

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 Blinatumomab for the treatment of Precursor B-Cell Acute Lymphoblastic Leukemia. Blinatumomab 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 Blinatumomab therapy or relapse after successful treatment with Blinatumomab. Evidence is emerging that the resistant to Blinatumomab or who relapse after Blinatumomab 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 Blinatumomab a second round of Blinatumomab 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 may 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, 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 Fab domain, a Fc domain, a second scFv domain, and a third scFv at the C-terminal, wherein 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, and wherein the antigen is a tumor antigen, an immune signaling antigen, or a combination thereof. In yet another aspect, the present disclosure refers to a tetra-specific antibody, comprising a tetra-specific antibody monomer as disclosed herein. In still another aspect, the present disclosure refers to an isolated nucleic acid sequence, encoding a tetra-

specific antibody monomer as disclosed herein. In still another aspect, the present disclosure refers to an expression vector, comprising the isolated nucleic acid sequence of the present disclosure. In still a further aspect, the present disclosure refers to a host cell comprising the isolated nucleic acid sequence of the present disclosure, wherein the host cell is a prokaryotic cell or a eukaryotic cell. In yet another aspect, the present disclosure refers to a method for producing a tetra-specific antibody as disclosed herein or a tetra-specific antibody monomer as disclosed herein, comprising culturing a host cell comprising an isolated nucleic acid sequence such that the DNA sequence encoding the tetra-specific antibody or monomer is expressed, and purifying said tetra-specific antibody or monomer, wherein the isolated nucleic acid sequence encodes an amino acid having a percentage homology to SEQ ID NO. 60, 62, 64, 66 or 68, wherein the percentage homology is not less than 98%. In yet another aspect, the present disclosure refers to a pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a tetra-specific antibody as disclosed herein. In yet another aspect, the present disclosure refers to the use of the tetra-specific antibody according to the present disclosure in the manufacture of a medicament for treating cancer wherein the antibody binds at least one tumor associated antigen and at least one immune signaling antigen.

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 Fab domain, a Fc domain, a second scFv 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 signalling 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 signalling 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 signalling 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 signalling 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 signalling 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.

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 Fab domain,

a Fc domain,

a second scFv domain, and

a third scFv at the C-terminal,

wherein 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, 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 Fab domain, the second scFv domain, and the third scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, CD3, CD28, 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 Fab domain, the second scFv domain, and the third scFv domain each independently has a binding specificity against an antigen selected from CD3, EGFRvIII, PD-L1, and 4-1BB.

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

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

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

7. The tetra-specific antibody monomer of Claim1, wherein the third scFv domain has a binding specificity against 4-1BB.

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

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

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. 02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, or 64, wherein the percentage homology is not less than 98%.

11. 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. 02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60, wherein the percentage homology is not less than 98%.

12. 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. 02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, or 60, wherein the percentage homology is not less than 98%.

13. The tetra-specific antibody monomer of Claim 1, wherein the monomer has a binding domain which is specific for CD3, a binding domain which is specific for a tumor associated antigen, and a third and fourth binding domains which are specific for two distinct immune checkpoint modulators.

14. The tetra-specific antibody monomer of Claim 13, wherein (i) the tumor associated antigen is ROR1, CEA, HER2, EGFR, EGFRvIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypimay-3, gpA33, GD2, TROP2, BCMA, CD19, CD20, CD33, CD123, CD22 or CD30; and/or (ii) the two distinct immune checkpoint modulators are selected from the group consisting of: PD-L1, PD-1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, Light, HVEM, CD73, and CD39.

15. The tetra-specific antibody monomer of Claim 14, wherein the monomer targets human ROR1, human CD19 or EGFRvIII as the tumor associated antigen, and wherein the monomer also carries an anti-human PD-L1, an anti-human 4-1BB and an anti-human CD3 binding domain.

16. The tetra-specific antibody monomer of Claim 1, wherein:

(a) the monomer comprises: 3 complementarity determining regions (CDRs) of SEQ ID NO: 2, 3CDRs of SEQ NO: 4, 3CDRs of SEQ NO: 50, 3CDRs of SEQ NO: 52, 3CDRs of SEQ NO: 14, 3CDRs of SEQ NO: 16, 3CDRs of SEQ NO: 22, and 3CDRs of SEQ NO: 24;

(b) the monomer comprises: 3 CDRs of SEQ ID NO: 30, 3CDRs of SEQ NO: 32, 3 CDRs of SEQ NO: 10, 3 CDRs of SEQ NO: 12, 3CDRs of SEQ NO: 38, 3 CDRs of SEQ NO: 40, 3CDRs of SEQ NO: 2, and 3 CDRs of SEQ NO: 4;

(c) the monomer comprises: 3 CDRs of SEQ ID NO: 26, 3CDRs of SEQ NO:28, 3 CDRs of SEQ NO:10, 3 CDRs of SEQ NO:12, 3CDRs of SEQ NO:38, 3 CDRs of SEQ NO:40, 3CDRs of SEQ NO:2, and 3 CDRs of SEQ NO:4; or

(d) the monomer comprises: 3 CDRs of SEQ ID NO:2, 3CDRs of SEQ NO:4, 3 CDRs of SEQ NO:54, 3 CDRs of SEQ NO:56, 3CDRs of SEQ NO:14, 3 CDRs of SEQ NO:16, 3CDRs of SEQ NO:26, and 3 CDRs of SEQ NO:28.

17. A tetra-specific antibody, comprising a tetra-specific antibody monomer of Claim 1.

18. The tetra -specific antibody of Claim 17, comprising an amino acid sequence having a percentage homology to SEQ ID NO. 66, and an amino acid sequence having a percentage homology to SEQ ID NO. 68, wherein the percentage homology is not less than 98%.

19. A tetra-specific antibody, comprising a tetra-specific antibody monomer of any one of claims 2 to 16.

20. An isolated nucleic acid sequence encoding a tetra-specific antibody monomer of Claim 1.

21. The isolated nucleic acid sequence of Claim 20 comprising an amino acid sequence having a percentage homology to SEQ ID NO. 02, 04, 06, 08, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 66 or 68 wherein the percentage homology is not less than 98%.

22. An expression vector, comprising the isolated nucleic acid sequence of Claim 20 or 21.

23. A host cell comprising the isolated nucleic acid sequence of Claim 20 or 21, wherein the host cell is a prokaryotic cell or a eukaryotic cell.

24. A method for producing a tetra-specific antibody according to Claim 17 or a tetra-specific antibody monomer according to Claim 1, comprising culturing a host cell comprising an isolated nucleic acid sequence such that the DNA sequence encoding the tetra-specific antibody or monomer is expressed, and purifying said tetra-specific antibody or monomer, wherein the isolated nucleic acid sequence encodes an amino acid having a percentage homology to SEQ ID NO. 60, 62, 64, 66 or 68, wherein the percentage homology is not less than 98%.

25. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a tetra-specific antibody of any one of Claims 17 to 19.

26. Use of the tetra-specific antibody according to any one of Claims 17 to 19 in the manufacture of a medicament for treating cancer wherein the antibody binds at least one tumor associated antigen and at least one immune signaling antigen.