

sequence having a percentage homology to SEQ ID NO: 24, wherein the percentage homology is not less than 98%.

In yet another aspect, the present disclosure refers to a tetra-specific antibody comprising 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 domain 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, 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, PD-L1, 4-1BB, and ROR1; and wherein the tetra-specific antibody comprises 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%.

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. 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, wherein the method comprises culturing a host cell capable of expressing the antibody or the monomer such that the tetra-specific antibody or monomer is expressed, and purifying said tetra-specific antibody or monomer, wherein the host cell capable of expressing the antibody or monomer is a host cell as disclosed herein. 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.

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 domain 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, wherein the first scFv domain, the Fab domain, the second scFv domain, and the third scFv domain each independently has a binding specificity against:

- (i) an antigen selected from CD3, PD-L1, 4-1BB, and ROR1; or
- (ii) an antigen selected from CD3, PD-L1, 4-1 BB, and CD19; or
- (iii) an antigen selected from CD3, PD-L1, 4-1 BB, and EGFRvIII; and

wherein the monomer comprises:

(a) 3 complementarity determining regions (CDRs) of SEQ ID NO: 30, 3 CDRs of SEQ NO: 32, 3 CDRs of SEQ NO: 10, 3 CDRs of SEQ NO: 12, 3 CDRs of SEQ NO: 38, 3 CDRs of SEQ NO: 40, 3 CDRs of SEQ NO: 2, and 3 CDRs of SEQ NO: 4; or

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

(c) 3 CDRs of SEQ ID NO:2, 3 CDRs of SEQ NO:4, 3 CDRs of SEQ NO:54, 3 CDRs of SEQ NO:56, 3 CDRs of SEQ NO:14, 3 CDRs of SEQ NO:16, 3 CDRs of SEQ NO:26, and 3 CDRs of SEQ NO:28; or

(d) 3 CDRs of SEQ ID NO: 2, 3 CDRs of SEQ NO: 4, 3 CDRs of SEQ NO: 50, 3 CDRs of SEQ NO: 52, 3 CDRs of SEQ NO: 14, 3 CDRs of SEQ NO: 16, 3 CDRs of SEQ NO: 22, and 3 CDRs of SEQ NO: 24.

2. 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 domain 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, wherein the first scFv domain, the Fab domain, the second scFv domain, and the third scFv domain each independently has a binding specificity against:

- (i) an antigen selected from CD3, PD-L1, 4-1BB, and ROR1; or
- (ii) an antigen selected from CD3, PD-L1, 4-1 BB, and CD19; or
- (iii) an antigen selected from CD3, PD-L1, 4-1 BB, and EGFRvIII; and

wherein the monomer comprises:

- a) an amino acid sequence having a percentage homology to SEQ ID NO: 30, an amino acid sequence having a percentage homology to SEQ ID NO: 32, an amino acid sequence having a percentage homology to SEQ ID NO: 10, an amino acid sequence having a percentage homology to SEQ ID NO: 12, an amino acid sequence having a percentage homology to SEQ ID NO: 38, an amino acid sequence having a percentage homology to SEQ ID NO: 40, an amino acid sequence having a percentage homology to SEQ ID NO: 2 and an amino acid sequence having a percentage homology to SEQ ID NO: 4, wherein the percentage homology is not less than 98%; or
- b) an amino acid sequence having a percentage homology to SEQ ID NO: 26, an amino acid sequence having a percentage homology to SEQ ID NO: 28, an amino acid sequence having a percentage homology to SEQ ID NO: 10, an amino acid sequence having a percentage homology to SEQ ID NO: 12, an amino acid sequence having a percentage homology to SEQ ID NO: 38, an amino acid sequence having a percentage homology to SEQ ID NO: 40, an amino acid sequence having a percentage homology to SEQ ID NO: 2 and an amino acid sequence having a percentage homology to SEQ ID NO: 4, wherein the percentage homology is not less than 98%; or
- c) an amino acid sequence having a percentage homology to SEQ ID NO: 2, an amino acid sequence having a percentage homology to SEQ ID NO: 4, an amino acid sequence having a percentage homology to SEQ ID NO: 54, an amino acid sequence having a percentage homology to SEQ ID NO: 56, an amino acid sequence having a percentage homology to SEQ ID NO: 14, an amino acid sequence having a percentage homology to SEQ ID NO: 16, an amino acid sequence having a percentage homology to SEQ ID NO: 26 and an amino acid sequence having a percentage homology to SEQ ID NO: 28, wherein the percentage homology is not less than 98%; or

- d) an amino acid sequence having a percentage homology to SEQ ID NO: 2, an amino acid sequence having a percentage homology to SEQ ID NO: 4, an amino acid sequence having a percentage homology to SEQ ID NO: 50, an amino acid sequence having a percentage homology to SEQ ID NO: 52, an amino acid sequence having a percentage homology to SEQ ID NO: 14, an amino acid sequence having a percentage homology to SEQ ID NO: 16, an amino acid sequence having a percentage homology to SEQ ID NO: 22 and an amino acid sequence having a percentage homology to SEQ ID NO: 24, wherein the percentage homology is not less than 98%.
3. The tetra-specific antibody monomer of Claim 1 or 2, wherein the first scFv domain has a binding specificity against CD3.
 4. The tetra-specific antibody monomer of Claim 1 or 2, wherein the Fab domain has a binding specificity against EGFRvIII.
 5. The tetra-specific antibody monomer of Claim 1 or 2, wherein the second scFv domain has a binding specificity against PD-L1.
 6. The tetra-specific antibody monomer of Claim 1 or 2, wherein the third scFv domain has a binding specificity against 4-1BB.
 7. The tetra-specific antibody monomer of Claim 1 or 2, 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.
 8. The tetra-specific antibody monomer of Claim 1 or 2, 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.
 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. 02, 04, 10, 12, 14, 16, 22, 24, 26, 28, 30, 32, 38, 40, 50, 52, 54 or 56, 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. 02, 04, 10, 12, 14, 16, 22, 24, 26, 28, 30, 32, 38, 40, 50, 52, 54 or 56, wherein the percentage homology is not less than 98%.
 11. A tetra-specific antibody comprising 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 domain 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, 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, PD-L1, 4-1BB, and ROR1; and

wherein the tetra-specific antibody comprises 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%.

12. A tetra-specific antibody, comprising a tetra-specific antibody monomer of any one of Claims 1 to 10.

13. An isolated nucleic acid sequence encoding a tetra-specific antibody monomer of Claim 1 or 2.

14. The isolated nucleic acid sequence of Claim 13 encoding a tetra-specific antibody monomer of Claim 1 and comprising a nucleic acid sequence encoding an amino acid sequence having a percentage homology to SEQ ID NO. 02, 04, 10, 12, 14, 16, 22, 24, 26, 28, 30, 32, 38, 40, 50, 52, 54, 56, 66 or 68 wherein the percentage homology is not less than 98%.

15. An expression vector, comprising the isolated nucleic acid sequence of Claim 13 or 14.

16. A host cell comprising the isolated nucleic acid sequence of Claim 13 or 14.

17. A host cell according to claim 16 wherein the host cell comprises an isolated nucleic acid sequence encoding an amino acid sequence having a percentage homology to SEQ ID NO. 66 and an isolated nucleic acid sequence encoding an amino acid sequence having a percentage homology to SEQ ID NO. 68, wherein the percentage homology is not less than 98%.

18. A host cell according to Claim 16 or 17 wherein the host cell is a prokaryotic cell.

19. A host cell according to Claim 16 or 17, wherein the host cell is a eukaryotic cell.

20. A method for producing a tetra-specific antibody according to Claim 11 or 12 or a tetra-specific antibody monomer according to Claim 1 or 2, wherein the method comprises culturing a host cell capable of expressing the antibody or the monomer such that the tetra-specific antibody or monomer is expressed, and purifying said tetra-specific antibody or monomer, wherein the host cell capable of expressing the antibody or monomer is a host cell according to any one of Claims 16 to 19.

21. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a tetra-specific antibody according to Claim 11 or 12.

22. Use of the tetra-specific antibody according to Claim 11 or 12 in the manufacture of a medicament for treating cancer.