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

Summary ~~of the Invention~~:

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

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

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

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

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

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

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

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

[0030a] In one aspect, there is provided a B7-H3 binding molecule, wherein said molecule is an isolated antibody, or an immunoreactive fragment thereof, which comprises a variable domain that specifically binds an extracellular domain of B7-H3, wherein said molecule:

(A) is internalized upon binding to B7-H3 expressed on the surface of a cancer cell; and

(B) comprises a variable domain that comprises or retains the binding specificity of:

(1) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 21), CDR_2 (SEQ ID NO: 23) and CDR_3 (SEQ ID NO: 25), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 29), CDR_2 (SEQ ID NO: 31) and CDR_3 (SEQ ID NO: 33);

(2) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 5), CDR_2 (SEQ ID NO: 7) and CDR_3 (SEQ ID NO: 9), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 13), CDR_2 (SEQ ID NO: 15) and CDR_3 (SEQ ID NO: 17); or

(3) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 37), CDR_2 (SEQ ID NO: 39) and CDR_3 (SEQ ID NO: 41), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 45), CDR_2 (SEQ ID NO: 47) and CDR_3 (SEQ ID NO: 49).

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

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

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

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

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

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

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

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

(1) F243L, R292P and Y300L;

(2) F243L, R292P and V305I;

(3) F243L, R292P and P396L; and

(4) R292P, V305I and P396L;

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

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

(2) F243L, R292P, V305I and P396L;

or

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

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

(A) F243L, R292P, and Y300L;

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

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

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

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

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

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

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

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

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

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

[0037a] In another aspect, there is provided a hybridoma that secretes the isolated antibody as defined above.

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

[0038a] In yet another aspect, there is provided a isolated nucleic acid molecule that encodes a polypeptide chain of the B7-H3 binding molecule as defined above.

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

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

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

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

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

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

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

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

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

[0044a] In one aspect, there is provided a pharmaceutical composition comprising (i) a therapeutically effective amount of the B7-H3 binding molecule as defined above and (ii) a pharmaceutically acceptable carrier.

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

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

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

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

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

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

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

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

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

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

[0050] The ~~invention~~disclosure further concerns the above-described use characterised in that the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a dhordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a

multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious unveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0050a] In one aspect, there is provided the use of the B7-H3 binding molecule as defined above or of the pharmaceutical composition as defined above in the preparation of a medicament for the treatment of cancer in a patient.

[0051] The ~~invention~~disclosure further concerns the use of any of the above-described antibodies or immunoreactive fragments or dual affinity retargeting reagents (DART™s) in the preparation of a medicament for the treatment or the prevention of cancer in a patient. The ~~invention~~disclosure further concerns such uses characterised in that the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a dhordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious unveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

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

What Is Claimed Is:

Claim 1. A B7-H3 binding molecule, wherein said molecule is an isolated antibody, or an immunoreactive fragment thereof, ~~or a diabody,~~ which comprises a variable domain that specifically binds an extracellular domain of B7-H3, wherein said molecule:

~~(A) binds to 4Ig and 2Ig isoforms of B7-H3 that is endogenously expressed on the surface of a cancer cell;~~

(A)~~(B)~~ is internalized upon binding to B7-H3 expressed on the surface of a cancer cell; and

(B)~~(C)~~ comprises a variable domain that comprises or retains the binding specificity of:

(1) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 21), CDR_2 (SEQ ID NO: 23) and CDR_3 (SEQ ID NO: 25), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 29), CDR_2 (SEQ ID NO: 31) and CDR_3 (SEQ ID NO: 33);

(2) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 5), CDR_2 (SEQ ID NO: 7) and CDR_3 (SEQ ID NO: 9), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 13), CDR_2 (SEQ ID NO: 15) and CDR_3 (SEQ ID NO: 17); or

(3) a light chain variable domain that comprises CDR_1 (SEQ ID NO: 37), CDR_2 (SEQ ID NO: 39) and CDR_3 (SEQ ID NO: 41), and a heavy chain variable domain that comprises CDR_1 (SEQ ID NO: 45), CDR_2 (SEQ ID NO: 47) and CDR_3 (SEQ ID NO: 49).

Claim 2. The B7-H3 binding molecule of claim 1, which is a humanized monoclonal antibody.

Claim 3. The B7-H3 binding molecule of any of claims 1-2, wherein said molecule comprises a variant human IgG1 Fc region, wherein said variant human IgG1 Fc region comprises at least one amino acid modification relative to a wild-

Claim 11. An isolated nucleic acid molecule that encodes a polypeptide chain of the B7-H3 binding molecule of any of claims 1-9.

~~Claim 12. The B7-H3 binding molecule of claim 1, wherein said molecule is a diabody comprising:~~

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

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

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

~~Claim 13. The B7-H3 binding molecule of claim 12, wherein said molecule other than B7-H3 is a hapten.~~

~~Claim 14. The B7-H3 binding molecule of claim 13, wherein said hapten is fluorescein isothiocyanate.~~

~~Claim 15. The B7-H3 binding molecule of claim 12, wherein said molecule other than B7-H3 is a T-Cell Receptor or the NKG2D receptor.~~

~~Claim 16. The B7-H3 binding molecule of claim 12, wherein said molecule other than B7-H3 is a tumor-associated antigen.~~

~~Claim 17. The B7-H3 binding molecule of claim 16, wherein said tumor-associated antigen is selected from the group consisting of A33; ADAM-9; ALCAM; BAGE; beta-catenin; CA125; Carboxypeptidase-M; CD103; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD36; CD40/CD154; CD45; CD46; CD5; CD56; CD79a/CD79b; CDK4; CEA; CTLA4; Cytokeratin-8; EGF-R; EphA2; ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; gp100; HER-2/neu; human-papillomavirus-E6; human-papillomavirus-E7; Integrin-Alpha-V-Beta-6; JAM-3; KID3; KID31; KSA-(17-1A); LUCA-2; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; N-acetylglucosaminyltransferase; Oncostatin-M;~~

~~p15; PIPA; PSA; PSMA; ROR1; sTn; TNF-ß receptor; TNF-α receptor; TNF-γ receptor; Transferrin Receptor; and VEGF receptor.~~

~~Claim 18. A nucleic acid molecule that encodes a polypeptide chain of the B7-H3 binding molecule of any of claims 12-17.~~

Claim 12~~19~~. A pharmaceutical composition comprising (i) a therapeutically effective amount of the B7-H3 binding molecule of any of claims 1-9 ~~or 12-17~~ and (ii) a pharmaceutically acceptable carrier.

Claim 13~~20~~. The pharmaceutical composition of claim 12~~19~~, which further comprises one or more additional anti-cancer agents.

Claim 14~~21~~. The pharmaceutical composition of claim 13~~20~~, wherein said additional anti-cancer agent is a chemotherapeutic agent, a radiation therapeutic agent, a hormonal therapeutic agent, a toxin or an immunotherapeutic agent.

Claim 15~~22~~. The pharmaceutical composition of claim 14~~20~~, wherein said additional anti-cancer agent is a toxin selected from the group consisting of: a taxane, a maytansinoid, an auristatin, a calicheamicin, an anthracycline, a CC-1065 analog, docetaxel, a cathepsin, ricin, gelonin, Pseudomonas exotoxin, diphtheria toxin, RNase, and a toxic radioisotope.

Claim 16~~23~~. The use of the B7-H3 binding molecule of any of claims 1-9 ~~or 12-17~~ or of the pharmaceutical composition of any of claims 12-15~~19-22~~ in the preparation of a medicament for the treatment of cancer in a patient.

Claim 17~~24~~. The use of the B7-H3 binding molecule of claim 16~~23~~, characterised in that said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a 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