

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 domain 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 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, 4-1BB, and PD-L1.

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 or 40, wherein the percentage homology is not less than 98%. In yet another aspect, the present disclosure refers to a tetra-specific antibody, comprising the tetra-specific antibody monomer as described herein, wherein the tetra-specific monomer comprises: (i) 3 complementarity determining regions (CDRs) of SEQ ID No. 26 and 3 CDRs of SEQ ID No. 28; (ii) 3 CDRs of SEQ ID No. 2 and 3 CDRs of SEQ ID No. 4; (iii) 3 CDRs of SEQ ID No. 14 and 3 CDRs of SEQ ID No. 16; and/or (iv) 3 CDRs of SEQ ID No. 10 and 3 CDRs of SEQ ID No. 12. 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. 38 or 40, 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 sequence having a percentage homology to SEQ ID NO. 38 and an amino acid sequence having a percentage homology to SEQ ID NO. 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

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 domain 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 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, 4-1BB, and PD-L1.

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

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

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

5. The tetra-specific antibody monomer of Claim 1, wherein the third scFv domain has a binding specificity against PD-L1.

6. 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, and wherein the third scFv domain has a binding specificity against PD-L1.

7. 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.

8. The tetra-specific antibody monomer of Claim 1, comprising an amino acid sequence having a percentage homology to SEQ ID No. 38 or 40, wherein the percentage homology is not less than 98%.

9. The tetra-specific antibody monomer of Claim 1, wherein the tetra-specific antibody monomer comprises a scFv domain comprising an amino acid sequence having a percentage homology to SEQ ID No. 2, 4, 6, 8, 10, 12, 26, 28, 30 or 32, wherein the percentage homology is not less than 98%.
10. The tetra-specific antibody monomer of Claim 1, wherein the tetra-specific antibody monomer comprises a Fab domain comprising an amino acid sequence having a percentage homology to SEQ ID NO. 2, 4, 6, 8, 10, 12, 26, 28, 30 or 32, wherein the percentage homology is not less than 90%.
11. The tetra-specific antibody monomer of Claim 1, wherein the monomer comprises: (i) 3 complementarity determining regions (CDRs) of SEQ ID No. 26 and 3 CDRs of SEQ ID No. 28; (ii) 3 CDRs of SEQ ID No. 2 and 3 CDRs of SEQ ID No. 4; (iii) 3 CDRs of SEQ ID No. 14 and 3 CDRs of SEQ ID No. 16; and/or (iv) 3 CDRs of SEQ ID No. 10 and 3 CDRs of SEQ ID No. 12.
12. The tetra-specific antibody monomer of Claim 11, wherein the monomer comprises: (i) 3 complementarity determining regions (CDRs) of SEQ ID No. 26 and 3 CDRs of SEQ ID No. 28; (ii) 3 CDRs of SEQ ID No. 2 and 3 CDRs of SEQ ID No. 4; (iii) 3 CDRs of SEQ ID No. 14 and 3 CDRs of SEQ ID No. 16; and (iv) 3 CDRs of SEQ ID No. 10 and 3 CDRs of SEQ ID No. 12.
13. A tetra-specific antibody, comprising the tetra-specific antibody monomer of Claim 11 or 12.
14. 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 or 40, wherein the percentage homology is not less than 98%.
15. An isolated nucleic acid sequence, encoding the tetra-specific antibody of Claim 14 comprising an amino acid sequence having a percentage homology to SEQ ID NO. 38 or 40, wherein the percentage homology is not less than 98%.
16. An expression vector, comprising the isolated nucleic acid sequence of Claim 15.
17. A host cell comprising the isolated nucleic acid sequence of Claim 15, wherein the host cell is a prokaryotic cell or a eukaryotic cell.
18. A method for producing a tetra-specific antibody, comprising culturing a host cell of Claim 17 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 sequence having a percentage homology to SEQ ID NO. 38 and an amino acid sequence having a percentage homology to SEQ ID NO. 40, wherein the percentage homology is not less than 98%.
19. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and the tetra-specific antibody of Claim 13 or 14.

20. Use of the tetra-specific antibody according to Claim 13 or 14 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.

21. Use of the tetra-specific antibody according to Claim 13 or 14 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.