[0042] Consequently, despite all prior advances, a need remains for improved compositions capable of more vigorously directing the body's immune system to attack cancer cells or pathogen-infected cells, especially at lower therapeutic concentrations. As described in detail below, the present invention addresses this need by providing Tri-Specific Binding Molecules that bind to: (1) an epitope of CD3, (2) an epitope of CD8, and (3) an epitope of a Disease-Associated Antigen that is expressed on a target cell (especially a cancer cell, or a pathogen-infected cell) and mediate coordinated binding of cytotoxic T cells to cells presenting the Disease-Associated Antigen.

Summary:

[0043] 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 Binding Domains may be selected such that the Tri-Specific Binding Molecules are capable of binding to any three different epitopes. Such epitopes may be epitopes of the same antigen or epitopes of two or three different antigens. The disclosure also provides a novel ROR1-binding antibody, as well as derivatives thereof and uses for such compositions.

[0044] The present disclosure particularly relates to the embodiment of such Tri-Specific Binding Molecules in which the three epitopes are selected such that one or two of such epitopes are epitope(s) of an immune system cell, and especially, a cytotoxic lymphocyte immune system cell (CTL), and in which the remaining epitope(s) are epitope(s) of a Disease-Associated Antigen. Such particularly preferred Tri-Specific Binding Molecules are capable of localizing a cytotoxic lymphocyte cell to a cell that expresses a Disease-Associated Antigen, and of thereby facilitating the killing of cells that express the Disease-Associated Antigen. The Disease-Associated Antigen may be a cancer antigen, or may be an antigen that is characteristic of a pathogen (*e.g.*, bacterial, fungal, viral or protozoan) infection. More particularly, the disclosure relates to such Tri-Specific Binding Molecules that are capable of mediating coordinated binding to: (1) an epitope of CD3, (2) an epitope of CD8, and (3) an epitope of a Disease-Associated Antigen. By binding to CD3 and CD8, and to the Disease-Associated Antigen, such molecules co-localize cytotoxic T cells to cells

presenting the Disease-Associated Antigen, leading to the activation of such T cells and the initiation of a cytotoxic response against cells expressing the Disease-Associated Antigen.

[0045] In detail, the disclosure provides a Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, wherein the binding 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) a Non-Diabody-Type 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 association of two CH2-CH3 Domains to one another.

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

[0045a] 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) 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);

(B) 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);

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

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

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

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

[0045b] 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) 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);

(B) 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);

(C) 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);

(D) 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;

(E) 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

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

[0045c] In yet another 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) a Receptor-Type Binding Domain; and

(III) a Fc Domain; wherein:

(A) 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

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

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

(B) 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);

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

(first polypeptide of Receptor-Type Binding Domain)—(Cysteine-Containing Domain);

(D) the fourth chain comprises, from the N-terminus to the C-terminus:

(second polypeptide of Receptor-Type Binding Domain)—(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, and the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II;

(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 first polypeptide of the Receptor-Type Binding Domain and the second polypeptide of the Receptor-Type Binding Domain associate to form a Receptor-Type Binding Domain, 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.

[0045d] In a further aspect, there is provided a Tri-Specific Binding Molecule, comprising four polypeptide chains, wherein:

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a 5T4 antigen (VLI);

(2) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a CD3 antigen (VHII);

(3) a first Cysteine-Containing Domain;

(4) a Heterodimer-Promoting Domain;

(5) a second Cysteine-Containing Domain; and

(6) CH2 and CH3 Domains of an IgG;

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the CD3 antigen (VLII);

(2) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the 5T4 antigen (VHI);

(3) a Cysteine-Containing Domain; and

(4) a Heterodimer-Promoting Domain, wherein the Heterodimer-Promoting Domain of the second polypeptide chain is complementary to the Heterodimer-Promoting Domain of the first polypeptide chain;

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

(1) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a CD8 antigen (VHIII);

(2) a CH1 Domain;

(3) a CH2-CH3 Domain of an IgG; and

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the CD8 antigen (VLIII); and

(2) a Cysteine-Containing Light Chain Constant Domain (CL);

wherein:

(i) the VLI and VHI Domains associate to form a Domain capable of binding the epitope of the 5T4 antigen;

(ii) the VLII and VHII Domains associate to form a Domain capable of binding the epitope of the CD3 antigen;

(iii) the VLIII and VHIII Domains associate to form a Domain capable of binding the epitope of the CD8 antigen;

(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.

[0046] The disclosure particularly concerns the embodiment of such Tri-Specific Binding Molecule, wherein one of Epitope I, Epitope II or Epitope III is an epitope of a cellular receptor.

[0047] The disclosure additionally concerns the embodiments of such Tri-Specific Binding Molecules, wherein one of Epitope I, Epitope II or Epitope III is an epitope of a Disease-Associated Antigen (and especially wherein the Disease-Associated Antigen is a cancer antigen that is arrayed on the surface of a cancer cell, or is a pathogen antigen that is arrayed on the surface of a pathogen or pathogen-infected cell).

[0048] The disclosure additionally concerns the embodiments 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.

[0049] The disclosure especially concerns the embodiments of such Tri-Specific Binding Molecules, wherein one of Epitope I, Epitope II or Epitope III is an epitope of CD3, a second of Epitope I, Epitope II or Epitope III is an epitope of CD8, and the third of Epitope I, Epitope II or Epitope III is an epitope of the Disease-Associated Antigen, and wherein the Antigen-Binding Domains I, II and III of the Tri-Specific Binding Molecules mediate coordinated binding of a cytotoxic T cell and a cell expressing the Disease-Associated Antigen. The disclosure particularly concerns the embodiments of such Tri-Specific Binding Molecules, wherein the CD3, the CD8 are arrayed on the surface of a T cell and wherein the Disease-Associated Antigen is arrayed on the surface of a cancer cell, pathogen or pathogen-infected cell, and wherein the immunospecific binding is sufficient to co-localize the CD3 and the CD8, and the Disease-Associated Antigen, thereby facilitating the activation of the CD8-arraying T cell against the Disease-Associated Antigen-arraying cell.

[0050] The disclosure additionally concerns the embodiments of above-described 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 the Epitope III, wherein the molecule comprises:

(A) a first polypeptide chain:

(I) 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) a first Cysteine-Containing Domain; and a Heterodimer-Promoting Domain; or

(b) a Cysteine-Containing Heterodimer-Promoting Domain;

(5) a second Cysteine-Containing Domain; and

(6) CH2 and CH3 Domains of an IgG;

or

(II) that comprises in the N-terminus to C-terminus direction:

(1) a first Cysteine-Containing Domain;

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to the third of the three epitopes (VL_{III}); and

(2) a Cysteine-Containing Light Chain Constant Domain (CL);

wherein:

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

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

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

(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.

[0051] The disclosure additionally concerns the embodiments of above-described 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.

[0052] The disclosure additionally concerns the embodiments of above-described 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:6**, 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:6**, such that the Fc Domain formed from their association exhibits altered FcγR-mediated effector function.

[0053] The disclosure additionally concerns the embodiments of above-described 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:7** and **SEQ ID NO:8**.

[0054] The disclosure additionally concerns the embodiments of above-described Tri-Specific Binding Molecules, wherein:

(A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD3, an epitope of CD8 and an epitope of the Disease-Associated Antigen;

(B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD3, an epitope of the Disease-Associated Antigen and an epitope of CD8;

(C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD8, an epitope of CD3, and an epitope of the Disease-Associated Antigen;

(D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD8, an epitope of the Disease-Associated Antigen and an epitope of CD3;

(E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Disease-Associated Antigen, an epitope of CD3, and an epitope of CD8; or

(F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Disease-Associated Antigen, an epitope of CD8, and an epitope of CD3.

[0055] The disclosure additionally concerns the embodiments of above-described Tri-Specific Binding Molecules, wherein:

(A) the epitope of CD3 is a CD3 epitope recognized by antibodyOKT3, M291, YTH12.5, CD3 mAb 1 or CD3 mAb 2; or

(B) the epitope of CD8 is a CD8 epitope recognized by antibody TRX2 or OKT8.

[0056] The disclosure additionally concerns a pharmaceutical composition that comprises the above-described Tri-Specific Binding Molecule and a pharmaceutically acceptable carrier, excipient or diluent.

[0057] The disclosure additionally concerns a method of treating cancer which comprises administering an effective amount of the above-described pharmaceutical composition to an individual in need thereof, wherein the Disease-Associated Antigen is the cancer antigen.

[0058] The disclosure additionally concerns a method of treating a disease-associated with the presence of a pathogen which comprises administering an effective amount of the pharmaceutical composition of claim 15 to an individual in need thereof, wherein the Disease-Associated Antigen is the pathogen antigen.

[0059] The disclosure additionally concerns an anti-ROR1 antibody, or ROR1-binding fragment, wherein the antibody comprises:

(A) a Light Chain Variable Domain that comprises a CDR_L1 having the sequence of **SEQ ID NO:117**, a CDR_L2 having the sequence of **SEQ ID NO:118**, and a CDR_L3 having the sequence of **SEQ ID NO:119**; and

(B) a Heavy Chain Variable Domain that comprises a CDR_H1 having the sequence of **SEQ ID NO:120**, a CDR_H2 having the sequence of **SEQ ID NO:121**, and a CDR_H3 having the sequence of **SEQ ID NO:122**.

[0060] The disclosure additionally concerns the embodiments of such anti-ROR1 antibody or ROR1-binding fragment thereof, wherein the antibody has a Light Chain Variable Domain having the sequence of **SEQ ID NO:51**. The disclosure additionally concerns the embodiments of such anti-ROR1 antibodies or ROR1-binding fragments thereof, wherein the antibody has a Heavy Chain Variable Domain having the sequence of **SEQ ID NO:52**, or both a Light Chain Variable Domain having the sequence of **SEQ ID NO:51** and a Heavy Chain Variable Domain having the sequence of **SEQ ID NO:52**.

[0061] The disclosure additionally concerns a diabody, BiTe or single-chain antibody that comprises the ROR1 binding fragment of any of such claims anti-ROR1 antibodies.

[0061a] In one aspect, there is provided a binding molecule capable of binding an epitope of CD3, comprising:

(A) a Light Chain Variable Domain comprising: a CDRL1 having the sequence RSSTGAVTTSNYAN, a CDRL2 having the sequence GTNKRAP, and a CDRL3 having the sequence ALWYSNLWV; and

(B) a Heavy Chain Variable Domain comprising: a CDRH1 having the sequence GFTFSTYAMN, a CDRH2 having the sequence RIRSKYNNYATYYADSVKG, and a CDRH3 having the sequence HKNFGNSYVTWFAY;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

[0061b] In another aspect, there is provided a binding molecule capable of binding an epitope of 5T4, comprising:

(A) a Light Chain Variable Domain comprising: a CDRL1 having the sequence RASQGISNYLA, a CDRL2 having the sequence RANRLQS, and a CDRL3 having the sequence LQYDDFPWT; or a CDRL1 having the sequence RSSQSIVYSNGNTYLE, a CDRL2 having the sequence KVSNRFS, and a CDRL3 having the sequence FQGSHVPFT; and

(B) a Heavy Chain Variable Domain comprising: a CDRH1 having the sequence GYTFTSFWMH, a CDRH2 having the sequence RIDPNRGGTEYNEKAKS, and a CDRH3 having the sequence GNPYYPMDY; or a CDRH1 having the sequence GYTFTSYWIT, a CDRH2 having the sequence DIYPGSGRANYNEKFKS, and a CDRH3 having the sequence YGPLFTTWDPNSYAMDY;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

[0061c] In yet another aspect, there is provided a binding molecule capable of binding an epitope of ROR1, comprising:

(A) a Light Chain Variable Domain comprising a CDRL1 having the sequence of SEQ ID NO:117, a CDRL2 having the sequence of SEQ ID NO:118, and a CDRL3 having the sequence of SEQ ID NO:119; and

(B) a Heavy Chain Variable Domain comprising a CDRH1 having the sequence of SEQ ID NO:120, a CDRH2 having the sequence of SEQ ID NO:121, and a CDRH3 having the sequence of SEQ ID NO:122;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

[0062] The disclosure additionally concerns a pharmaceutical composition that comprises any of the above-described anti-ROR1 antibodies or ROR1-binding fragments thereof and a pharmaceutically acceptable carrier, excipient or diluent. The disclosure additionally concerns a method of treating cancer which comprises administering an effective amount of such a pharmaceutical composition to an individual in need thereof.

[0062a] 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 or any of the above-described binding molecules.

[0062b] In another aspect, there is provided a pharmaceutical composition comprising any of the above-described Tri-Specific Binding Molecules or any of the above-described binding molecules, and a pharmaceutically acceptable carrier, excipient or diluent.

[0062c] In yet another aspect, there is provided any of the above-described Tri-Specific Binding Molecules, any of the above-described binding molecules, or a composition as described above, for use as a medicament.

[0062d] In a further aspect, there is provided use of any of the above-described Tri-Specific Binding Molecules, any of the above-described binding molecules, or a composition as described above, in the manufacture of a medicament for treating cancer or a pathogen infection.

Brief Description of the Figures:

[0063] **Figures 1A-1B** show diagrammatic representation of the Domains of DART™ diabodies. **Figure 1A** shows a diagrammatic representation of the Domains of a basic DART™ diabody. **Figure 1B** provides a schematic of a covalently bonded diabody composed of two polypeptide chains, each having a Heterodimer-Promoting Domain VL and VH domains that recognize the same epitope are shown using the same shading.

[0064] **Figures 2A-2B** provide a schematic of covalently bonded diabodies composed of two polypeptide chains, each having a CH2 and CH3 Domain (**Figure 2A**) or in which only one has a CH2 and CH3 Domain (**Figure 2B**), such that the associated chains form an Fc Domain that comprises all or part of a naturally occurring Fc Domain. VL and VH domains that recognize the same epitope are shown using the same shading.

[0065] **Figures 3A-3C** provide schematics showing tetravalent diabodies composed of two pairs of polypeptide chains. The pairs are different, thus resulting in a bi-specific molecule that is bivalent with respect to each of two epitopes, in which one is an epitope of DR5 and the other is an epitope of a molecule present on the surface of an effector cell. One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form an Fc Domain that comprises all or part of a naturally occurring Fc Domain. VL and VH domains that recognize the same epitope are shown using the same shading. Only one pair of epitopes (shown with the same shading) is capable of binding to DR5. **Figure 3A** shows an Ig diabody. **Figure 3B** shows an Ig diabody, which contains E-coil and K-coil heterodimer-promoting domains. **Figure 3C**, shows an Fc-DART™ diabody that contains antibody CH1 and CL domains. The notation "**VL1**" and "**VH1**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "first" epitope. Similarly, the notation "**VL2**" and "**VH2**"denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "second" epitope.

[0066] **Figures 4A-4L** provide a diagrammatic representation of the Domains of preferred Tri-Specific Binding Molecules. **Figures 4A** and **4B**, respectively, illustrate

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) 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);

(B) 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);

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

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

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

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

(B) 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);

(C) 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);

(D) 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;

(E) 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

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

Claim 4. The Tri-Specific Binding Molecule of claim 3, 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 5. 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) a Receptor-Type Binding Domain; and

(III) a Fc Domain; wherein:

(A) 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

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

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

(B) 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);

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

(first polypeptide of Receptor-Type Binding Domain)—(Cysteine-Containing Domain);

(D) the fourth chain comprises, from the N-terminus to the C-terminus:

(second polypeptide of Receptor-Type Binding Domain)—(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, and the VH_{II} Domain is a Heavy Chain Variable Domain of an immunoglobulin capable of binding to Epitope II;

(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 first polypeptide of the Receptor-Type Binding Domain and the second polypeptide of the Receptor-Type Binding Domain associate to form a Receptor-Type Binding Domain, 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 6. The Tri-Specific Binding Molecule of any one of claims 1, 2 and 5, 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 7. The Tri-Specific Binding Molecule of any one of claims 1-6, 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 8. The Tri-Specific Binding Molecule of any one of claims 1-7, 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 9. The Tri-Specific Binding Molecule of any one of claims 1-8, 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:6, and the Fc Domain formed from their association exhibits altered FcγR-mediated effector function.

Claim 10. The Tri-Specific Binding Molecule of any one of claims 1-9, 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 11. The Tri-Specific Binding Molecule of any one of claims 1-10, wherein:
the Linker 1 comprises the sequence of SEQ ID NO: 1;

the Linker 2 comprises the sequence of SEQ ID NO: 2 or 131;

the Heterodimer-Promoting Domain comprises the sequence of SEQ ID NO: 3, 4, 115 or 116;

the Linker 3 comprises the sequence of SEQ ID NO: 5 or GGG;

the Linker 4 comprises the sequence of SEQ ID NO: 15, 16, 131 or 132, or GGC or GGG;

the CH2-CH3 Domain comprises the sequence of SEQ ID NO: 7 or 8;

the CH1-Hinge Domain comprises the sequence of SEQ ID NO: 9 or 10;

the Cysteine-Containing Domain comprises the sequence of SEQ ID NO: 2, 5, 9, 10, 11, 12, 13, 14, 127, 128, 129, 130 or 133; or

combination of the foregoing.

Claim 12. The Tri-Specific Binding Molecule of claim 11, wherein the Heterodimer-Promoting Domain of SEQ ID NO: 115 or 116 is present when Linker 2 comprises the sequence of SEQ ID NO: 131, or the Heterodimer-Promoting Domain of SEQ ID NO: 3, 4, 115 or 116 is present when Linker 2 comprises the sequence of SEQ ID NO: 2.

Claim 13. The Tri-Specific Binding Molecule of any one of claims 1-12, wherein one of Epitope I, Epitope II or Epitope III is an epitope of:

an effector cell chosen from CD2, CD3, CD16, CD19, CD20, CD22, CD32B, CD64, B cell Receptor (BCR), T cell Receptor (TCR), or NKG2D Receptor; or

CD8; or

a Disease-Associated Antigen.

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

(A) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD3, an epitope of CD8 and an epitope of the Disease-Associated Antigen;

(B) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD3, an epitope of the Disease-Associated Antigen and an epitope of CD8;

(C) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD8, an epitope of CD3, and an epitope of the Disease-Associated Antigen;

(D) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of CD8, an epitope of the Disease-Associated Antigen and an epitope of CD3;

(E) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Disease-Associated Antigen, an epitope of CD3, and an epitope of CD8;

or

(F) the Epitope I, Epitope II and Epitope III are, respectively, an epitope of the Disease-Associated Antigen, an epitope of CD8, and an epitope of CD3.

Claim 15. The Tri-Specific Binding Molecule of claim 13 or claim 14, wherein one of Epitope I, Epitope II or Epitope III is an epitope of CD3 and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of CD3 comprises one or more of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 101 and 102.

Claim 16. The Tri-Specific Binding Molecule of any one of claims 13-15, wherein one of Epitope I, Epitope II or Epitope III is an epitope of CD8 and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of CD8 comprises one or more of SEQ ID NO: 29, 30, 31 and 32.

Claim 17. The Tri-Specific Binding Molecule of any one of claims 13-16, wherein the Disease-Associated Antigen is a cancer antigen that is arrayed on the surface of a cancer cell.

Claim 18. The Tri-Specific Binding Molecule of claim 17, wherein the cancer antigen is colon cancer antigen 19.9; gastric cancer mucin antigen 4.2; colorectal carcinoma antigen A33; ADAM-9; AFP oncofetal antigen-alpha-fetoprotein; B7-H3; BAGE; beta-catenin; CA125; Carboxypeptidase M; B1; CD5; CD19; CD20; CD22; CD23; CD25; CD27; CD28; CD30; CD33; CD36; CD40/CD154; CD45; CD56; CD46; CD52; CD79a/CD79b; CD103; CD317; CDK4; CEA carcinoembryonic antigen; CEACAM5 and CEACAM6; CO-43 (blood group Leb) and CO-514 (blood group Lea); CTLA-1and CTLA-4; Cytokeratin 8; DR5; E1 series (blood group B); EGF-R epidermal growth factor receptor; Ephrin receptors (and in particular EphA2); Erb (ErbB1; ErbB3; ErbB4); lung adenocarcinoma antigen F3; antigen FC10.2; GAGE (GAGE-1; GAGE-2); GD2/GD3/GD49/GM2/GM3; GICA 19-9; gp37 (human leukemia T cell antigen); gp75 (melanoma antigen); gp100; HER-2/neu; human B-lymphoma antigen-CD20; human milk fat globule antigen; human papillomavirus-E6/human papillomavirus-E7; HMW-MAA (high molecular weight melanoma antigen); I antigen (differentiation antigen); I(Ma) as found in gastric adenocarcinomas; Integrin Alpha-V-Beta-6 IntegrinP6 (ITGB6); Interleukin-13 Receptor α2 (IL13Ra2); JAM-3; KID3; KID31; KS 1/4 pan-carcinoma antigen; KS 1/4 pan-carcinoma antigen; KSA (17-1A); human lung carcinoma antigens L6 and L20; LEA; LUCA-2; M1:22:25:8, M18, M39; MAGE (MAGE-1; MAGE-3); MART; Myl, MUC-1; MUM-1; N-acetylglucosaminyltransferase; neoglycoprotein; NS-10; OFA-1 and OFA-2; Oncostatin M (Oncostatin Receptor Beta); ρ15; PSA (prostate specific antigen); PSMA; PEMA (polymorphic epithelial mucin antigen); PIPA; prostatic acid phosphate; R24; ROR1; SSEA-1, SSEA-3 and SSEA-4; sTn; T cell receptor derived peptide; T5A7; Tissue Antigens 37; TAG-72; TL5 (blood group A); TNF-

receptor (TNF-a receptor, TNF-B receptor; or TNF-y receptor); TRA-1-85 (blood group H); Transferrin Receptor; TSTA tumor-specific transplantation antigen; VEGF-R or 5T4.

Claim 19. The Tri-Specific Binding Molecule of claim 18, wherein one of Epitope I, Epitope II or Epitope III is an epitope of B7-H3, A33, 5T4 or ROR1 and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of B7-H3, A33, 5T4 or ROR1 comprises one or more of SEQ ID NO: 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57 and 58.

Claim 20. The Tri-Specific Binding Molecule of any one of claims 13-16, wherein the Disease-Associated Antigen is a pathogen antigen that is arrayed on the surface of a pathogen or pathogen-infected cell.

Claim 21. The Tri-Specific Binding Molecule of claim 20, wherein:

the pathogen is a virus, and/or

the pathogen is human immunodeficiency virus (HIV) or respiratory syncytial virus (RSV), and/or

the pathogen antigen is HIV gp41, HIV gp120 or RSV glycoprotein F.

Claim 22. The Tri-Specific Binding Molecule of claim 21, wherein one of Epitope I, Epitope II or Epitope III is an epitope of HIV gp41, HIV gp120 or RSV glycoprotein F and the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of HIV gp41, HIV gp120 or RSV glycoprotein F comprises one or more of SEQ ID NO: 33, 34, 35, 36, 37 and 38.

Claim 23. The Tri-Specific Binding Molecule of any one of claims 20-22, wherein: Epitope I is an epitope of HIV, Epitope II is an epitope of CD3, and Epitope III is an epitope of CD8, or Epitope I is an epitope of CD3, Epitope II is an epitope of HIV, and Epitope III is an epitope of CD8.

Claim 24. A Tri-Specific Binding Molecule, comprising four polypeptide chains, wherein:

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a 5T4 antigen (VLI);

(2) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a CD3 antigen (VHII);

(3) a first Cysteine-Containing Domain;

(4) a Heterodimer-Promoting Domain;

(5) a second Cysteine-Containing Domain; and

(6) CH2 and CH3 Domains of an IgG;

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the CD3 antigen (VLII);

(2) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the 5T4 antigen (VHI);

(3) a Cysteine-Containing Domain; and

(4) a Heterodimer-Promoting Domain, wherein the Heterodimer-Promoting Domain of the second polypeptide chain is complementary to the Heterodimer-Promoting Domain of the first polypeptide chain;

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

(1) a Heavy Chain Variable Domain of an immunoglobulin capable of binding to an epitope of a CD8 antigen (VHIII);

(2) a CH1 Domain;

(3) a CH2-CH3 Domain of an IgG; and

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

(1) a Light Chain Variable Domain of an immunoglobulin capable of binding to the epitope of the CD8 antigen (VLIII); and

(2) a Cysteine-Containing Light Chain Constant Domain (CL);

wherein:

(i) the VLI and VHI Domains associate to form a Domain capable of binding the epitope of the 5T4 antigen;

(ii) the VLII and VHII Domains associate to form a Domain capable of binding the epitope of the CD3 antigen;

(iii) the VLIII and VHIII Domains associate to form a Domain capable of binding the epitope of the CD8 antigen;

(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.

Claim 25. The Tri-Specific Binding Molecule of claim 24, wherein:

the VL1 comprises: a CDRL1 having the sequence RASQGISNYLA, a CDRL2 having the sequence RANRLQS, and a CDRL3 having the sequence LQYDDFPWT; and

the VH1 comprises: a CDRH1 having the sequence GYTFTSFWMH, a CDRH2 having the sequence RIDPNRGGTEYNEKAKS, and a CDRH3 having the sequence GNPYYPMDY.

Claim 26. The Tri-Specific Binding Molecule of claim 24 or 25, wherein:

the VL1 comprises the sequence of SEQ ID NO: 47 and the VH1 comprises the sequence of SEQ ID NO: 48; the VLII comprises the sequence of SEQ ID NO: 26 and the VHII comprises the sequence of SEQ ID NO: 27 or 28; the VLIII comprises the sequence of SEQ ID NO: 29 and the VHIII comprises the sequence of SEQ ID NO: 30; the VLIII comprises the sequence of SEQ ID NO: 31 and the VHIII comprises the sequence of SEQ ID NO: 32; or combination of the foregoing.

Claim 27. The Tri-Specific Binding Molecule of any one of claims 24-26, wherein: the first Cysteine-Containing Domain of the first polypeptide chain comprises the sequence of SEQ ID NO:2 and the Heterodimer-Promoting Domain of the first polypeptide chain comprises the sequence SEQ ID NO: 3; the first Cysteine-Containing Domain of the second polypeptide chain comprises the sequence of SEQ ID NO:2 and the Heterodimer-Promoting Domain of the second polypeptide chain comprises the sequence SEQ ID NO: 4; the CH1 Domain of the third polypeptide chain comprises the sequence of SEQ ID NO: 9; the CL Domain of the fourth polypeptide chain comprises the sequence of SEQ ID NO: 13; the CH2 and CH3 Domains of the first polypeptide

chain and third polypeptide chain differ from one another and each have an amino acid sequence of SEQ ID NO:7 or SEQ ID NO: 8; or combination of the foregoing.

Claim 28. The Tri-Specific Binding Molecule of any one of claims 24-27, wherein: the first polypeptide chain comprises the sequence of SEQ ID NO: 103; the second polypeptide chain comprises the sequence of SEQ ID NO: 104; the third polypeptide chain comprises the sequence of SEQ ID NO: 105; and the fourth polypeptide chain comprises the sequence of SEQ ID NO: 106.

Claim 29. A binding molecule capable of binding an epitope of CD3, comprising:

(A) a Light Chain Variable Domain comprising: a CDRL1 having the sequence RSSTGAVTTSNYAN, a CDRL2 having the sequence GTNKRAP, and a CDRL3 having the sequence ALWYSNLWV; and

(B) a Heavy Chain Variable Domain comprising: a CDRH1 having the sequence GFTFSTYAMN, a CDRH2 having the sequence RIRSKYNNYATYYADSVKG, and a CDRH3 having the sequence HKNFGNSYVTWFAY;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

Claim 30. The binding molecule of claim 29, wherein: the Light Chain Variable Domain comprises the sequence of SEQ ID NO:101 and the Heavy Chain Variable Domain comprises the sequence of SEQ ID NO:102.

Claim 31. A binding molecule capable of binding an epitope of 5T4, comprising:

(A) a Light Chain Variable Domain comprising: a CDRL1 having the sequence RASQGISNYLA, a CDRL2 having the sequence RANRLQS, and a CDRL3 having the sequence LQYDDFPWT; or a CDRL1 having the sequence RSSQSIVYSNGNTYLE, a CDRL2

having the sequence KVSNRFS, and a CDRL3 having the sequence FQGSHVPFT; and

(B) a Heavy Chain Variable Domain comprising: a CDRH1 having the sequence GYTFTSFWMH, a CDRH2 having the sequence RIDPNRGGTEYNEKAKS, and a CDRH3 having the sequence GNPYYPMDY; or a CDRH1 having the sequence GYTFTSYWIT, a CDRH2 having the sequence DIYPGSGRANYNEKFKS, and a CDRH3 having the sequence YGPLFTTWDPNSYAMDY;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

Claim 32. The binding molecule of claim 31, wherein: the Light Chain Variable Domain comprises the sequence of SEQ ID NO:47 and the Heavy Chain Variable Domain comprises the sequence of SEQ ID NO:48; or the Light Chain Variable Domain comprises the sequence of SEQ ID NO:49 and the Heavy Chain Variable Domain comprises the sequence of SEQ ID NO:50.

Claim 33. A binding molecule capable of binding an epitope of ROR1, comprising:

(A) a Light Chain Variable Domain comprising a CDRL1 having the sequence of SEQ ID NO:117, a CDRL2 having the sequence of SEQ ID NO:118, and a CDRL3 having the sequence of SEQ ID NO:119; and

(B) a Heavy Chain Variable Domain comprising a CDRH1 having the sequence of SEQ ID NO:120, a CDRH2 having the sequence of SEQ ID NO:121, and a CDRH3 having the sequence of SEQ ID NO:122;

wherein the binding molecule is an antibody, antibody fragment, single-chain antibody, diabody, BiTe or Tri-Specific Binding Molecule.

Claim 34. The binding molecule of claim 33, wherein the Light Chain Variable Domain comprises the sequence of SEQ ID NO:51 and the Heavy Chain Variable Domain comprises the sequence of SEQ ID NO:52.

Claim 35. A nucleic acid composition comprising polynucleotides that encode polypeptide chains of the Tri-Specific Binding Molecule of any one of claims 1-28 or the binding molecule of any one of claims 29-34.

Claim 36. A pharmaceutical composition comprising the Tri-Specific Binding Molecule of any one of claims 1-28 or the binding molecule of any one of claims 29-34, and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 37. A Tri-Specific Binding Molecule of any one of claims 1-28, a binding molecule of any one of claims 29-34, or a composition of claim 36, for use as a medicament.

Claim 38. Use of a Tri-Specific Binding Molecule of any one of claims 1-28, a binding molecule of any one of claims 29-34, or a composition of claim 36, in the manufacture of a medicament for treating cancer or a pathogen infection.