[0037] 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 chains each possessing a VH1, VL2, VH2, and VL2 Domain.

[0038] However, despite all prior advances, a need remains for compositions that could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to this and other goals.

Summary:

[0039] The present disclosure relates to Tri-Specific Binding Molecules, which are multi-chain polypeptide molecules that possess three Binding Domains and are thus capable of mediating coordinated binding to three epitopes. The Tri-Specific Binding Molecule is preferably characterized in possessing binding domains that permit it to immunospecifically bind to: (1) an epitope of a first Cancer Antigen, (2) an epitope of a second Cancer Antigen, and (3) an epitope of a molecule that is expressed on the surface of an immune system effector cell, and are thus capable of localizing an immune system effector cell to a cell that expresses a Cancer Antigen, so as to thereby facilitate the killing of such cancer cell.

[0040] In detail, the disclosure provides a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, said Epitopes being Epitope I, Epitope II, and Epitope III, wherein two of three epitopes are epitopes of Cancer Antigen(s), and the third of said epitopes is an epitope of an Effector Cell Antigen.

[0041] The disclosure particularly concerns the embodiment of such Tri-Specific Binding Molecule wherein the molecule comprises four different polypeptide chains covalently complexed together and comprises:

(I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that

is capable of immunospecifically binding to an Epitope II present on a second antigen, wherein the Antigen-Binding Domain I and the Antigen-Binding Domain II are both Diabody-Type Binding Domains;

(II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(III) an Fc Domain that is formed by the complexing of two CH2-CH3 Domains to one another;

wherein one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and the third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen, and wherein the Antigen-Binding Domains I, II and III of the Binding Molecules mediate coordinated binding of an immune system effector cell expressing the Effector Cell Antigen and a cancer cell expressing the first and second Cancer Antigens.

[0041a] In one aspect, there is provided a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:
four different polypeptide chains covalently complexed together, and:

(I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(III) a Fc Domain, wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, from the N-terminus to the C-terminus:

(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) a fourth polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{III} Domain)—(Cysteine-Containing Domain);

(F) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III}

Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III: and

(G) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

[0041b] In another aspect, there is provided a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising: three different polypeptide chains covalently complexed together, and:

(I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(III) a Fc Domain; wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain); or

(Cysteine-Containing Domain 1)—(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain 2)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III;

(F) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III}

Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain; and

(G) the first, second and third antigens are the same antigen, or are independently the same or different from another of the antigens.

[0042] The disclosure particularly concerns the embodiment of such Tri-Specific Binding Molecules wherein the Fc Domain is capable of binding to an Fc Receptor arrayed on the surface of a cell.

[0043] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Effector Cell Antigen is arrayed on the surface of an effector cell and wherein the Cancer Antigens are arrayed on the surface of a cancer cell, and wherein the immunospecific binding is sufficient to co-localize the Effector Cell Antigen, and the Cancer Antigens, thereby facilitating the activation of the effector cell against the cancer cell.

[0044] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Effector Cell Antigen is selected from the group consisting of: CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, the B cell Receptor (BCR), the T cell Receptor (TCR), and the NKG2D Receptor.

[0045] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the first and second Cancer Antigens are independently selected from the group consisting of: colon cancer antigen 19.9; a gastric cancer

mucin; antigen 4.2; glycoprotein A33 (gpA33); ADAM-9; gastric cancer antigen AH6; ALCAM; malignant human lymphocyte antigen APO-1; cancer antigen B1; B7-H3; beta-catenin; blood group ALe^b/Le^y; Burkitt's lymphoma antigen-38.13, colonic adenocarcinoma antigen C14; ovarian carcinoma antigen CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD30 ; CD33; CD36; CD45; CD46; CD52; CD79a/CD79b; CD103; CD317; CDK4; carcinoembryonic antigen (CEA); CEACAM5; CEACAM6; CO17-1A; CO-43 (blood group Le^b); CO-514 (blood group Le^a); CTA-1; CTLA4; Cytokeratin 8; antigen D1.1; antigen $D_1$56-22; DR5; E_1 series (blood group B); EGFR (Epidermal Growth Factor Receptor); Ephrin receptor A2 (EphA2); ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; lung adenocarcinoma antigen F3; antigen FC10.2; G49, ganglioside GD2; ganglioside GD3; ganglioside GM2; ganglioside GM3; G_{D2}; G_{D3}; GICA 19-9; G_{M2}; gp100; human leukemia T cell antigen Gp37; melanoma antigen gp75; gpA33; HER2 antigen ($p185^{HER2}$); human milk fat globule antigen (HMFG); human papillomavirus-E6/human papillomavirus-E7; high molecular weight melanoma antigen (HMW-MAA); I antigen (differentiation antigen) I(Ma); Integrin Alpha-V-Beta-6 Integrinβ6 (ITGB6); Interleukin-13; Receptor α2 (IL13Rα2); JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; human lung carcinoma antigens L6 and L20; LEA; LUCA-2; M1:22:25:8; M18; M39; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; Myl; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; melanoma-associated antigen p97; polymorphic epithelial mucin (PEM); polymorphic epithelial mucin antigen (PEMA); PIPA; prostate-specific antigen (PSA); prostate-specific membrane antigen (PSMA); prostatic acid phosphate; R_{24}; ROR1; sphingolipids; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; T_5A_7; TAG-72; TL5 (blood group A); TNF-α receptor; TNF-ß receptor; TNF-γ receptor; TRA-1-85 (blood group H); Transferrin Receptor; tumor-specific transplantation antigen (TSTA), oncofetal antigen-alpha-fetoprotein (AFP); VEGF; VEGFR, VEP8; VEP9; VIM-D5; and Y hapten, Le^y.

[0046] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the first and second Cancer Antigens are selected from the group consisting of: CD2, CD317, CEACAM5, CEACAM6, DR5, EphA2, gpA33, Her2, B7-H3; EGF, EGFR, VEGF and VEGFR.

[0047] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the Non-Diabody-Type Binding Domain III comprises the Fab-Type Binding Domain (VL_{III}/VH_{III}) that is capable of immunospecifically binding to an Epitope III, wherein the molecule comprises:

(A) a first polypeptide chain that comprises in the N-terminus to C-terminus direction:

(1) a light chain variable Domain of an immunoglobulin capable of binding to a first of the three epitopes (VL_I);

(2) a heavy chain variable Domain of an immunoglobulin capable of binding to a second of the three epitopes (VH_{II});

(3) a Heterodimer-Promoting Domain; and

(4) CH2 and CH3 Domains of an IgG;

(B) a second polypeptide chain that comprises, in the N-terminus to C-terminus direction:

(1) a light chain variable Domain of an immunoglobulin capable of binding to the second of the three epitopes (VL_{II});

(2) a heavy chain variable Domain of an immunoglobulin capable of binding to the first of the three epitopes (VH_I); and

(3) a complementary Heterodimer-Promoting Domain;

(C) a third polypeptide chain that comprises, in the N-terminus to C-terminus direction:

(1) a heavy chain variable Domain of an immunoglobulin capable of binding to a third of the three epitopes (VH_{III}); and

(2) a CH1 Domain, a Hinge Domain, and a CH2-CH3 Domain of an IgG;

and

(D) a fourth polypeptide chain that comprises, in the N-terminus to C-terminus direction:

(1) a light chain variable Domain of an immunoglobulin capable of binding to the third of the three epitopes (VL_{III}); and

(2) a light chain constant Domain (CL);

wherein:

(i) the VL_I and VH_I Domains associate to form a Domain capable of binding the first epitope;

(ii) the VL_{II} and VH_{II} Domains associate to form a Domain capable of binding the second epitope;

(iii) the VL_{III} and VH_{III} Domains associate to form a Domain capable of binding the third epitope;

(iv) the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form an Fc Domain;

(v) the first and second polypeptide chains are covalently bonded to one another;

(vi) the first and third polypeptide chains are covalently bonded to one another; and

(vii) the third and fourth polypeptide chains are covalently bonded to one another.

[0048] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

(A) the Heterodimer-Promoting Domain is an E-coil and the complementary Heterodimer-Promoting Domain is a K-coil; or

(B) the Heterodimer-Promoting Domain is a K-coil and the complementary Heterodimer-Promoting Domain is an E-coil.

[0049] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

(A) the CH2-CH3 Domains of the first and third polypeptide chains each have the sequence of **SEQ ID NO:1**, such that the Fc Domain formed from their association exhibits normal FcγR-mediated effector function; or

(B) the CH2-CH3 Domain of the first and third polypeptide chains comprise at least one amino acid substitution, relative to the sequence of **SEQ ID NO:1**, such that the Fc Domain formed from their association exhibits altered FcγR-mediated effector function.

[0050] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the at least one amino acid substitution comprises at least one amino acid substitution selected from the group consisting of: L235V, F243L, R292P, Y300L, V305I, and P396L, wherein the numbering is that of the EU index as in Kabat.

[0051] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the at least one amino acid substitution comprises:

(A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;

(B) at least two substitutions selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) at least three substitutions 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 four substitutions selected from the group consisting of:

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

(2) F243L, R292P, V305I and P396L;

or

(E) at least the five substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L, V305I and P396L; and

(2) L235V, F243L, R292P, Y300L and P396L.

[0052] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein the CH2-CH3 Domain of the first and third polypeptide chains differ from one another and have an amino acid sequence selected from the group consisting of **SEQ ID NO:52** and **SEQ ID NO:53**.

[0053] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

(A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the second Cancer Antigen and an epitope of the Effector Cell Antigen;

(B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the second Cancer Antigen;

(C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of second Cancer Antigen, an epitope of the first Cancer Antigen, and an epitope of the Effector Cell Antigen;

(D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the second Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the first Cancer Antigen;

(E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of the first Cancer Antigen, and an epitope of the second Cancer Antigen;

and

(F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of second Cancer Antigen, and an epitope of the first Cancer Antigen.

[0054] The disclosure additionally concerns the embodiment of such Tri-Specific Binding Molecules wherein:

(A) the epitope of an Effector Cell Antigen is a CD2 epitope recognized by antibody Lo-CD2a;

(B) the epitope of an Effector Cell Antigen is a CD3 epitope recognized by antibody OKT3, M291, YTH12.5, Anti-CD3 mAb 1 or Anti-CD3 mAb 2;

(C) the epitope of an Effector Cell Antigen is a CD16 epitope recognized by antibody 3G8 or A9;

(D) the epitope of an Effector Cell Antigen is a CD19 epitope recognized by antibody MD1342, MEDI-551, blinatumomab or HD37;

(E) the epitope of an Effector Cell Antigen is a CD20 epitope recognized by antibody rituximab, ibritumomab, ofatumumab, and tositumomab;

(F) the epitope of an Effector Cell Antigen is a CD22 epitope recognized by antibody epratuzumab;

(G) the epitope of an Effector Cell Antigen is a CD32B epitope recognized by antibody CD32B mAb 1;

(H) the epitope of an Effector Cell Antigen is a CD64 epitope recognized by antibody CD64 mAb 1;

(I) the epitope of an Effector Cell Antigen is a BCR/CD79 epitope recognized by antibody CD79 mAb 1;

(J) the epitope of an Effector Cell Antigen is a TCR epitope recognized by antibody BMA 031;

or

(K) the epitope of an Effector Cell Antigen is a NKG2D Receptor epitope recognized by antibody KYK-2.0.

[0054a] In one aspect, there is provided a nucleic acid composition comprising polynucleotides that encode polypeptide chains of any of the above-described Tri-Specific Binding Molecules.

[0055] In another aspect, there is provided a pharmaceutical composition comprising any of the above-described Tri-Specific Binding Molecules, and a pharmaceutically acceptable carrier, excipient or diluent.

[0056] In another aspect, there is provided any of the above-described Tri-Specific Binding Molecules, or a composition as above-described, for use as a medicament. In yet another aspect, there is provided use of any of the above-described Tri-Specific Binding Molecules or a composition as above-described in the manufacture of a medicament to treat a cancer.

[0057] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules wherein 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 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, 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 uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0058] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules wherein the cancer is acolorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer or a rectal cancer.

[0059] The disclosure additionally concerns the embodiment of such pharmaceutical compositions or such Tri-Specific Binding Molecules the cancer is acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

What Is Claimed Is:

Claim 1. A Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising: four different polypeptide chains covalently complexed together, and:

(I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(III) a Fc Domain, wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{II} Domain)—(Linker 1)—(VH_{I} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, from the N-terminus to the C-terminus:

(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) a fourth polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{III} Domain)—(Cysteine-Containing Domain);

(F) the VL_{I} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_{I} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III: and

(G) the VL_{I} Domain and the VH_{I} Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain.

Claim 2. The Tri-Specific Binding Molecule of claim 1, wherein the Cysteine-Containing Domain of the third polypeptide chain is chosen from a CH1 Domain and/or a Hinge Domain and the Cysteine-Containing Domain of the fourth polypeptide chain is a CL Domain.

Claim 3. The Tri-Specific Binding Molecule of claim 1 or claim 2, wherein (i) the first polypeptide chain and the second polypeptide chain are covalently bonded to one another; (ii) the first polypeptide chain and the third polypeptide chain are covalently bonded to one another; and (iii) the third polypeptide chain and the fourth polypeptide chain are covalently bonded to one another.

Claim 4. A Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising: three different polypeptide chains covalently complexed together, and:

(I) an Antigen-Binding Domain I that is capable of immunospecifically binding to an Epitope I present on a first antigen, and an Antigen-Binding Domain II that is capable of immunospecifically binding to an Epitope II present on a second antigen;

(II) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and

(III) a Fc Domain; wherein:

(A) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen, a second of Epitope I, Epitope II or Epitope III is an epitope of a first Cancer Antigen, and a third of Epitope I, Epitope II or Epitope III is an epitope of a second Cancer Antigen;

(B) a first polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain)—(Linker 3)—(CH2-CH3 Domain); or

(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain); or

(Cysteine-Containing Domain)—(CH2-CH3 Domain)—(Linker 4)—(VL_I Domain)—(Linker 1)—(VH_{II} Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(C) a second polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{II} Domain)—(Linker 1)—(VH_I Domain)—(Linker 2)—(Heterodimer-Promoting Domain);

(D) a third polypeptide chain comprises, from the N-terminus to the C-terminus:

(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain); or

(Cysteine-Containing Domain 1)—(VL_{III} Domain)—(Flexible Linker)—(VH_{III} Domain)—(Cysteine-Containing Domain 2)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

(E) the VL_I Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VH_I Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope I, the VL_{II} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II, the VL_{III} Domain is a Light Chain Variable Domain of an immunoglobulin capable of binding to Epitope III, and the VH_{III} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope III;

(F) the VL_I Domain and the VH_I Domain associate to form the Antigen-Binding Domain I, the VL_{II} Domain and the VH_{II} Domain associate to form the Antigen-Binding Domain II, the VL_{III} Domain and the VH_{III} Domain associate to form the Antigen-Binding Domain III, and the CH2-CH3 Domain of the first polypeptide chain and the CH2-CH3 Domain of the third polypeptide chain associate to form the Fc Domain; and

(G) the first, second and third antigens are the same antigen, or are independently the same or different from another of the antigens.

Claim 5. The Tri-Specific Binding Molecule of claim 4, wherein the Cysteine-Containing Domain 1 of the third polypeptide chain is a CL Domain and the Cysteine-Containing Domain 2 of the third polypeptide chain is chosen from a CH1 Domain and/or a Hinge Domain.

Claim 6. The Tri-Specific Binding Molecule of any one of claims 1-5, wherein:

the Linker 2 comprises a cysteine residue, or

the Heterodimer-Promoting Domain comprises a cysteine residue, or

the Linker 2 comprises a cysteine residue and the Heterodimer-Promoting Domain comprises a cysteine residue.

Claim 7. The Tri-Specific Binding Molecule of any one of claims 1-6, wherein:

the CH2-CH3 Domain of the first polypeptide chain is a knob-bearing CH2-CH3 Domain and the CH2-CH3 Domain of the third polypeptide chain is a hole-bearing CH2-CH3 Domain; or

the CH2-CH3 Domain of the third polypeptide chain is a knob-bearing CH2-CH3 Domain and the CH2-CH3 Domain of the first polypeptide chain is a hole-bearing CH2-CH3 Domain.

Claim 8. The Tri-Specific Binding Molecule of any one of claims 1-7, wherein the CH2-CH3 Domain of the first polypeptide chain and the third polypeptide chain comprise at least one amino acid substitution, relative to the sequence of SEQ ID NO:1, and the Fc Domain formed from their association exhibits altered FcγR-mediated effector function.

Claim 9. The Tri-Specific Binding Molecule of any one of claims 1-8, wherein:

the Heterodimer-Promoting Domain on the first polypeptide chain is an E-coil Domain and the Heterodimer-Promoting Domain on the second polypeptide chain is a K-coil Domain; or

the Heterodimer-Promoting Domain on the first polypeptide chain is an K-coil Domain and the Heterodimer-Promoting Domain on the second polypeptide chain is a E-coil Domain.

Claim 10. The Tri-Specific Binding Molecule of any one of claims 1-9, wherein: the Linker 1 comprises the sequence of SEQ ID NO: 33;

the Linker 2 comprises the sequence of SEQ ID NO: 34 or 47;

the Heterodimer-Promoting Domain comprises the sequence of SEQ ID NO: 35, 36, 37, 38, 39, 40, 41 or 42;

the Linker 3 comprises the sequence of SEQ ID NO: 46, 47, 48, 49, 50, 51, 152, or GCG or GGG;

the Linker 4 comprises the sequence of SEQ ID NO: 50 or 152;

the CH2-CH3 Domain comprises the sequence of SEQ ID NO: 52 or 53;

the CH1-Hinge Domain comprises the sequence of SEQ ID NO: 207, 208 or 209;

the Cysteine-Containing Domain comprises the sequence of SEQ ID NO: 34, 36, 38, 48, 210 or 211; or

combination of the foregoing.

Claim 11. The Tri-Specific Binding Molecule of claim 10, wherein the Heterodimer-Promoting Domain of SEQ ID NO: 41 or 42 is present when Linker 2 comprises the sequence of SEQ ID NO: 47, or the Heterodimer-Promoting Domain of SEQ ID NO: 39, 40, 41 or 42 is present when Linker 2 comprises the sequence of SEQ ID NO: 34.

Claim 12. The Tri-Specific Binding Molecule of any one of claims 1-11, wherein:

(A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the second Cancer Antigen and an epitope of the Effector Cell Antigen;

(B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the first Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the second Cancer Antigen;

(C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of second Cancer Antigen, an epitope of the first Cancer Antigen, and an epitope of the Effector Cell Antigen;

(D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the second Cancer Antigen, an epitope of the Effector Cell Antigen and an epitope of the first Cancer Antigen;

(E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of the first Cancer Antigen, and an epitope of the second Cancer Antigen; and

(F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Effector Cell Antigen, an epitope of second Cancer Antigen, and an epitope of the first Cancer Antigen.

Claim 13. The Tri-Specific Binding Molecule of any one of claims 1-12, wherein the Effector Cell Antigen is CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, B cell Receptor (BCR), T cell Receptor (TCR), or NKG2D Receptor.

Claim 14. The Tri-Specific Binding Molecule of any of claims 1-13, wherein:

(A) the epitope of an Effector Cell Antigen is a CD2 epitope recognized by antibody Lo-CD2a;

(B) the epitope of an Effector Cell Antigen is a CD3 epitope recognized by antibody OKT3, M291, YTH12.5, Anti-CD3 mAb 1 or Anti-CD3 mAb 2;

(C) the epitope of an Effector Cell Antigen is a CD16 epitope recognized by antibody 3G8 or A9;

(D) the epitope of an Effector Cell Antigen is a CD19 epitope recognized by antibody MD1342, MEDI-551, blinatumomab or HD37;

(E) the epitope of an Effector Cell Antigen is a CD20 epitope recognized by antibody rituximab, ibritumomab, ofatumumab, and tositumomab;

(F) the epitope of an Effector Cell Antigen is a CD22 epitope recognized by antibody epratuzumab;

(G) the epitope of an Effector Cell Antigen is a CD32B epitope recognized by antibody CD32B mAb 1;

(H) the epitope of an Effector Cell Antigen is a CD64 epitope recognized by antibody CD64 mAb 1;

(I) the epitope of an Effector Cell Antigen is a BCR/CD79 epitope recognized by antibody CD79 mAb 1;

(J) the epitope of an Effector Cell Antigen is a TCR epitope recognized by antibody BMA 031;

(K) the epitope of an Effector Cell Antigen is a NKG2D Receptor epitope recognized by antibody KYK-2.0; or

(L) one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope comprises one or more of SEQ ID NO: 102-104, 108, 112, 114 and 115-137.

Claim 15. The Tri-Specific Binding Molecule of any one of claims 1-14, wherein the first Cancer Antigen and the second Cancer Antigen are independently chosen from: colon cancer antigen 19.9; a gastric cancer mucin; antigen 4.2; glycoprotein A33 (gpA33); ADAM-9; gastric cancer antigen AH6; ALCAM; malignant human lymphocyte antigen

APO-1; cancer antigen B1; B7-H3; beta-catenin; blood group ALeb/Ley; Burkitt's lymphoma antigen-38.13, colonic adenocarcinoma antigen C14; ovarian carcinoma antigen CA125; Carboxypeptidase M; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD30 ; CD33; CD36; CD45; CD46; CD52 ; CD79a/CD79b; CD103; CD317 ; CDK4; carcinoembryonic antigen (CEA); CEACAM5; CEACAM6; CO17-1A; CO-43 (blood group Leb); CO-514 (blood group Lea); CTA-1; CTLA4; Cytokeratin 8; antigen D1.1; antigen D156-22; DR5; E1 series (blood group B); EGFR (Epidermal Growth Factor Receptor); Ephrin receptor A2 (EphA2); ErbB1; ErbB3; ErbB4; GAGE-1; GAGE-2; GD2/GD3/GM2; lung adenocarcinoma antigen F3; antigen FC10.2; G49, ganglioside GD2; ganglioside GD3; *ganglioside GM2*; *ganglioside GM3*; *GD2*; *GD3*; *GICA 19-9*; *GM2*; gp100; human leukemia T cell antigen Gp37; melanoma antigen gp75; gpA33; HER2 antigen (p185HER2); human milk fat globule antigen (HMFG); human papillomavirus-E6/human papillomavirus-E7; high molecular weight melanoma antigen (HMW-MAA); I antigen (differentiation antigen) I(Ma); Integrin Alpha-V-Beta-6 Integrinβ6 (ITGB6); Interleukin-13; Receptor α2 (IL13Rα2); JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; human lung carcinoma antigens L6 and L20; LEA; LUCA-2; M1:22:25:8; M18; M39; MAGE-1; MAGE-3; MART; MUC-1; MUM-1; Myl; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1; OFA-2; Oncostatin M; p15; melanoma-associated antigen p97; polymorphic epithelial mucin (PEM); polymorphic epithelial mucin antigen (PEMA); PIPA; prostate-specific antigen (PSA); prostate-specific membrane antigen (PSMA); prostatic acid phosphate; R24; ROR1; sphingolipids; SSEA-1; SSEA-3; SSEA-4; sTn; T cell receptor derived peptide; T5A7; TAG-72; TL5 (blood group A); TNF-α receptor; TNF-ß receptor; TNF-γ receptor; TRA-1-85 (blood group H); Transferrin Receptor; tumor-specific transplantation antigen (TSTA), oncofetal

antigen-alpha-fetoprotein (AFP); VEGF, VEGFR; VEP8; VEP9; VIM-D5; and Y hapten, Ley.

Claim 16. The Tri-Specific Binding Molecule of claim 15, wherein one of Epitope I, Epitope II or Epitope III is an epitope of an Effector Cell Antigen and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope comprises one or more of SEQ ID NO: 3, 8, 13, 18, 23, 25, 27, 29, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98, 153, 158, 163, 167, 172, 177, 181, 186, 191-206, 302-306 and 308-316.

Claim 17. A nucleic acid composition comprising polynucleotides that encode polypeptide chains of the Tri-Specific Binding Molecule of any one of claims 1-16.

Claim 18. A pharmaceutical composition comprising the Tri-Specific Binding Molecule of any one of claims 1-16, and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 19. A Tri-Specific Binding Molecule of any one of claims 1-16, or a composition of claim 18, for use as a medicament.

Claim 20. Use of a Tri-Specific Binding Molecule of any one of claims 1-16 or a composition of claim 18 in the manufacture of a medicament to treat a cancer.

Claim 21. The use of claim 20, wherein the cancer is characterized by the presence of a cancer 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 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 uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, or a uterine cancer.

Claim 22. The use of claim 20 or claim 21, wherein the cancer is a colorectal cancer, a hepatocellular carcinoma, a glioma, a kidney cancer, a breast cancer, a multiple myeloma, bladder cancer, a neuroblastoma, a sarcoma, a non-Hodgkin's lymphoma, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer, a rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphoma (NHL), mantel cell leukemia (MCL), small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.