

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:

- (I) four different polypeptide chains covalently complexed together;
- (II) 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;
- (III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and
- (IV) a Fc Domain, wherein:
 - (A) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:
(VL_I Domain)—(Linker 1)—(VH_I 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, in the N-terminus to C-terminus direction:

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

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

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

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

(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:

(I) three different polypeptide chains covalently complexed together;

(II) 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;

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

(IV) a Fc Domain; wherein:

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

(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, in the N-terminus to C-terminus direction:

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

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

(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:

(I) four different polypeptide chains covalently complexed together;

(II) 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;

(III) a Receptor-Type Binding Domain; and

(IV) a Fc Domain; wherein:

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

(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, in the N-terminus to C-terminus direction:

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

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

(first polypeptide of Receptor-Type Binding Domain)—(Cysteine-Containing Domain) —(CH2-CH3 Domain);

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

(second polypeptide of Receptor-Type Binding 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, 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);

[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] Disclosed is 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] Disclosed is 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] Disclosed is 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.

[0062b] 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.

[0062c] In yet another aspect, there is provided any of the above-described Tri-Specific 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, 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.

[00197] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary humanized anti-A33 (**gpA33 mAb 1**) antibody is (**SEQ ID NO:46**):

QVQLQQSGPE LVKPGASVKI SCKASGYTFS GSWMNWVKQR PGQGLEWIGR
IYPGDGETNY NGKFKDKATL TADKSSTAY MELSSLTSVD SAVYFCARIY
GNNVYFDVWG AGTTVTVSS

(f) 5T4 Tumor Antigen

[00198] The 5T4 tumor antigen is another illustrative Disease-Associated Antigen. The amino acid sequence of the Light Chain Variable Domain of an exemplary humanized anti-5T4 mAb 1 antibody ("**5T4 mAb 1**") is (**SEQ ID NO:47**):

DIQMTQSPSS LSASVGDRV ITCRASQG IS NYLAWFQQKP GKAPKSLIYR
ANRLQSGVPS RFGSGSGTD FTLTIS SLQP EDVATYYCLQ YDDFPWTFGQ
GTKLEIK

[00199] The amino acid sequence of the Heavy Chain Variable Domain of such exemplary humanized 5T4 mAb 1 is (**SEQ ID NO:48**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SFWMHWVRQA PGQGLEWMGR
IDPNRGGTEY NEKAKSRVTM TADKSTSTAY MELSSLRSED TAVYYCAGGN
PYPFMDYWGQ GTTVTVSS

[00200] The amino acid sequence of the Light Chain Variable Domain of a second exemplary humanized 5T4 mAb 2 antibody ("**5T4 mAb 2**") is (**SEQ ID NO:49**):

DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV YSNGNTYLEW YLQKPGQSPK
LLIYKVS NRF SGVPDRFSGS GS GTDFTLKI SRVEAEDLGV YYCFQGS HVP
FTFGSGTKLE IK

[00201] The amino acid sequence of the Heavy Chain Variable Domain of such second exemplary humanized 5T4 mAb 2 is (**SEQ ID NO:50**):

QVQLQQPGAE LVKPGASVKM SCKASGYTFT SYWITWVKQR PGQGLEWIGD
IYPGSGRANY NEKFKSKATL TVDTSSSTAY MQLSSLTSED SAVYNCARYG
PLFTTVVDPN SYAMDYWGQG TSVTVSS

(g) ROR1 Antigen

[00202] The ROR1 tumor antigen is another illustrative Disease-Associated Antigen. Exemplary anti-ROR1 antibodies include antibody 2A2 (WO 2010/124188), R11 (WO 2012/075158) and R12 (WO 2012/075158).

What Is Claimed Is:

Claim 1. A Tri-Specific Binding Molecule capable of immunospecifically binding to three different epitopes, comprising:

- (I) four different polypeptide chains covalently complexed together;
- (II) 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;
- (III) an Antigen-Binding Domain III that is capable of immunospecifically binding to an Epitope III present on a third antigen; and
- (IV) a Fc Domain, wherein:
 - (A) a first polypeptide chain comprises, in the N-terminus to C-terminus direction:

(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, in the N-terminus to
C-terminus direction:

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

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

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

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

(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:

(I) three different polypeptide chains covalently complexed together;

(II) 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;

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

(IV) a Fc Domain; wherein:

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

(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, in the N-terminus to C-terminus direction:

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

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

(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:

(I) four different polypeptide chains covalently complexed together;

(II) 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;

(III) a Receptor-Type Binding Domain; and

(IV) a Fc Domain; wherein:

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

(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, in the N-terminus to C-terminus direction:

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

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

(first polypeptide of Receptor-Type Binding Domain)—(Cysteine-Containing Domain)—(CH2-CH3 Domain);

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

(second polypeptide of Receptor-Type Binding 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, 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 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; or

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.

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 the six CDRs of SEQ ID NO: 17 and 18; 19 and 20; 21 and 22; 23 and 25; 24 and 25; 26 and 27; 26 and 28; or 101 and 102; or 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 the six CDRs of SEQ ID NO: 29 and 30; or 31 and 32; or 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; ALCAM; 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-1 and 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 Integrin β 6 (ITGB6); Interleukin-13 Receptor α 2 (IL13R α 2); JAM-3; KID3; KID31; 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); p15; PSA (prostate specific antigen); PSMA; PEMA (polymorphic epithelial mucin antigen); PIPA; prostatic acid phosphatase; 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- α receptor, TNF- β receptor; or TNF- γ receptor); TRA-1-85 (blood group H); Transferrin Receptor; TSTA tumor-specific transplantation antigen; VEGF-R; Y hapten, Le^y, 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 the six CDRs of SEQ ID NO: 39 and 40; 41 and 42; 43 and 44; 45 and 46; 47 and 48; 49 and 50; 51 and 52; 53 and 54; 55 and 56; or 57 and 58; or 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 the six CDRs of SEQ ID NO: 33 and 34; 35 and 36; or 37 and 38; or 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.

(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 VL_I comprises: a CDRL1 having the sequence RASQGISNYLA, a CDRL2 having the sequence RANRLQS, and a CDRL3 having the sequence LQYDDFPWT; and
- the VH_I 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 VL_I comprises the sequence of SEQ ID NO: 47 and the VH_I 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. The Tri-Specific Binding Molecule of claim 15, wherein the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of CD3 comprises:

(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.

Claim 30. The Tri-Specific 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. The Tri-Specific Binding Molecule of claim 19, wherein the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of 5T4 comprises:

(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 YGPLFTTWDPN SYAMDY.

Claim 32. The Tri-Specific 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. The Tri-Specific Binding Molecule of claim 19, wherein the Antigen-Binding Domain that is capable of immunospecifically binding to the epitope of ROR1 comprises:

(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.

- Claim 34. The Tri-Specific 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 1-34.
- Claim 36. A pharmaceutical composition comprising the Tri-Specific Binding Molecule of any one of claims 1-34, and a pharmaceutically acceptable carrier, excipient or diluent.
- Claim 37. A Tri-Specific Binding Molecule of any one of claims 1-34, 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 1-34, or a composition of claim 36, in the manufacture of a medicament for treating cancer or a pathogen infection.