

combination of antibodies. (Hodi et al., 2016, *Lancet Oncol.* 17(11):1558-1568, Hellman et al., 2018, *Cancer Cell* 33(5): 853-861). However, as many clinical trials have shown a great benefit of treating cancer patients with monoclonal antibodies that are specific for one or more immune checkpoint molecules data has emerged that only those patients with a high mutational burden that generates a novel T cell epitope(s) which is recognized by antigen-specific T cells show a clinical response (Snyder et al., 2014, *NEJM* 371:2189-2199). Those patients that have a low tumor mutational load mostly do not show an objective clinical response (Snyder et al., 2014, *NEJM* 371:2189-2199, Hellman et al., 2018, *Cancer Cell* 33(5): 853-861).

In recent years other groups have developed an alternate approach that does not require the presence of neoepitope presentation by antigen-presenting cells to activate T cells. One example is the development of a bi-specific antibody where the binding domain of an antibody which is specific for a tumor associated antigen, e.g., CD19, is linked to an antibody binding domain specific for CD3 on T cells thus creating a bi-specific T cell engager or BiTe molecule. In 2014, the FDA approved a bi-specific antibody called Blinatumumab for the treatment of Precursor B-Cell Acute Lymphoblastic Leukemia. Blinatumumab links the scFv specific for CD19 expressed on leukemic cells with the scFv specific for CD3 expressed on T cells (Bejnjamin and Stein 2016, *Ther Adv Hematol* 7(3):142-146). However, despite an initial response rate of >50% in patients with relapsed or refractory ALL many patients are resistant to Blinatumumab therapy or relapse after successful treatment with Blinatumumab. Evidence is emerging that the resistant to Blinatumumab or who relapse after Blinatumumab treatment is attributable to the expression of immune checkpoint inhibitory molecules expressed on tumor cells such as PD-L1 that drives an inhibitory signal through PD-1 expressed on activated T cells (Feucht et al., 2016, *Oncotarget* 7(47):76902-76919). In a case study of a patient who was resistant to therapy with Blinatumumab a second round of Blinatumumab therapy was performed but with the addition of a monoclonal antibody, pembrolizumab (Keytruda, Merck), which specific for PD-1 and blocks the interaction of T cell-expressed PD-1 with tumor cell expressed PD-L1 resulted in a dramatic response and reduction of tumor cells in the bone marrow from 45% to less than 5% in this one patient (Feucht et al., 2016, *Oncotarget* 7(47):76902-76919). These results show that combining a bi-specific BiTe molecule with one or more monoclonal antibodies can significantly increase clinical activity compared to either agent alone.

SUMMARY

The present disclosure provides, among others, tetra-specific antibody monomers, antibodies containing tetra-specific monomers, antigen-binding fragments thereof, multi-specific antibodies, immuno-conjugates comprising the disclosed antibodies or monomers, methods of making disclosed monomers, antigen-binding fragments, and antibodies, and methods of using the disclosed molecules for treating cancer.

In one aspect, the present disclosure refers to a tetra-specific antibody monomer having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal, a first scFv domain at the N-terminal, a second scFv domain a Fab domain, a Fc domain, and a third scFv at the C-terminal, wherein the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each has a binding specificity against a different antigen, and wherein the antigen is a tumor antigen, an immune signaling antigen, or a combination thereof.

In another aspect, the present disclosure refers to a tetra-specific antibody, comprising the tetra-specific antibody monomer as described herein, wherein the tetra-specific antibody comprises an amino acid sequence having a percentage homology to SEQ ID NO. 38 and 40, wherein the percentage homology is not less than 98%. In yet another aspect, the present disclosure refers to an isolated nucleic acid sequence, encoding the tetra-specific antibody as described herein comprising an amino acid sequence having a percentage homology to SEQ ID NO. 37 and 39, wherein the percentage homology is not less than 98%.

In yet another aspect, the present disclosure refers to an expression vector, comprising the isolated nucleic acid sequence as described herein. In yet another aspect, the present disclosure refers to a host cell comprising the isolated nucleic acid sequence as described herein, wherein the host cell is a prokaryotic cell or a eukaryotic cell. In a further aspect, the present disclosure refers to a method for producing a tetra-specific antibody, comprising culturing a host cell as described herein comprising an isolated nucleic acid sequence such that the DNA sequence encoding the tetra-specific antibody is expressed, and purifying said tetra-specific antibody, wherein the isolated nucleic acid sequence encodes an amino acid having a percentage homology to SEQ ID NO. 37-40, wherein the percentage homology is not less than 98%.

In a further aspect, the present disclosure refers to a pharmaceutical composition, comprising a pharmaceutically acceptable carrier and the tetraspecific antibody as described herein. In a further aspect, the present disclosure refers to the use of the tetra-specific antibody as described herein in the manufacture of a medicament for treating a human subject with a cancer, wherein an effective amount of the tetra-specific antibody is to be administered to the subject. In yet another aspect, the present disclosure refers to the use of the tetra-specific antibody as described herein and an effective amount of a therapeutic agent in the manufacture of a combination therapy for treating a human subject with a cancer, wherein an effective amount of the therapeutic agent is to be co-administered, and wherein the therapeutic agent comprises an antibody, a chemotherapy agent, an enzyme, an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a check point inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, an inhibitor of PD1, PD-L1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, CD3, CD19 or a combination thereof.

In one example, the application provides tetra-specific antibody monomers. In one example, the tetra-specific antibody monomer has a N-terminal and a C-terminal and include in tandem from the N-terminal to the C-terminal, a first scFv domain at the N-terminal, a second scFv domain, a Fab domain, a Fc domain, and a third scFv at the C-terminal. The first scFv domain, the Fab domain, the second scFv domain, and the third scFv domain each has a binding specificity against a different antigen.

In one example, the antigen includes a tumor antigen, an immune signaling antigen, or a combination thereof. In one example, the first scFv domain, the Fab domain, the second scFv domain, and the third scFv domain each has a binding specificity against a tumor antigen or an immune signaling antigen. In one example, the first scFv domain has a binding specificity against a tumor antigen. In one example, the first scFv domain has a binding specificity against an immune signaling antigen. In one example, the second scFv domain has a binding specificity against a tumor antigen. In one example, the second scFv domain has a binding specificity against an immune signaling antigen. In one example, the Fab domain has a binding specificity against a tumor antigen. In one example, the Fab domain has a binding

specificity against an immune signaling antigen. In one example, the third scFv domain has a binding specificity against a tumor antigen. In one example, the third scFv domain has a binding specificity against a tumor antigen.

In one example, the tetra-specific monomer includes the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain, each independently has a binding specificity against an antigen selected from CD19, CD3, CD137, 4-1BB, PD-L1, ROR1, CD28, 41BB, CEA, HER2, EGFRvIII, EGFR, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypimay-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD20, CD33, CD123, CD22, CD30, PD1, OX40, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, LIGHT, HVEM, CSF1R, CD73, and CD39. In one example, the scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each independently has a binding specificity against tumor specific antigens including, but not limited to, CD19, CD3, CD137, ROR1, CEA, HER2, EGFR, EGFRvIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypimay-3, gpA33, GD2, TROP2, BCMA, CD20, CD33, CD123, CD22, CD30, or immune checkpoint modulators including, without limitation, PD-L1, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, Light, HVEM, CD73, CD39, etc. In one example, one set of scFv domain may specifically bind to an immune checkpoint modulators or a tumor antigen. The scFv specific to CD3 component may be on either C or N terminal of heavy or light chains.

In one example, the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each independently has a binding specificity against an antigen selected from CD19, CD3, CD137M, PD-L1, and 4-1BB. In one example, the first scFv domain has a binding specificity against CD19. In one example, the second scFv domain has a binding specificity against CD3. In one example, the Fab domain has a binding specificity against 4-1BB or CD137. In one example, the third scFv domain has a binding specificity against PD-L1.

In one example, the first scFv domain has a binding specificity against CD19, the second scFv domain has a binding specificity against CD3, the Fab domain has a binding specificity against 4-1BB, and the third scFv domain has a binding specificity against PD-L1. In one example, the first scFv domain has a binding specificity against CD19, the second scFv domain has a binding specificity against CD3, the Fab domain has a binding specificity against CD137, and the third scFv domain has a binding specificity against PD-L1.

The scFv domain may include a linker linking the scFv domain to the heavy chain or light chain of the antibody. In one example, the linker may include more than 10 amino acids. In one example, the linker may include more than 15 amino acids long. In one example, the linker may include less than 20 amino acids.

In one example, the linker may comprises a gly-gly-gly-gly-ser (G4S)_n linker, and n may be an integral between 1 to 20. For example, n may be 2, 4, or 6. In one example, the first scFv domain, the second scFv domain, or the third scFv domain may comprise a gly-gly-gly-gly-ser (G4S)_n linker, wherein n is 2 or 4.

Fc domain may be humanized. In one example, the Fc domain is a human IgG1 Fc.

In one example, the application provides a tetra-specific antibody monomers having an amino acid sequence having a percentage homology to SEQ ID NO. 38 and 39. The percentage homology is not less than 70%, 80%, 90%, 95%, 98% or 99%.

The application further provides antigen-binding fragments. In one example, the application provides scFv domains. In one example, the scFv domain has an amino acid sequence having a percentage homology to SEQ ID No. 2, 4, 6, 8, 10, 12, 26, 28, 30, 32, wherein the percentage homology is not less than 70%, 80%, 90%, 95%, 98% or 99%. In one example, the application provides Fab domains. In one example, the Fab domain includes an amino acid sequence having a percentage homology to SEQ ID NO. 1-12,26-32, wherein the percentage homology is not less than 70%, 80%, 90%, 95%, 98% or 99%. The antigen-binding fragments disclosed herein may be used to construct the tetra-specific antibody monomers or multi-specific antibodies.

In one example, the application provides multi-specific antibodies. In one example, the multi-specific antibody includes tetra-specific antibody monomers. In one example, the multi-specific antibody includes two tetra-specific antibody monomers disclosed herein. As each tetra-specific antibody monomer has four antigen-binding domains, the multi-specific antibody disclosed may include 8 antigen-binding domains. In one example, the antigen binding domains in such multi-specific antibody each independently has a binding specificity against a different antigen therefor providing an octa-specific antibody. In one example, the multi-specific antibody is a penta-specific antibody. In one example, the multi-specific antibody is a penta-specific antibody. In one example, the multi-specific antibody is a penta-specific antibody a hexa-specific antibody. In one example, the multi-specific antibody is a penta-specific antibody a hepta-specific antibody.

In one example, the multi-specific antibody includes a dimer of a tetra-specific antibody monomer therefor providing a tetra-specific antibody. In one example, the application provides an isolated, purified, or non-natural existing multi-specific antibodies. In one example, the application provides a tetra-specific antibody having an amino acid sequence having a percentage homology to SEQ ID No. 37-40. The percentage homology is not less than 70%, 80%, 90%, 95%, 98% or 99%.

The application further provides isolated nucleic acid sequence encoding the tetra-specific antibody monomers, the multi-specific antibodies, or the antigen-binding fragments thereof. In one example, the nucleic acid encodes an amino acid sequence having a percentage homology to the tetra-specific antibody monomer having a SEQ ID No. 37, 38. The percentage homology is not less than 70%, 80%, 90%, 95%, 98% or 99%.

The application further provides expression vectors and host cells comprising the nucleic acid sequences disclosed herein. In one example, the host cell includes the expression vector. The host cell may be a prokaryotic cell or a eukaryotic cell.

The application further provides immuno-conjugates. In one example, the immuno-conjugate includes a cytotoxic agent or an imaging agent linked to the multi-specific antibody disclosed herein through a linker.

The linker may be cleavable or non-cleavable. In one example, the linker may include a covalent bond such as an ester bond, an ether bond, an amid bond, a disulphide bond, an imide bond, a sulfone bond, a phosphate bond, a phosphorus ester bond, a peptide bond, or a combination thereof. In one example, the linker comprises a hydrophobic poly(ethylene glycol) linker.

The cytotoxic agent may include a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent from class of calicheamicin, an antimetabolic agent, a toxin, a radioactive isotope, a therapeutic agent, or a combination thereof. In one example, the cytotoxic agent comprises a calicheamicin, ozogamicin, monomethyl auristatin E, emtansine, a derivative or a combination thereof.

The imaging agent may be any compound useful for imaging purpose. In one example, the imaging agent may be radionuclide, a fluorescent agent, a quantum dots, or a combination thereof.

The application further provides pharmaceutical composition. In one example, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and the tetra-specific antibody monomer disclosed herein. In one example, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and the multi-specific antibody disclosed herein. In one example, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and the antigen-binding fragment disclosed herein. In one example, the pharmaceutical composition comprises a pharmaceutically acceptable carrier and the immuno-conjugate disclosed herein.

In one example, the pharmaceutical composition further includes a therapeutic agent. Example therapeutic agents include without limitation a radioisotope, radionuclide, a toxin, a chemotherapeutic agent, an antibody, an enzyme, or a combination thereof. In one example, the therapeutic agent comprises an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, or a combination thereof.

In one example, the therapeutic agent comprises a check point inhibitor. In one example, the therapeutic agent comprises an inhibitor of PD1, PDL1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, a derivative or a combination thereof.

In a further example, the application provides methods for making the tetra-specific antibody monomers, the multi-specific antibodies, the antigen-binding fragments thereof, and immuno-conjugates thereof. In one example, the method includes the steps of culturing the host cell containing the nucleic acid sequences disclosed herein such that the DNA sequence encoding the antibody is expressed and purifying the antibody. In one example, the antibody is a tetra-specific antibody.

In a further example, the application provides methods of using the tetra-specific antibody monomers, the multi-specific antibodies, the antigen-binding fragments thereof, and immuno-conjugates thereof for cancer treatment. In one example, the method includes the step of administering tetra-specific antibody monomers, the multi-specific antibodies, the antigen-binding fragments thereof, and immuno-conjugates thereof, or pharmaceutical composition thereof to a subject in need of such treatment. In

one example, the method includes the step of administering to the subject an effective amount of the tetra-specific antibody.

In one example, the method includes directly injecting into the tumour site an effective amount of multi-specific monomers, multi-specific antibodies, the immuno-conjugates, the antigen-binding fragment thereof.

Varieties of cancer may be prevented or treated. In one example, the cancer may have cells expressing ROR1, CEA, HER2, EGFR, EGFR VIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypimay-3, gpA33, GD2, TROP2, NKG2D, BCMA, PD-L1, 4-1BB, CD3, CD19, CD20, CD33, CD137, CD123, CD22, or CD30. Example cancers include without limitation breast cancer, colorectal cancer, anal cancer, pancreatic cancer, gallbladder cancer, bile duct cancer, head and neck cancer, nasopharyngeal cancer, skin cancer, melanoma, ovarian cancer, prostate cancer, urethral cancer, lung cancer, non-small lung cell cancer, small cell lung cancer, brain tumor, glioma, neuroblastoma, esophageal cancer, gastric cancer, liver cancer, kidney cancer, bladder cancer, cervical cancer, endometrial cancer, thyroid cancer, eye cancer, sarcoma, bone cancer, leukemia, myeloma or lymphoma.

In one example, the method may further include co-administering an effective amount of a therapeutic agent. In one example, the therapeutic agent may include an antibody, a chemotherapy agent, an enzyme, or a combination thereof. In one example, the therapeutic agent may include an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, or a combination thereof. In one example, the therapeutic agent may include a check point inhibitor. In one example, the therapeutic agent may include an inhibitor of PD1, PD-L1, cd19, cd3, cd137, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, a derivative or a combination thereof.

In one example, the therapeutic agent may include capecitabine, cisplatin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Mustine, vincristine, procarbazine, prednisolone, bleomycin, vinblastine, dacarbazine, etoposide, Epirubicin, pemetrexed, folinic acid, gemcitabine, oxaliplatin, irinotecan, topotecan, camptothecin, docetaxel, paclitaxel, , fulvestrant, tamoxifen, letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, fadrozole, erlotinib, lapatinib, dasatinib, gefitinib, osimertinib, vandertanib, afatinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib, sorafenib, nab-palitaxel, Everolimus, temsirolimus, Dabrafenib, vemurafenib, trametinib, vintafolide, apatinib, crizotinib, periforsine, olaparib, Bortezomib, tofacitinib, trastuzumab, a derivative or a combination thereof.

The subject may be a human. In one example, the subject may be suffering from cancer. The application further provides solutions comprising an effective concentration of the multi-specific antibodies, monomers, or immuno-conjugates disclosed herein. In one example, the solution is blood plasma in a subject.

The objectives and advantages of the disclosure may become apparent from the following detailed description of example examples thereof in connection with the accompanying drawings.

Still other examples may become readily apparent to those skilled in the art from the following detailed description, wherein are described examples by way of illustrating the best mode contemplated. As may be realized, other and different examples are possible and the examples' several details are capable of modifications in various obvious respects, all without departing from their spirit and the scope. Accordingly, the drawings and detailed description are to be regarded as illustrative in nature and not as restrictive.

BRIEF DESCRIPTION OF THE FIGURES

The foregoing and other features of this disclosure may become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying tables and figures. Understanding that these tables and figures depict only several embodiments arranged in accordance with the disclosure and are, therefore, not to be considered limiting of its scope, the disclosure may be described with additional specificity and detail through use of the accompanying drawings, in which:

FIGURE 1 is a diagram of the general format of a guided navigation control (GNC) tetra-specific antibody.

FIGURE 2 depicts experiment results showing re-directed T cell cytotoxicity (RTCC) assay with PBMC (peripheral blood mononuclear cells) as effectors and B-Acute Lymphoblastic Leukemia (B-ALL) cell line Kasumi-2 as targets

FIGURE 3 depicts experiment results showing proliferation of CD8+ T cells induced by tetra-specific GNC antibodies

FIGURE 4 depicts experiment results showing proliferation of CD4+ T cells induced by tetra-specific GNC antibodies

FIGURE 5 depicts experiment results showing secretion of Gamma Interferon from PBMC induced by tetra-specific GNC antibodies

FIGURE 6 depicts experiment results showing secretion of Granzyme B from PBMC induced by tetra-specific GNC antibodies

FIGURE 7 shows example tetra-specific antibodies with CD19 tumor antigen recognition domain.

FIGURE 8 provides a list of example tetra-specific antibodies disclosed herein.

CLAIMS

What is claimed is:

1. A tetra-specific antibody monomer having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal,

a first scFv domain at the N-terminal,

a second scFv domain

a Fab domain,

a Fc domain, and

a third scFv at the C-terminal,

wherein the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each has a binding specificity against a different antigen, and wherein the antigen is a tumor antigen, an immune signaling antigen, or a combination thereof.

2. The tetra-specific antibody monomer of Claim 1, wherein the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, CD3, CD28, 41BB, CEA, HER2, EGFRvIII, EGFR, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypimay-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD19, CD20, CD33, CD123, CD22, CD30, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, LIGHT, HVEM, CSF1R, CD73, and CD39, and wherein the Fc domain comprises a human IgG Fc domain.

3. The tetra-specific antibody monomer of Claim 1, wherein the first scFv domain, the second scFv domain, the Fab domain, and the third scFv domain each independently has a binding specificity against an antigen selected from CD19, CD3, CD137, 4-1BB, and PD-L1.

4. The tetra-specific antibody monomer of Claim 1, wherein the first scFv domain has a binding specificity against CD19.

5. The tetra-specific antibody monomer of Claim1, wherein the second scFv domain has a binding specificity against CD3.

6. The tetra-specific antibody monomer of Claim 1, wherein the Fab domain has a binding specificity against 4-1BB or CD137.

7. The tetra-specific antibody monomer of Claim1, wherein the third scFv domain has a binding specificity against PD-L1.

8. The tetra-specific antibody monomer of Claim 1, wherein the first scFv domain has a binding specificity against CD19, wherein the second scFv domain has a binding specificity against CD3, wherein

the Fab domain has a binding specificity against 4-1BB or CD137, and wherein the third scFv domain has a binding specificity against PD-L1.

9. The tetra-specific antibody monomer of Claim 1, wherein the first scFv domain, the second scFv domain, or the third scFv domain comprises a gly-gly-gly-gly-ser (G4S)_n linker, wherein n is 2, 3 or 4.

10. The tetra-specific antibody monomer of Claim 1, comprising an amino acid sequence having a percentage homology to SEQ ID No. 37-40, wherein the percentage homology is not less than 98%.

11. The scFv domain of the tetra-specific antibody monomer of Claim 1, comprising an amino acid sequence having a percentage homology to SEQ ID No. 2, 4, 6, 8, 10, 12, 26, 28, 30, 32, wherein the percentage homology is not less than 98%.

12. The Fab domain of the tetra-specific antibody monomer of Claim 1, comprising an amino acid sequence having a percentage homology to SEQ ID NO. 1-12, 26-32, wherein the percentage homology is not less than 90%.

13. A tetra-specific antibody, comprising the tetra-specific antibody monomer of Claim 1, wherein the tetra-specific antibody comprises an amino acid sequence having a percentage homology to SEQ ID NO. 38 and 40, wherein the percentage homology is not less than 98%.

14. An isolated nucleic acid sequence, encoding the tetra-specific antibody of Claim 13 comprising an amino acid sequence having a percentage homology to SEQ ID NO. 37 and 39, wherein the percentage homology is not less than 98%.

15. An expression vector, comprising the isolated nucleic acid sequence of Claim 14.

16. A host cell comprising the isolated nucleic acid sequence of Claim 14, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

17. A method for producing a tetra-specific antibody, comprising culturing a host cell of Claim 16 comprising an isolated nucleic acid sequence such that the DNA sequence encoding the tetra-specific antibody is expressed, and purifying said tetra-specific antibody, wherein the isolated nucleic acid sequence encodes an amino acid having a percentage homology to SEQ ID NO. 37-40, wherein the percentage homology is not less than 98%.

18. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and the tetra-specific antibody of Claim 13.

19. Use of the tetra-specific antibody according to Claim 13 in the manufacture of a medicament for treating a human subject with a cancer, wherein an effective amount of the tetra-specific antibody is to be administered to the subject.

20. Use of the tetra-specific antibody according to Claim 13 and an effective amount of a therapeutic agent in the manufacture of a combination therapy for treating a human subject with a cancer, wherein an effective amount of the therapeutic agent is to be co-administered, and wherein the therapeutic

agent comprises an antibody, a chemotherapy agent, an enzyme, an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a check point inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, an inhibitor of PD1, PD-L1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, CD3, CD19 or a combination thereof.