising sequences encoding such molecules or polypeptides, and other agents as described herein.

[00322] As provided herein, the B7-H3-binding molecules of the present invention, comprising the anti-B7-H3-VL and/or VH Domains provided herein, have the ability to bind B7-H3 present on the surface of a cell and induce antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) and/or mediate redirected cell killing (*e.g.*, redirected T-cell cytotoxicity). Without meaning to be bound by any mechanism of action, the **B7-H3-ADC** molecules of the present invention are internalized upon binding to B7-H3 expressed by a tumor cell and mediate the killing of the tumor cell via the action of the conjugated cytotoxin.

[00323] Thus, B7-H3-binding molecules of the present invention, comprising the anti-B7-H3-VL and/or VH Domains provided herein, have the ability to treat any disease or condition associated with or characterized by the expression of B7-H3. As discussed above, B7-H3 is an onco-embryonic antigen expressed in numerous blood and solid malignancies that is associated with high-grade tumors exhibiting a less-differentiated morphology, and is correlated with poor clinical outcomes. Thus, without limitation, the B7-H3-binding molecules of the present invention may be employed in the diagnosis or treatment of cancer, particularly a cancer characterized by the expression of B7-H3.

[00324] The cancers that may be treated by the B7-H3-binding molecules of the present invention include 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, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a glioblastoma, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia (*e.g.*, an acute myeloid leukemia), a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer (*e.g.*, a non-small-cell lung cancer (NSCLC)), a medulloblastoma, a melanoma, a meningioma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood (including neuroblastoma and rhabdomyosarcoma), a soft-tissue sarcoma, a squamous cell cancer (*e.g.*, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer (*e.g.*, a thyroid metastatic cancer), and a uterine cancer.

[00325] In particular, B7-H3-binding molecules of the present invention may be used in the treatment of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer (NSCLC), acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, mesothelioma pharyngeal cancer, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, small round blue cell tumors of childhood (including neuroblastoma and rhabdomyosarcoma), squamous cell cancer (*e.g.*, squamous cell cancer of the head and neck (SCCHN), testicular cancer, thyroid cancer (*e.g.*, thyroid metastatic cancer), and uterine cancer.

[00326] The bispecific B7-H3-binding molecules of the present invention augment the cancer therapy provided by B7-H3 by promoting the redirected killing of tumor cells that

express the second specificity of such molecules (*e.g.*, CD2, CD3, CD8, CD16, the T Cell Receptor (TCR), NKG2D, *etc.*). Such B7-H3-binding molecules are particularly useful for the treatment of cancer.

[00327] In addition to their utility in therapy, the B7-H3-binding molecules of the present invention may be detectably labeled and used in the diagnosis of cancer or in the imaging of tumors and tumor cells.

XIII. Pharmaceutical Compositions

[00328] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the B7-H3-binding molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the B7-H3-binding molecules of the present invention and a pharmaceutically acceptable carrier. The invention also encompasses such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor-specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00329] In a specific embodiment, the term "**pharmaceutically acceptable**" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "**carrier**" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00330] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a B7-H3-binding molecule of the present invention, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00331] The present invention provides kits that can be used in the above methods. A kit can comprise any of the B7-H3-binding molecules of the present invention, including **B7-H3-ADC**. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers.

XIV. Methods of Administration

[00332] 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 fusion protein or a conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or a conjugated molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00333] 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.*

[00334] Methods of administering a molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the B7-H3-binding molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. 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. Patents No. 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.

[00335] The invention also provides that preparations of the B7-H3-binding molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the B7-H3-binding molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

[00336] The lyophilized preparations of the B7-H3-binding molecules of the present invention should be stored at between 2°C and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such B7-H3-binding molecules when provided in liquid form are supplied in a hermetically sealed container.

[00337] The amount of such preparations of the invention that will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder

can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00338] As used herein, an "**effective amount**" of a pharmaceutical composition is an amount sufficient to effect beneficial or desired results including, without limitation, clinical results such as decreasing symptoms resulting from the disease, attenuating a symptom of infection (*e.g.*, viral load, fever, pain, sepsis, etc.) or a symptom of cancer (e.g., the proliferation, of cancer cells, tumor presence, tumor metastases, *etc.*), thereby increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication such as via targeting and/or internalization, delaying the progression of the disease, and/ or prolonging survival of individuals.

[00339] 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 the effect of) viral presence and to reduce and /or delay the development of the viral disease, either directly or indirectly. In some embodiments, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more chemotherapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art.

[00340] For the B7-H3-binding molecules encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. For the B7-H3-binding molecules encompassed by the invention, the dosage administered to a patient is typically from about 0.01 μg/kg to about 30 mg/kg or more of the subject's body weight.

[00341] The dosage and frequency of administration of a B7-H3-binding molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00342] The dosage of a B7-H3-binding molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00343] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00344] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327).

[00345] Treatment of a subject with a therapeutically or prophylactically effective amount of a B7-H3-binding molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody 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. The pharmaceutical compositions of the invention can be administered once a day with such administration occurring once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc*. Alternatively, the pharmaceutical compositions of the invention can be administered twice a day with such administration occurring once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc*. Alternatively, the pharmaceutical compositions of the invention can be administered three times a day with such administration occurring once a

week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year, *etc*. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

XV. Embodiments of the Invention

[00346] The invention particularly concerns the following embodiments (E_A and E_B):

E_A1. An anti-B7-H3 antibody drug conjugate (**B7-H3-ADC**) that comprises the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n},$$

wherein:

Ab is an antibody that binds to B7-H3 that comprises a humanized Variable Heavy Chain (VH) Domain and a humanized Variable Light Chain (VL) Domain, or is a B7-H3-binding fragment thereof, and;

D is a cytotoxic drug moiety;

LM is a bond or a Linker Molecule that covalently links Ab and D;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

E_A2. The **B7-H3-ADC** of E_A1, wherein:

(A) (i) said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:99**, and

(ii) said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:104**;

or

(B) (i) said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:20**, and

(ii) said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:21**;

or

(C) (i) said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:30**, and

(ii) said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:31**.

EA3. The **B7-H3-ADC** of EA1, wherein said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:99** and said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:104**.

EA4. The **B7-H3-ADC** of EA1, wherein said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:20** and said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

EA5. The **B7-H3-ADC** of EA1, wherein said humanized VL Domain comprises the amino acid sequence of **SEQ ID NO:30** and said humanized VH Domain comprises the amino acid sequence of **SEQ ID NO:31**.

EA6. The **B7-H3-ADC** of any one of EA1-EA5, wherein said **Ab** is an antibody.

EA7. The **B7-H3-ADC** of any one of E1- EA5, wherein said **Ab** is an antigen binding fragment of an antibody.

EA8. The **B7-H3-ADC** of any one of EA1-EA7, wherein said **B7-H3-ADC** comprises an Fc Domain of a human IgG.

EA9. The **B7-H3-ADC** of EA8, wherein said human IgG is a human IgG1, IgG2, IgG3, or IgG4.

EA10. The **B7-H3-ADC** of EA8 or EA9, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

EA11. The **B7-H3-ADC** of EA10, wherein said modifications that reduces the affinity of the variant Fc Domain for an FcγR comprise the substitution of

L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.

E_A12. The **B7-H3-ADC** of E_A10 or E_A11, wherein said modifications that that enhances the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

E_A13. The **B7-H3-ADC** of any one of E_A1-E_A12, wherein at least one of said **LM** is a Linker Molecule.

E_A14. The **B7-H3-ADC** of E_A13, wherein said **LM** Linker Molecule is a peptidic linker.

E_A15. The **B7-H3-ADC** of E_A13, wherein said **LM** Linker Molecule is a cleavable linker.

E_A16. The **B7-H3-ADC** of E_A15, wherein said molecule comprises the formula:

$$\mathbf{Ab - [V\text{-}(W)_k\text{-}(X)_1\text{-}A] - D}$$

wherein:

V is said cleavable **LM** Linker Molecule,

$\mathbf{(W)_k\text{-}(X)_1\text{-}A}$ is an elongated, self-eliminating spacer system, that self-eliminates via a 1,(4+2n)-elimination,

W and **X** are each a 1,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula $(\mathbf{Y})_m$, wherein **Y** is a 1,(4+2n) electronic cascade spacer, or a group of formula **U**, being a cyclisation elimination spacer,

k, 1 and m are independently an integer of 0 (included) to 5 (included),

n is an integer of 0 (included) to 10 (included),

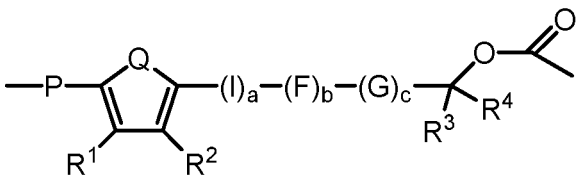
with the provisos that:

when **A** is $(\mathbf{Y})_m$: then $k+l+m \geq 1$, and

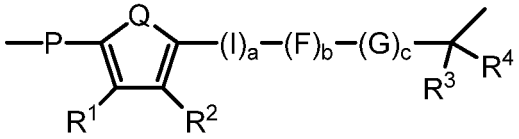
if $k+l+m=1$, then $n>1$;

when **A** is **U**: then $k+1 \geq 1$.

W, **X**, and **Y** are independently selected from compounds having the formula:



or the formula:

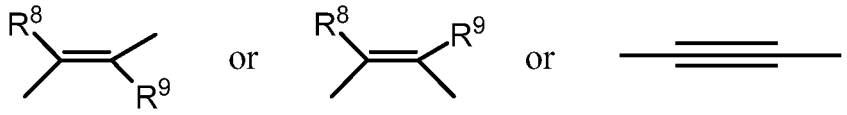


wherein: Q is $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$

P is NR^7, O or S

a, b, and c are independently an integer of 0 (included) to 5 (included);

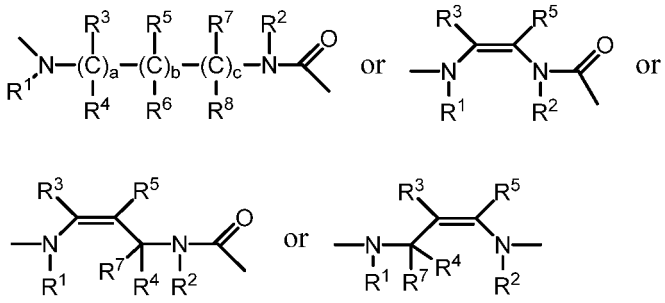
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are independently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being

connected to one another to form one or more aliphatic or aromatic cyclic structures;

U is selected from compounds having the formula:



wherein:

a, b and c are independently selected to be an integer of 0 or 1; provided that a + b + c = 2 or 3;

R^1 and/or R^2 independently represent H, C1-6 alkyl, said alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_X), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_X$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)ORx), sulphinyl (S(=O)Rx), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7 and R^8 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl

group, and two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 are optionally connected to one another to form one or more aliphatic or aromatic cyclic structures.

E_A17. The **B7-H3-ADC** of E_A16, wherein said **LM** Linker Molecule comprises:

(1) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(2) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(3) p-ammocinnamyloxycarbonyl;

(4) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl;

(5) p-amino-benzyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(6) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(7) p-aminophenylpentadienyloxycarbonyl;

(8) p-aminophenylpentadienyloxycarbonyl-p-arninocinnamyloxycarbonyl;

(9) p-aminophenylpentadienyloxycarbonyl-paminobenzyloxycarbonyl;

(10) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl;

(11) p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(12) p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(13) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino) ethyl(methylamino)carbonyl;

(14) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(15) p-aminobenzyloxycarbonyl-p-arninocinnamyloxycarbonyl (methylamino)ethyl(methylamino)-carbonyl;

(16) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(17) p-aminobenzyloxycarbonyl-p-aminobenzyl;

(18) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl -p-aminobenzyl;

(19) p-aminocinnamyl;

(20) p-aminocinnamyloxycarbonyl-p-aminobenzyl;

(21) p-aminobenzyloxycarbonyl-p-aminocinnamyl;

(22) p-amino-cinnamyloxycarbonyl-p-aminocinnamyl;

(23) p-aminophenylpentadienyl;

(24) p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl;

(25) p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl;

or

(26) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

EA18. The **B7-H3-ADC** of any one of EA13-EA17, wherein said **LM** Linker Molecule is conjugated to the side chain of an amino acid of a polypeptide chain of **Ab** and binds said **Ab** to a molecule of said cytotoxic drug moiety **D**.

EA19. The **B7-H3-ADC** of any one of EA1-EA18, wherein said cytotoxic drug moiety **D** comprises a cytotoxin, a radioisotope, an immunomodulator, a cytokine, a lymphokine, a chemokine, a growth factor, a tumor necrosis factor, a hormone, a hormone antagonist, an enzyme, an oligonucleotide, a DNA, an RNA, an siRNA, an RNAi, a microRNA, a photoactive therapeutic agent, an anti-angiogenic agent, a pro-apoptotic agent, a peptide, a lipid, a carbohydrate, a chelating agent, or combinations thereof.

EA20. The **B7-H3-ADC** of EA19, wherein said cytotoxic drug moiety **D** comprises a cytotoxin and is selected from the group consisting of a tubulysin, an auristatin, a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, and a duocarmycin.

EA21. The **B7-H3-ADC** of EA19, wherein said cytotoxic drug moiety **D** comprises a tubulysin cytotoxin and is selected from the group consisting of tubulysin A, tubulysin B, tubulysin C, and tubulysin D.

EA22. The **B7-H3-ADC** of EA19, wherein said cytotoxic drug moiety **D** comprises an auristatin cytotoxin and is selected from the group consisting of MMAE (N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine) and MMAF (N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine).

E_A23. The **B7-H3-ADC** of E_A19, wherein said cytotoxic drug moiety **D** comprises a maytansinoid cytotoxin and is selected from the group consisting of Mytansine, DM1 and DM4.

E_A24. The **B7-H3-ADC** of E_A19, wherein said cytotoxic drug moiety **D** comprises a calicheamicin cytotoxin and is selected from the group consisting of calicheamicin γ1, calicheamicin β1Br, calicheamicin γ1Br, calicheamicin α2I, calicheamicin α3I, calicheamicin β1I, calicheamicin γ1I, and calicheamicin Δ1I.

E_A25. The **B7-H3-ADC** of E_A19, wherein said cytotoxic drug moiety **D** comprises a pyrrolobenzodiazepine cytotoxin and is selected from the group consisting of vadastuximab talirine, SJG-136, SG2000, SG2285 and SG2274.

E_A26. The **B7-H3-ADC** of E_A19, wherein said cytotoxic drug moiety **D** comprises a duocarmycin cytotoxin and is selected from the group consisting of duocarmycin A, duocarmycin B1, doucarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin (U-80244) and spiro-duocarmycin (DUBA).

E_A27. The **B7-H3-ADC** of any of E_A1-E_A26, wherein said LM Linker Molecule is covalently linked to said Ab via reduced inter-chain disulfides.

E_A28. A pharmaceutical composition that comprises an effective amount of the **B7-H3-ADC** of any of E_A1-E_A27 and a pharmaceutically acceptable carrier, excipient or diluent.

E_A29. Use of the **B7-H3-ADC** of any one of E_A1-E_A27 or the pharmaceutical composition of E_A28 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

E_A30. The use of E_A29, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

E_A31. The use of E_A30, wherein said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an

astrocytic tumor, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a glioblastoma, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia (*e.g.*, an acute myeloid leukemia), a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer (*e.g.*, a non-small-cell lung cancer (NSCLC)), a medulloblastoma, a melanoma, a meningioma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood (including neuroblastoma and rhabdomyosarcoma), a soft-tissue sarcoma, a squamous cell cancer (*e.g.*, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer (*e.g.*, a thyroid metastatic cancer), and a uterine cancer.

EA31. The use of EA30, wherein said cancer is selected from the group consisting: of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer (NSCLC), acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, mesothelioma pharyngeal cancer,

non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, small round blue cell tumors of childhood (including neuroblastoma and rhabdomyosarcoma), squamous cell cancer (*e.g.*, squamous cell cancer of the head and neck (SCCHN), testicular cancer, thyroid cancer (*e.g.*, a thyroid metastatic cancer), and uterine cancer.

E_B1. A B7-H3-binding molecule that comprises a Variable Light Chain (VL) Domain that comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, and a Variable Heavy Chain (VH) Domain that comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, wherein:

(1) said CDR_H1 Domain comprises the amino acid sequence of **SEQ ID NO:27**;

(2) said CDR_H2 Domain comprises the amino acid sequence of **SEQ ID NO:28**; and

(3) said CDR_H3 Domain comprises the amino acid sequence of **SEQ ID NO:29**.

E_B2. The B7-H3-binding molecule of E_B1, that comprises said VL Domain that comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, and said VH Domain that comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, wherein:

(1) said CDR_L1 Domain comprises the amino acid sequence of **SEQ ID NO:23**;

(2) said CDR_L2 Domain comprises the amino acid sequence of **SEQ ID NO:24**; and

(3) said CDR_L3 Domain comprises the amino acid sequence of **SEQ ID NO:25**.

E_B3. The B7-H3-binding molecule of E_B1, that comprises said VL Domain that comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, and said VH Domain that comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, wherein said domains are selected from the group consisting of:

(1) a CDR_H1 Domain comprising the amino acid sequence of **SEQ ID NO:27**;

(2) a CDR_H2 Domain comprising the amino acid sequence of **SEQ ID NO:28**;

(3) a CDR_H3 Domain comprising the amino acid sequence of **SEQ ID NO:29**;

(4) a CDR_L1 Domain comprising the amino acid sequence of **SEQ ID NO:23**;

(5) a CDR_L2 Domain comprising the amino acid sequence of **SEQ ID NO:24**; and

(6) a CDR_L3 Domain comprising the amino acid sequence of **SEQ ID NO:25**.

E_B4. The B7-H3-binding molecule of any one of E_B1-E_B3, wherein said VH Domain comprises the amino acid sequence of **SEQ ID NO:26** or **SEQ ID NO:31**.

E_B5. The B7-H3-binding molecule of any one of E_B1-E_B4, wherein said VL Domain comprises the amino acid sequence of **SEQ ID NO:22** or **SEQ ID NO:30**.

E_B6. A B7-H3-binding molecule that comprises a VL Domain and a VH Domain, wherein said VL Domain comprises the amino acid sequence of **SEQ ID NO:20**.

E_B7. A B7-H3-binding molecule that comprises a VL Domain and a VH Domain, wherein said VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

E_B8. A B7-H3-binding molecule that comprises a VL Domain and a VH Domain, wherein said VL Domain comprises the amino acid of **SEQ ID NO:20** and said VH Domain comprises the amino acid sequence of **SEQ ID NO:21**.

E_B9. The B7-H3-binding molecule of any one of E_B1-E_B8, wherein said molecule is an antibody or antigen binding fragment thereof.

E_B10. The B7-H3-binding molecule of any one of E_B1-E_B8, wherein said molecule is:

(a) a bispecific antibody; or

(b) a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or

(c) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more polypeptide chains.

E_B11. The B7-H3-binding molecule of any one of E_B1-E_B10, wherein said molecule comprises an Fc Domain.

E_B12. The B7-H3-binding molecule of E_B10, wherein said molecule is a diabody and comprises an Albumin-Binding Domain (ABD).

E_B13. The B7-H3-binding molecule of E_B11, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

E_B14. The B7-H3-binding molecule of E_B13, wherein said modifications that reduces the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.

E_B15. The B7-H3-binding molecule of any one of E_B13 or E_B14, wherein said modifications that that enhances the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

E_B16. The B7-H3-binding molecule of any one of E_B1-E_B15, wherein said molecule is bispecific and comprises two epitope-binding sites capable of immunospecific binding to an epitope of B7-H3 and two epitope-binding

sites capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

E_B17. The B7-H3-binding molecule of any one of E_B1-E_B15, wherein said molecule is bispecific and comprises one epitope-binding site capable of immunospecific binding to an epitope of B7-H3 and one epitope-binding site capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

E_B18. The B7-H3-binding molecule of any one of E_B1- E_B15, wherein said molecule is trispecific and comprises:

(a) one epitope-binding site capable of immunospecific binding to an epitope of B7-H3;

(b) one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and

(c) one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell.

E_B19. The B7-H3-binding molecule of any one of E_B1-E_B8, wherein said molecule is capable of simultaneously binding to B7-H3 and a molecule present on the surface of an effector cell.

E_B20. The B7-H3-binding molecule of any one of E_B16- E_B18, wherein said molecule present on the surface of an effector cell is CD2, CD3, CD8, TCR, or NKG2D.

E_B21. The B7-H3-binding molecule of any one of E16-E_B20, wherein said effector cell is a cytotoxic T-cell, or a Natural Killer (NK) cell.

E_B22. The B7-H3-binding molecule of any of E_B16-E_B21, wherein said molecule present on the surface of an effector cell is CD3.

E_B23. The B7-H3-binding molecule of E_B18, wherein said first molecule present on the surface of an effector cell is CD3 and said second molecule present on the surface of an effector cell is CD8.

E_B24. The B7-H3-binding molecule of any one of E_B16-E_B23, wherein said molecule mediates coordinated binding of a cell expressing B7-H3 and a cytotoxic T cell.

E_B25. A pharmaceutical composition that comprises an effective amount of the B7-H3-binding molecule of any of E_B1-E_B24 and a pharmaceutically acceptable carrier, excipient or diluent.

E_B26. Use of the B7-H3-binding molecule of any one of E_B1-E_B24 or the pharmaceutical composition of E_B26 in the treatment of a disease or condition associated with or characterized by the expression of B7-H3.

E_B27. The use of E_B26, wherein said disease or condition associated with or characterized by the expression of B7-H3 is cancer.

E_B28. The use of E_B27, wherein said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a glioblastoma, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia (*e.g.*, an acute myeloid leukemia), a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer (*e.g.*, a non-small-cell lung cancer (NSCLC)), a medulloblastoma, a melanoma, a

meningioma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood (including neuroblastoma and rhabdomyosarcoma), a soft-tissue sarcoma, a squamous cell cancer (*e.g.*, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer (*e.g.*, a thyroid metastatic cancer), and a uterine cancer

E_B29. The use of E_B27, wherein said cancer is selected from the group consisting: of adrenal cancer, bladder cancer, breast cancer, colorectal cancer, gastric cancer, glioblastoma, kidney cancer, non-small-cell lung cancer (NSCLC), acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, hairy cell leukemia, Burkett's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, mesothelioma pharyngeal cancer, non-Hodgkin's lymphoma, small lymphocytic lymphoma, multiple myeloma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, renal cell carcinoma, small round blue cell tumors of childhood (including neuroblastoma and rhabdomyosarcoma), squamous cell cancer (*e.g.*, squamous cell cancer of the head and neck (SCCHN), testicular cancer, thyroid cancer (*e.g.*, thyroid metastatic cancer), and uterine cancer.

EXAMPLES

[00347] Having now generally described the invention, the same will be more readily understood through reference to the following Examples. The following examples illustrate various methods for compositions in the diagnostic or treatment methods of the invention. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Example 1
Generation, Humanization and Characterization of Anti-B7-H3 Antibodies

[00348] Monoclonal antibodies were generated via immunization of mice with viable human fetal progenitor cells or tumor initiating/cancer step-like cells (CSLCs) as previously described (Loo *et al.* (2007) "The glycotope-specific RAV12 monoclonal antibody induces oncosis in vitro and has antitumor activity against gastrointestinal adenocarcinoma tumor xenografts in vivo" Mol Cancer Ther; 6: 856-65). An IHC screen for cancer-specific mAbs identified a panel of anti-B7-H3 (CD276) reactive mAbs with highly differential tumor-versus-normal tissue binding. A subset of anti-B7-H3 antibodies that were efficiently internalized was identified using an internalization assay performed in a 5 day assay using a saporin-conjugated anti-mouse Fab at 1:1 or 10:1 Fab-ZAP:Test mAb ratio according to the manufacturers protocol (Advanced Targeting Systems). As shown in **Figure 7**, a number of anti-B7-H3 antibodies including the anti-B7-H3 antibodies designated "**mAb-C**," and "**mAb-D**" were efficiently internalized.

[00349] The above-described murine anti-B7-H3 mAbs: **mAb-B**, **mAb-C** and **mAb-D** are used to form humanized VL and VH Domains in which the CDR_Ls and CDR_Hs of their domains are fused to human framework domains. The humanized VH and VL domains are then used to generate humanized light chains having a kappa light chain constant region (*i.e.*, **SEQ ID NO:1**) and IgG1 CH1, hinge, and Fc Domains (*i.e.*, **SEQ ID NOs:3, 7, 12**). The humanized antibodies were designated "**hmAb-B**," "**hmAb-C**," and "**hmAb-D**."

[00350] The amino acid sequences of the humanized VL and VH Domains are provided above. It will be noted that the CDRs of **hmAb-B** may be modified to generate alternative humanized VL and VH domains as described above. The amino acid sequence of the entire humanized light and heavy chains of **hmAb-C** and **hmAb-D** are provided above.

[00351] The binding kinetics of the humanized antibodies was investigated using Biacore analysis in which a soluble human or cyno B7-H3 (4Ig)-His tag fusion protein (shB7-H3-His or scB7-H3-His, respectively) was passed over immobilized antibodies. Briefly, each humanized antibody was captured on immobilized a Fab2 goat anti-human Fc surface and was incubated with shB7-H3-His or scB7-H3-his (6.25-100Nm), and the kinetics of binding were determined via Biacore analysis. The calculated k_a, k_d and K_D from these studies using a bivalent binding fit are presented in **Table 6.** The results demonstrate

that the humanized antibodies bind by human and cynomolgus monkey B7-H3 with a range of affinities.

Table 6						
	Human			Cyno		
Antibody	K_a ($x10^4$)	K_d ($x10^{-4}$)	K_D (nM)	K_a ($x10^4$)	K_d ($x10^{-4}$)	K_D (nM)
hmAb-B	11.0	0.12	0.11	7.1	2.9	4.1
hmAb-C	16	34	21.3	6.4	31	48.4
hmAb-D	3.4	22	62.9	1.35	77	592.3

[00352] The tissue cross-reactivity of the humanized antibodies was examined by immunohistochemistry (IHC). **Table 7** summarizes the findings for several IHC studies performed on normal human tissues, human tumor tissues, human cancer cell lines, and CHO cell lines expressing or not expressing B7-H3 using the humanized anti-B7-H3 antibodies at the indicated antibody concentrations. The scoring criteria for these studies is provided in **Table 8**.

Table 7				
Tissue	**Sample ID**	**hmAb-B 0.313 ug/ml**	**hmAb-C 0.625 ug/ml**	**hmAb-D 2.5 ug/ml**
Colon	MG06-CHTN-94F	epi 2+ (c) occasional	lamina propria cells 1+ (c) very rare	-
Liver	ILS11103A	hepatocytes 3+ (m) occasional to frequent	hepatocytes 2-3+ (m) rare to occasional	hepatocytes 1+ (m) rare
Kidney	ILS11119D	epi 1+ (c) rare	-	-
Pancreas	ILS10266	fibril 3+ (c) rare	endo 1+ (c,m) very rare	-
Lung	MG06-CHTN-85-A-2	pneumocytes 1+ (c) occasional	-	-
Heart	MG06-CHTN-76B	endo 2+ (c) rare to occasional	endo 1+ (c) very rare	-
Skin	MG03-St.Agn-50B	epi 2+ (c) occasional	squamous epi 1+ (c,m) rare	-
Adrenal	MG04-St.Agn-22B-A	4+ (m,c) frequent	epi 3+ (m,c) occasional to frequent	epi 2+ (c,m) rare to occasional
Head and Neck CA (squamous)	VNM00340-D03	2	2	1
	VNM00302-D01	3	2	1
	ILS7068-D04	3	2	1
	ILS2073-D01	3	3	2

Table 7				
Tissue	**Sample ID**	**hmAb-B 0.313 ug/ml**	**hmAb-C 0.625 ug/ml**	**hmAb-D 2.5 ug/ml**
Lung CA (NSCLC)	ILS7115-C	3	2	1
	ILS7253-C	2	1	1
	ILS2153-G	3	2	1
	ILS-11149- C	2	1 (BV) only	1
Hs700T ABC = 2.1e6	91812	4+ (c,m) frequent	4+ (m > c) frequent	2-3+ (m)
NCI-H1703 ABC = 8.1e5	033115-1	3-4+ (c) frequent	2-3+ (c,m) occasional to frequent	±
CHO +B7H3 Cl 31 ABC = 4.9e6	32113	2+ (c) frequent	3-4+ (c, m) frequent	3+ (m, c)
CHO +B7H3 Cl 32 ABC = 2.2e5	31813	3+ (c) frequent	1-2+ (c) rare to occasional	±
CHO - B7-H3	060414-2	-	-	-
c: cytoplasm	m: membrane	epi: epithelium	Tu: tumor	BV: blood vessel

Table 8			
Normal Tissue Scoring Criteria:		**Tumor Scoring Criteria:**	
-	negative	0 (negative):	no staining
±	equivocal	1 (weak):	1-100% of specific staining cells with 1+ staining intensity or 1-20% of specific staining cells with 2+ staining intensity
1+	weak		
2+	moderate	2 (moderate):	2+ staining intensity in 21-79% of specific staining cells or a 3+ staining intensity in 1-49% of specific staining cells
3+	strong		
4+	very strong	3 (strong):	2+ staining intensity in 80-100% of specific staining cells or 3+ staining intensity in ≥ 50% of specific staining cells.

[00353] These results demonstrate that all the humanized antibodies exhibit binding to numerous B7-H3 positive tumor cells. **hmAb-B** exhibits the greatest tumor reactivity under the conditions tested but also exhibited normal tissue reactivity to liver hepatocytes and adrenal tissue. **hmAb-C** exhibits somewhat reduced tumor reactivity as compared to **hmAb-B**, but also exhibits substantially less reactivity with normal liver hepatocytes, and also reactivity on fewer independent samples. **hmAb-D** exhibits overall reduced reactivity on tumor and normal tissues. The antibodies show comparable cross-reactivity with

cynomolgus monkey tissues although **hmAb-D** binds with less intensity in these IHC studies. To minimize off target toxicity **hmAb-C** and **hmAb-D** may be preferred for generation of **B7-H3-ADC** molecules of the invention.

Example 2
Production of B7-H3-ADC

[00354] The above-described murine anti-B7-H3 mAbs: **mAb-A**, **mAb-B**, **mAb-C** and **mAb-D** were used to form chimeric antibodies in which the VL Domain of such antibodies was fused to a human Light Chain Kappa Constant Region (**SEQ ID NO:1**), and in which the VH Domain of such antibodies was fused to human IgG1 CH1-Hinge-CH2-CH3 Constant Region (**SEQ ID NOs:3, 7, and 12**, respectively). The chimericized antibodies ("**chmAb-A**," "**chmAb-B**," "**chmAb-C**," and "**chmAb-D**") were converted to **B7-H3-ADC** via cysteine-conjugation to a B7-H3 binding domain thereof with the cleavable auristatin E linker/payload "**vc-MMAE**" (Concortis Biosystems), as discussed above.

Example 3
B7-H3-ADCs Exhibit Potent *in vitro* Activity

[00355] In order to demonstrate the anti-tumor activity of the **B7-H3-ADC** of the present invention, the above-described **B7-H3-ADC** (MMAE) were incubated at concentrations ranging from 1-100,000 pM with B7-H3-expressing JIMT-1 breast cancer cells, MDA-MB-468 breast cancer cells, A375.52 melanoma cells, Calu-6 non-small cell lung cancer cells, NCI-H1703 non-small cell lung cancer cells, NCI-H1975 non-small cell lung cancer cells, PA-1 ovarian cancer cells, Hs700T pancreatic cancer cells, DU145 prostate cancer cells, or B7-H3-negative Raji B Cell lymphoma cells. *in vitro* cytotoxicity was quantified after 7 days. Briefly, **B7-H3-ADCs** and controls are diluted and plated into microtiter plates, 5000 cells are added to each well and incubated at 37C for 4-7 days. Alamar Blue Reagent (*e.g.*, BioRad/ThermoFisher/Invitrogen) is added to the plates and read according to the manufacturer's protocol. The number of antibody binding sites present on these cells was determined using a Bangs QFACS™ Kit.

[00356] The cytotoxicity curves from this study are presented in **Figures 8A-8J**. The IC50 values were determined and are provided in **Table 9**. The results of these studies demonstrate that each of the internalizing anti-B7-H3 antibodies tested exhibited dose-dependent cytotoxicity *in vitro* against B7-H3-expressing tumor cells. The antibodies

exhibited a range of potencies. The relative potency in these assays was: **chmAb-C** > **chmAb-B** > **chmAb-D** > **chmAb-A**.

Table 9

B7-H3-ADC	Cell Line								
	Breast Cancer		Melanoma	Non-Small Cell Lung Cancer			Ovarian Cancer	Pancreatic Cancer	Prostate Cancer
	JIMT-1	MDA-MB-468	A375.52	Calu-6	NCI-H1703	NCI-H1975	PA-1	Hs700T	DU145
	Antibody Binding Sites per Cell (x 10^5)								
	11	4.2	7.5	8.5	8.1	4.8	6.1	21	2.4
	IC50 (pM)								
chmAb-A B7-H3 ADC	9100	8095	703	995	1517	26976	8326	607	20153
chmAb-B B7-H3 ADC	221	352	153	59	90	31	555	159	3770
chmAb-C B7-H3 ADC	124	201	267	30	43	16	409	109	465
chmAb-D B7-H3 ADC	735	1383	887	171	219	162	1795	303	2587

Example 4
B7-H3-ADCs Exhibit Potent *in vivo* Activity

[00357] In order to further demonstrate the anti-tumor activity of the **B7-H3-ADC** of the present invention, the above-described **chmAb-B B7-H3-ADC**, **chmAb-C B7-H3-ADC**, and/or **chmAb-D B7-H3-ADC** (MMAE) molecules were evaluated for *in vivo* toxicity in a CD1 nude mouse model using different tumor cell lines. In brief, ~5 x 10^6 tumor cells (suspended in 1:1 media and MATRIGEL®) were subcutaneously implanted into the flank of the CD1 nude mice (Charles River Laboratories). When tumors had reached a volume of approximately 150 mm^3, the mice were randomized and **B7-H3-ADC** or control vehicle was administered intraperitoneally. In these studies, one dose of the **B7-H3-ADC** or control vehicle was administered (qdx1). Tumors were measured twice weekly by orthogonal measurements with electronic calipers, with tumor volumes calculated as: (length x width x height)/2. The tumor volume (relative to control) was determined ("T/C"). A finding that the tumor volume of treated animals had decreased to ≤ 5 mm^3 during the study period was considered to denote a Complete Response ("CR").

in vivo Activity Against MDA-MB-468 Breast Cancer Tumor Cells

[00358] The results of this study with respect to mammary fat pad implanted MDA-MB-468 breast cancer tumor cells are presented in **Table 10** and in **Figure 9**, and show responsiveness against the MDA-MB-468 tumor cells.

Table 10

Treatment (Initial Dose on Day 30)	Dose (mg/kg)	T/C	CR	Response
chmAb-B B7-H3 ADC	10	4	6/7	Highly Active
chmAb-C B7-H3 ADC	10	20	4/7	Highly Active
chmAb-D B7-H3 ADC	10	8	1/7	Highly Active

in vivo Activity Against NCI-H1703 Non-Small Cell Lung Cancer Tumor Cells

[00359] The results of this study with respect to subcutaneously implanted NCI-H1703 non-small cell lung cancer tumor cells are presented in **Table 11** and in **Figures 10A-10C**, and show responsiveness against the NCI-H1703 tumor cells.

Table 11

Treatment (Initial Dose on Day 52)	Dose (mg/kg)	T/C	CR	Response
chmAb-B B7-H3-ADC	10	28	5/7	Highly Active
	3	22	3/7	Highly Active
	1	74	0/7	Active
chmAb-C B7-H3-ADC	10	0	6/7	Highly Active
	3	11	5/7	Highly Active
	1	70	0/7	Active
chmAb-D B7-H3-ADC	10	32	5/7	Highly Active
	3	4	6/7	Highly Active
	1	76	0/7	Active

in vivo Activity Against PA-1 Ovarian Cancer Tumor Cells

[00360] The results of this study with respect to subcutaneously implanted PA-1 ovarian cancer tumor cells are presented in **Table 12** and in **Figures 11A-11C**, and show responsiveness against the PA-1 tumor cells.

Table 12

Treatment (Initial Dose on Day 42)	Dose (mg/kg)	T/C	CR	Response
chmAb-B B7-H3-ADC	10	0	6/7	Highly Active
	3	65	0/7	Active
	1	105	0/7	Not Active
chmAb-C B7-H3-ADC	10	37	3/7	Highly Active
	3	76	1/7	Active
	1	93	0/7	Not Active
chmAb-D B7-H3-ADC	10	11	7/7	Highly Active
	3	57	1/7	Active
	1	113	0/7	Not Active

in vivo Activity Against Calu-6 Non-Small Cell Lung Cancer Tumor Cells

[00361] The results of this study with respect to subcutaneously implanted Calu-6 non-small cell lung cancer tumor cells are presented in **Table 13** and in **Figures 12A-12C**, and show responsiveness against the Calu-6 tumor cells.

Table 13

Treatment (Initial Dose on Day 20)	Dose (mg/kg)	T/C	CR	Response
chmAb-B B7-H3-ADC	10	15	3/7	Highly Active
	3	35	0/7	Active
	1	64	0/7	Active
chmAb-C B7-H3-ADC	10	1	3/7	Highly Active
	3	87	0/7	Not Active
	1	68	0/7	Active
chmAb-D B7-H3-ADC	10	39	2/7	Highly Active
	3	43	0/7	Active
	1	54	0/7	Active

in vivo Activity Against A375.S2 Melanoma Tumor Cells

[00362] The results of this study with respect to subcutaneously implanted A375.S2 melanoma tumor cells are presented in **Table 14** and in **Figures 13A-13C**, and show responsiveness against the A375.S2 melanoma cells.

Table 14

Treatment (Initial Dose on Day 20)	Dose (mg/kg)	T/C	CR	Response
chmAb-B B7-H3-ADC	10	3	2/7	Highly Active
	3	13	0/7	Highly Active
	1	65	0/7	Active
chmAb-C B7-H3-ADC	10	4	1/7	Highly Active
	3	23	0/7	Highly Active
	1	70	0/7	Active
chmAb-D B7-H3-ADC	10	26	0/7	Highly Active
	3	7	0/7	Highly Active
	1	80	0/7	Active

[00363] The results of these studies demonstrate that each of the **B7-H3-ADC**s tested exhibited significant dose-dependent in vivo anti-tumor activity toward B7-H3-positive tumors in murine xenograft models of breast, lung and ovarian cancers as well as melanoma.

[00364] The pharmacokinetics of the above **B7-H3-ADC** (MMAE) molecules was evaluated in non-tumor bearing CD1 nude mice by administering such molecules intraperitoneally at a single dose of 5 mg/kg. Blood samples were collected over the course of 10 days and sandwich ELISAs were performed on the sera to quantify total antibody and intact **B7-H3-ADC** concentrations.

[00365] Representative results of this study, with respect to **chmAb-B B7-H3 ADC**, **chmAb-C B7-H3 ADC**, and **chmAb-D B7-H3 ADC**, are presented in **Figures 14A-14C** and in **Table 15**, and show that the **B7-H3-ADC** molecules were highly stable, exhibiting half-lives of approximately 2.2-3.6 days. The half-life of the conjugates was comparable to that of the unconjugated molecules, demonstrating that **B7-H3-ADC** molecules are highly stable in mice.

Table 15

B7-H3-ADC	**Total Anti-B7-H3 Antibody**		**Intact B7-H3-ADC***	
	$T_{1/2}$ (hours)	**AUC (hr*ng/mL)**	**$T_{1/2}$ (hours)**	**AUC (hr*ng/mL)**
chmAb-B B7-H3 ADC	114.1	4,796,235	58.9	4,032,575
chmAb-C B7-H3 ADC	75.9	2,698,831	52.6	2,201,893
chmAb-D B7-H3 ADC	177.2	5,162,024	87.3	3,502,158

*MMAE conjugate

Example 5
B7-H3-ADC Having Cleavable Linker-Duocarmycin Moiety

[00366] A **B7-H3-ADC** is constructed ("**hmAb-C B7-H3-ADC**") having an exemplary duocarmycin moiety (DUBA) linked to an amino acid residue of the **Ab** portion thereof via a cleavable linker conjugated to the antibody via reduced inter-chain disulfides, as described above (see **Schemes 9A-9I**) and in Elgersma, R.C. *et al.* (2014) "*Design, Synthesis, and Evaluation of Linker-Duocarmycin Payloads: Toward Selection of HER2-Targeting Antibody–Drug Conjugate SYD985*," Mol. Pharmaceut. 12:1813-1835 (see, also WO 02/083180; WO 2010/062171; WO 2011/133039; WO 2015/104359; and WO 2015/185142). The average Drug to antibody Ratio (DAR) is about 2-4, typically about 2.7. It will be understood that the exact DAR may vary for each preparation. The order of the steps of the synthesis may be varied as desired. Preferably, the method used will be that of **Schemes 9A-9I**, as described above, and the linker-DUBA is conjugated to the antibody via reduced inter-chain disulfides.

Example 6
B7-H3-ADC Having Cleavable Linker-Duocarmycin Moiety Retains Biological Activity

[00367] The above-described **hmAb-C B7-H3-ADC** (having an exemplary duocarmycin moiety (DUBA) linked to an amino acid residue of the **Ab** portion thereof via a cleavable linker) ("**hmAb-C-DUBA**") was incubated with cells for 7 days and viability

was determined using an Alamar blue assay essentially as described above. As shown in **Figures 15A-15C**, the **hmAb-C-DUBA** construct retained biological activity, as evidenced by its cytotoxic activity on B7-H3 postive tumor cells. Similar results were observed for the above-described **chmAb-C** linked to duocarmycin ("**chmAb-C-DUBA**").

[00368] In this study and additional studies described below, a molecule that binds an unrelated antigen (CD20) conjugated to DUBA ("**Ctrl-DUBA**") was used as a non-binding control ADC to account for non-specific activity in vivo due to rodent-specific carboxyesterase CES1c present in rodent plasma.

Example 7
B7-H3-ADC Exhibits Potent Anti-Tumor Activity *in vivo*

[00369] A multidose study was undertaken in order to assess the *in vivo* efficacy of the molecule. Calu-6 non-small cell lung carcinoma cells were subcutaneously implanted into groups of mice (n=5) essentially as described above, which then received doses of **hmAb-C-DUBA** (1 mg/kg x 3, 3 mg/kg x 3, or 6 mg/kg x 3) at Day 24, 31, 38 and 45 (shown by arrows) post inoculation, and the animals were evaluated for tumor volume (essentially as described above) for up to 62 days. As shown in **Figure 16**, all three tested doses of **hmAb-C-DUBA** proved to be effective in reducing or eliminating tumor volume. Calu-6 cells exhibited an IHC score of 2+ and the Antibody Binding Sites per Cell (ABC) is reported in **Table 9**.

[00370] In a second *in vivo* study (performed essentially as described above), Calu-6 non-small cell lung carcinoma cells were subcutaneously implanted into groups of mice (n=7), which then received a single dose of **hmAb-C-DUBA** or Ctrl-DUBA (3 mg/kg or 10 mg/kg) at Day 20 (shown by arrow). **Table 16** and **Figure 17** summarize the results, and show that the provision of **hmAb-C-DUBA** significantly decreased tumor volume.

Table 16

Treatment	Dose – QW (mg/kg)	Tumor Volume Treatment/Control %	Complete Remission
hmAb-C-DUBA	10	8	0/7
hmAb-C-DUBA	3	41	0/7
Ctrl-DUBA	10	71	0/7
Ctrl-DUBA	3	71	0/7

[00371] In a third *in vivo* study (performed essentially as described above), PA-1 ovarian carcinoma cells were subcutaneously implanted into groups of mice (n=6), which

then received a single dose of **hmAb-C-DUBA** or Ctrl-DUBA (1 mg/kg, 6 mg/kg or 10 mg/kg) at Day 25 (shown by arrow). **Table 17** and **Figure 18** summarize the results, and show that the provision of **hmAb-C-DUBA** significantly decreased tumor volume, and achieved complete remission of up to half the treated animals.

Table 17			
Treatment	**Dose – QW (mg/kg)**	**Tumor Volume Treatment/Control %**	**Complete Remission**
hmAb-C-DUBA	10	11	3/6
hmAb-C-DUBA	6	9	2/6
hmAb-C-DUBA	3	57	1/6
Ctrl-DUBA	10	84	0/6
Ctrl-DUBA	6	89	0/6
Ctrl-DUBA	3	111	0/6

[00372] Potent *in vivo* activity was also observed against A375.S2 melanoma cells. Such cells were subcutaneously implanted into groups of mice (n=7) (essentially as described above), which then received a single dose of **hmAb-C-DUBA** or Ctrl-DUBA (1 mg/kg or 3 mg/kg) at Day 25 (shown by arrow). **Table 18** and **Figure 19** summarize the results, and show that the provision of **hmAb-C-DUBA** significantly decreased tumor volume, and achieved complete remission in 5/7 treated animals at the higher dose tested.

Table 18			
Treatment	**Dose – QW (mg/kg)**	**Tumor Volume Treatment/Control %**	**Complete Remission**
hmAb-C-DUBA	3	1	5/7
hmAb-C-DUBA	1	16	1/7
Ctrl-DUBA	3	33	0/7
Ctrl-DUBA	1	70	1/7

[00373] Potent *in vivo* activity was observed against MDA-MB468 breast carcinoma cells. Such cells were implanted into the mammary fat pads of groups of mice (n=5) (essentially as described above), which then received either a single dose of **hmAb-C-DUBA** or Ctrl-DUBA (3 mg/kg or 6 mg/kg) at Day 70 or three doses of **hmAb-C-DUBA** or Ctrl-DUBA (3 mg/kg (shown by arrows). The animals were evaluated for tumor volume (essentially as described above) for up to 110 days. MDA-MB468 cells exhibited an IHC score of 2+, and the ABC is reported in **Table 9**. **Table 19** and **Figures 20A-20D** summarize the results. **Figure 20A** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 6 mg/kg (single dose). **Figure 20B** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 3 mg/kg (single dose). **Figure 20C** shows results for vehicle, **hmAb-C-**

DUBA or Ctrl-DUBA at 3 mg/kg (three doses). **Figure 20D** shows all of the results on a single graph. The data show that the provision of **hmAb-C-DUBA** significantly decreased tumor volume, and achieved complete remission in 4/5 treated animals at the higher dose tested, and that provision of replicate doses markedly improved the treatment outcome.

Table 19

Treatment	Dose – QW (mg/kg)	Tumor Volume Treatment/Control %	Complete Remission
hmAb-C-DUBA	6	1	4/5
hmAb-C-DUBA	3	51	1/5
hmAb-C-DUBA	3 x 3doses	2	3/5
Ctrl-DUBA	6	41	0/5
Ctrl-DUBA	3	43	0/5
Ctrl-DUBA	3 x 3 doses	53	0/5

[00374] In a further study, xenographs of PA-1 ovarian carcinoma cells (~5 x 10^6 tumor cells suspended in 1:1 media and MATRIGEL®) were subcutaneously introduced into of groups of mice which then received a dose of **hmAb-C-DUBA** or Ctrl-DUBA (either a single dose of 3 mg/kg, 6 mg/kg or 10 mg/kg) at day 24 post-inoculation, or two doses of 10 mg/kg **hmAb-C-DUBA** (at days 24 and 28 post-inoculation) or four doses of 6 mg/kg **hmAb-C-DUBA** (at days 24, 28, 31 and 35 post-inoculation). The animals were evaluated for tumor volume for up to 70 days (essentially as described above). PA-1 cells exhibited an IHC score of 2+, and the ABC is reported in **Table 9**. **Figures 21A-21D** summarize the results. **Figure 21A** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 10 mg/kg (single or double dose). **Figure 21B** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 6 mg/kg (single or quadruple dose). **Figure 21C** shows results for vehicle, **hmAb-C-DUBA** or Ctrl-DUBA at 3 mg/kg (single doses). **Figure 21D** shows all of the results on a single graph. The data show that the provision of **hmAb-C-DUBA** significantly decreased tumor volume in treated animals.

Example 8
Pharmacokinetics of B7-H3-ADC

[00375] The pharmacokinetics of the above-described **chmAb-C-DUBA** was investigated using a log/linear plot of total IgG or intact ADC curve in mice (n=3) that had each received a single intravenous dose of **chmAb-C-DUBA** (5 mg/kg). The results are shown in **Figure 22**.

[00376] The pharmacokinetics of **hmAb-C-DUBA** was investigated using a log/linear plot of total IgG or intact ADC curve in cynomolgus monkeys that had each received a

single intravenous dose of **hmAb-C-DUBA** (1 mg/kg (1 male; 1 female), 3 mg/kg (1 male; 1 female), 10 mg/kg (1 male; 1 female) or 27 mg/kg (2 males; 2 females)). The results are shown in **Figure 23A** (total IgG) and **Figure 23B** (intact ADC).

[00377] In these studies, total IgG was determined by ELISA. Briefly, Serum samples, standards and controls were captured on microtiter plates coated with goat anti-human IgG (H+L). Following washing, plates were incubated with peroxidase-conjugated goat anti-human IgG Fc. Following washing, plates were developed with 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate, the reaction was stopped with phosphoric acid, and the plates were read at 405 nM. Total IgG in the test samples was calculated from the standard curve. Intact ADC was also determined by ELISA. Briefly, Mouse anti-duocarmycin mAb was immobilized onto microtiter plates. Following washing, plates were incubated with peroxidase-conjugated goat anti-human IgG Fc. Following washing, plates were developed with 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate, the reaction was stopped with phosphoric acid, and the plates were read at 405 nM. Intact ADC in the test samples was calculated from the standard curve.

[00378] The pharmacokinetic parameters for the murine 5 mg/kg and the cynomolgus monkey 3 mg/kg and 10 mg/kg doses were deduced by comparing such data and are summarized in **Table 20** (wherein AUC Last denotes area under curve from the origin to the last data point). Exposure in mouse of intact ADC is limited due to rodent-specific carboxyesterase CES1c. These data indicate a large therapeutic index in a preclinical setting.

Table 20

Species	Dose (mg/kg)	$T_{1/2}$ (hr)	C_{max} (ng/ml)	AUC Last (hr*µg/ml)
Mouse	5	ND	5909	45
Cyno	3	62.7	113484	3798
Cyno	10	57.3	330983	17978

Example 9
Characterization of Anti-B7-H3 Diabodies

[00379] The B7-H3 x CD3 bispecific two-chain and three-chain diabodies are evaluated to determine their ability to mediate redirected cell killing and/or cytokine release from target cells expressing cell surface B7-H3. Redirected cell killing is examined using a cytotoxic T lymphocyte (CTL) assay. Briefly, B7-H3 x CD3 bispecific diabodies (or a

negative control diabody that binds an irrelevant antigen instead of B7-H3) are incubated for 24 hours with effector pan T-cells and target B7-H3-expressing tumor cells at an effector to target cell ratio of 10:1. The percentage cytotoxicity (*i.e.*, cell killing) is determined by measuring the release of lactate dehydrogenase (LDH) into the media by damaged cells. Cytokine release is examined using a similar format. Briefly, B7-H3 x CD3 bispecific diabodies (or a negative control diabody lacking an B7-H3-binding site) are incubated for 24 hours with effector PBMC cells alone or in the presence of target tumor cells (*e.g.*, SK-MES-1 lung carcinoma cells) at an effector to target cell ratio of 10:1 or 30:1 and the release of IFNγ, TNF-α, and IL-10 cytokines is determined. The analysis shows the ability of the B7-H3 x CD3 bispecific diabodies to mediate redirected cell killing and cytokine release.

[00380] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

WHAT IS CLAIMED IS:

Claim 1. An anti-B7-H3 antibody drug conjugate (**B7-H3-ADC**) that comprises the formula:

$$\textbf{Ab-(LM)}_{\textbf{m}}\textbf{-(D)}_{\textbf{n}},$$

wherein:

Ab is a humanized B7-H3 antibody or B7-H3 binding fragment thereof that binds to B7-H3 and comprises:

(i) the CDR_L1 sequence RASESIYSYLA, the CDR_L2 sequence NTKTLPE and the CDR_L3 sequence QHHYGTPPWT in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYGMS, the CDR_H2 sequence TINSGGSNTYY PDSLKG and the CDR_H3 sequence HDGGAMDY in its Variable Heavy Chain (VH) domain;

D is a cytotoxic drug moiety comprising a duocarmycin cytotoxin;

LM comprises at least one bond or a Linker Molecule that covalently links Ab and D;

m is an integer between 0 and n and denotes the number of Linker Molecules of the **B7-H3-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic drug moieties covalently linked to the **B7-H3-ADC** molecule.

Claim 2. The **B7-H3-ADC** of claim 1, wherein said Ab comprises:

(i) a humanized Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20**; and

(ii) a humanized Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**.

Claim 3. The **B7-H3-ADC** of claim 1 or claim 2, wherein said **Ab** further comprises an Fc domain of a human IgG.

Claim 4. The **B7-H3-ADC** of claim 3, wherein said human IgG is a human IgG1, IgG2, IgG3, or IgG4.

Claim 5. The **B7-H3-ADC** of claim 3 or claim 4, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

Claim 6. The **B7-H3-ADC** of claim 5, wherein said modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

Claim 7. The **B7-H3-ADC** of claim 5 or claim 6, wherein said modifications that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

Claim 8. The **B7-H3-ADC** of any one of claims 1-7, wherein at least one of said **LM** moieties is a Linker Molecule.

Claim 9. The **B7-H3-ADC** of claim 8, wherein said **LM** Linker Molecule comprises a cleavable linker.

Claim 10. The **B7-H3-ADC** of claim 9, wherein said cleavable linker is a peptidic linker.

Claim 11. The **B7-H3-ADC** of claim 10, wherein said peptidic linker is a valine-citrulline dipeptide linker.

Claim 12. The **B7-H3-ADC** of any one of claims 9-11, wherein said **LM** Linker Molecule further comprises a self-eliminating spacer between the cleavable linker and **D**.

Claim 13. The **B7-H3-ADC** of claim 12, wherein said self-eliminating spacer comprises a para-aminobenzyloxycarbonyl moiety.

Claim 14. The **B7-H3-ADC** of any one of claims 9-13, wherein said Linker Molecule further comprises a maleimide linker moiety between the cleavable linker and **Ab**.

Claim 15. The **B7-H3-ADC** of claim 8, wherein said conjugate comprises the formula:

Ab – [V-(W)$_k$-(X)$_1$-A] – D, and **LM** comprises the formula:

[V-(W)$_k$-(X)$_1$-A],

wherein:

V is a cleavable linker,

(W)$_k$-(X)$_1$-A is an elongated, self-eliminating spacer system, that self-eliminates via a l,(4+2n)-elimination,

W and **X** are each a l,(4+2n) electronic cascade spacer, being the same or different,

A is either a spacer group of formula (**Y**)$_m$, wherein **Y** is a l,(4+2n) electronic cascade spacer, or a group of formula **U**, being a cyclisation elimination spacer,

k, 1 and m are independently an integer of 0 (included) to 5 (included),

n is an integer of 0 (included) to 10 (included),

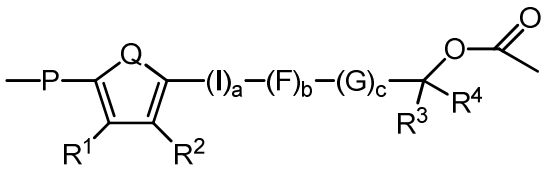
with the provisos that:

when **A** is (**Y**)$_m$: then k+l+m ≥ 1, and

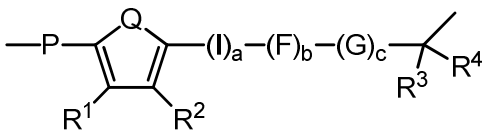
if k+l+m=l, then n>l;

when **A** is **U**: then k+1 ≥ 1.

W, **X**, and **Y** are independently selected from compounds having the formula:



or the formula:

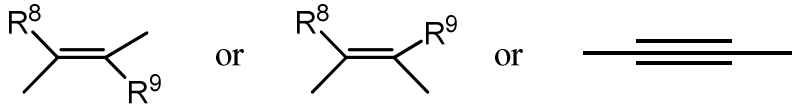


wherein: Q is $-R^5C=CR^6-$, S, O, NR^5, $-R^5C=N-$, or $-N=CR^5-$

P is NR^7, O or S

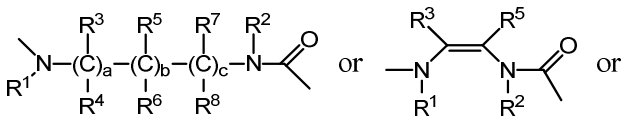
a, b, and c are independently an integer of 0 (included) to 5 (included);

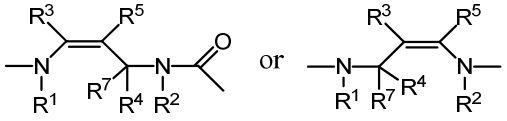
I, F and G are independently selected from compounds having the formula:



wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, and R^9 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), di-substituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are independently selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, or R^9 optionally being connected to one another to form one or more aliphatic or aromatic cyclic structures;

U is selected from compounds having the formula:





wherein:

a, b and c are independently selected to be an integer of 0 or 1; provided that a + b + c = 2 or 3;

R^1 and/or R^2 independently represent H, C1-6 alkyl, the alkyl being optionally substituted with one or more of the following groups: hydroxy (OH), ether (OR_x), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_X), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_X$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate (S(=O)ORx), sulphinyl (S(=O)Rx), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group; and

R^3, R^4, R^5, R^6, R^7 and R^8 independently represent H, C_{1-6} alkyl, C_{3-20} heterocyclyl, C_{5-20} aryl, C_{1-6} alkoxy, hydroxy (OH), amino (NH_2), mono-substituted amino (NR_xH), disubstituted amino ($NR_x^1R_x^2$), nitro (NO_2), halogen, CF_3, CN, $CONH_2$, SO_2Me, CONHMe, cyclic C_{1-5} alkylamino, imidazolyl, C_{1-6} alkylpiperazinyl, morpholino, thiol (SH), thioether (SR_x), tetrazole, carboxy (COOH), carboxylate ($COOR_x$), sulphoxy ($S(=O)_2OH$), sulphonate ($S(=O)_2OR_x$), sulphonyl ($S(=O)_2R_x$), sulphixy (S(=O)OH), sulphinate ($S(=O)OR_x$), sulphinyl ($S(=O)R_x$), phosphonooxy ($OP(=O)(OH)_2$), and phosphate ($OP(=O)(OR_x)_2$), where R_x, R_x^1 and R_x^2 are selected from a C_{1-6} alkyl group, a C_{3-20} heterocyclyl group or a C_{5-20} aryl group, and two or more of the substituents R^1, R^2, R^3, R^4, R^5, R^6, R^7, or R^8 are optionally connected to one another to form one or more aliphatic or aromatic cyclic structures.

Claim 16. The **B7-H3-ADC** of claim 15, wherein said **LM** linker molecule comprises:

(1) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(2) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl;

(3) p-ammocinnamyloxycarbonyl;

(4) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl;

(5) p-amino-benzyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(6) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl;

(7) p-aminophenylpentadienyloxycarbonyl;

(8) p-aminophenylpentadienyloxycarbonyl-p-arninocinnamyloxycarbonyl;

(9) p-aminophenylpentadienyloxycarbonyl-paminobenzyloxycarbonyl;

(10) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyloxycarbonyl;

(11) p-aminobenzyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(12) p-aminocinnamyloxycarbonyl(methylamino)ethyl(methylamino) carbonyl;

(13) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl(methylamino) ethyl(methylamino)carbonyl;

(14) p-aminocinnamyloxycarbonyl-p-aminobenzyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(15) p-aminobenzyloxycarbonyl-p-arninocinnamyloxycarbonyl (methylamino)ethyl(methylamino)-carbonyl;

(16) p-aminocinnamyloxycarbonyl-p-aminocinnamyloxycarbonyl (methylamino)ethyl(methylamino)carbonyl;

(17) p-aminobenzyloxycarbonyl-p-aminobenzyl;

(18) p-aminobenzyloxycarbonyl-p-aminobenzyloxycarbonyl -p-aminobenzyl;

(19) p-aminocinnamyl;

(20) p-aminocinnamyloxycarbonyl-p-aminobenzyl;

(21) p-aminobenzyloxycarbonyl-p-aminocinnamyl;

(22) p-amino-cinnamyloxycarbonyl-p-aminocinnamyl;

(23) p-aminophenylpentadienyl;

(24) p-aminophenylpentadienyloxycarbonyl-p-aminocinnamyl;

(25) p-aminophenylpentadienyloxycarbonyl-p-aminobenzyl;

or

(26) p-aminophenylpentadienyloxycarbonyl-p-aminophenylpentadienyl.

Claim 17. The **B7-H3-ADC** of any one of claims 8-16, wherein said **LM** Linker Molecule is conjugated to the side chain of an amino acid of a polypeptide chain of **Ab** and binds the **Ab** to a molecule of the cytotoxic drug moiety **D**.

Claim 18. The **B7-H3-ADC** of any one of claims 1-17, wherein said duocarmycin cytotoxin is selected from the group consisting of: duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, CC-1065, adozelesin, bizelesin, carzelesin (U-80244), *seco*-duocarmycin and spiro-duocarmycin (DUBA).

Claim 19. The **B7-H3-ADC** of claim 18, wherein said duocarmycin cytotoxin is *seco*-duocarmycin.

Claim 20. The **B7-H3-ADC** of any of claims 1-19, wherein said LM Linker Molecule is covalently linked to the **Ab** via reduced inter-chain disulfides.

Claim 21. The **B7-H3-ADC** of any of claims 1-14 and 17-20, wherein said **Ab** comprises:

(i) a light chain comprising the Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20** and a CL Kappa Domain of **SEQ ID NO:1**; and

(ii) a heavy chain comprising the Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**, a CH1 Domain of **SEQ ID NO:3**, a Hinge Domain of **SEQ ID NO:7** and a Fc Domain comprising a CH2-CH3 Domain of **SEQ ID NO:12**;

said **D** comprises *seco*-duocarmycin; and

said **LM** comprises a Linker Molecule comprising a maleimide linker moiety, a valine-citrulline dipeptide linker, and a para-aminobenzyloxycarbonyl moiety.

Claim 22. A B7-H3 binding molecule, wherein said B7-H3 binding molecule is an antibody, a diabody, or an epitope-binding fragment thereof, which is capable of binding to B7-H3, wherein said B7-H3 binding molecule comprises:

(i) a humanized Variable Light Chain (VL) domain comprising the amino acid sequence of **SEQ ID NO:20**; and

(ii) a humanized Variable Heavy Chain (VH) domain comprising the amino acid sequence of **SEQ ID NO:21**.

Claim 23. The B7-H3 binding molecule of claim 22, which further comprises an Fc Domain of a human IgG.

Claim 24. The B7-H3 binding molecule of claim 23, wherein said human IgG is a human IgG1, IgG2, IgG3, or IgG4.

Claim 25. The B7-H3 binding molecule of claim 23 or claim 24, wherein said Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduces the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Domain.

Claim 26. The B7-H3 binding molecule of claim 25, wherein said modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

Claim 27. The B7-H3 binding molecule of claim 25 or claim 26, wherein said modifications that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

Claim 28. A pharmaceutical composition that comprises an effective amount of the **B7-H3-ADC** of any one of claims 1-21, or the B7-H3 binding molecule of

any one of claims 22-27, and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 29. The **B7-H3-ADC** of any one of claims 1-21, the B7-H3 binding molecule of any one of claims 22-27, or the pharmaceutical composition of claim 28 for use as a medicament.

Claim 30. Use of the **B7-H3-ADC** of any one of claims 1-21, the B7-H3 binding molecule of any one of claims 22-27, or the pharmaceutical composition of claim 28, in the manufacture of a medicament for treating cancer.

Claim 31. The use of claim 30, wherein said cancer is selected from the group consisting of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, an adrenal cancer, a bladder cancer, a bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a B-cell cancer, 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, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, a glioblastoma, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, an acute myeloid leukemia, a chronic lymphocytic leukemia, a chronic myeloid leukemia, a hairy cell leukemia, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a Burkett's lymphoma, a diffuse large B cell lymphoma, a follicular lymphoma, a mantle cell lymphoma, a marginal zone lymphoma, a non-Hodgkin's lymphoma, a small lymphocytic lymphoma, a lung cancer, a non-small-cell lung cancer (NSCLC), a medulloblastoma, a melanoma, a meningioma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a

parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a renal cell carcinoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood, a soft-tissue sarcoma, a squamous cell cancer, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer, a thyroid metastatic cancer, and a uterine cancer.

Claim 32. The use of claim 30, wherein said cancer is selected from the group consisting of: an acute myeloid leukemia, a non-small cell lung cancer, a neuroblastoma, a squamous cell cancer of the head and neck, a prostate cancer and a thyroid metastatic cancer.

Claim 33. The use of claim 30, wherein the cancer is a prostate cancer.