

CLAIMS

What is claimed is:

1. A multi-specific antibody-like protein having a N-terminus and a C-terminus, comprising, a first monomer, comprising, from the N-terminus to the C-terminus, a first binding monomer, a CH1 domain, a first hinge, a first CH2 domain, and a first CH3 domain, wherein the first monomer comprises optionally a first binding domain (D1) linked to the N-terminus, a fourth binding domain (D4) linked to the C-terminus, or both,

a second monomer, comprising, from the N-terminus to the C-terminus, a second binding monomer, a CL domain, a second hinge, a second CH2 domain, and a second CH3 domain, wherein the second monomer comprises optionally a second binding domain (D2) linked to the N-terminus, a fifth binding domain (D5) linked to the C-terminus, or both,

wherein the first binding monomer and a second binding monomer are configured to form a dimer,

wherein the first monomer and the second monomer are covalently paired through at least one disulfide bond between the CH1 domain and the CL domain and at least one disulfide bond between the first hinge and the second hinge, and

wherein the multi-specific antibody-like protein is at least bi-specific.

2. The multi-specific antibody-like protein of Claim 1, wherein the first binding monomer comprises a VH domain, a second binding monomer comprises a VL domain, and wherein the VH, CH1, VL, CL domains form a Fab region as a third binding domain (D3).

3. The multi-specific antibody-like protein of Claim 1, wherein the first binding monomer and the second binding monomer form a NKG2D receptor as a third binding domain (D3).

4. The multi-specific antibody-like protein of Claim 1, wherein the first CH3 domain is configured to form a hole structure, wherein the second CH3 domain is configured to form a knob structure, and wherein the first CH2 and CH3 domains and the second CH2 and CH3 domains are configured to heterodimerize to form a complementary Fc domain.

5. The multi-specific antibody-like protein of Claim 1, wherein the D1, D2, D4, and D5 is independently a scFv domain, a VHH domain, a receptor, or a ligand.

6. The multi-specific antibody-like protein of Claim 1, wherein the D1, D2, D3, D4, and D5 each independently has a binding specificity against an antigen selected, EGFR, HER2, HER3, EGFRvIII, ROR1, CD3, CD28, CEA, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33,

GD2, TROP2, NKG2D, NKG2D ligand, BCMA, CD19, CD20, CD33, CD123, CD22, CD30, PD-L1, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, CD40L, VISTA, ICOS, BTLA, LIGHT, HVEM, CSF1R, CD73, CD39, CLDN18.2, DLL3, HLA-G, FcRH5, GPRC5D, LIV-1, MUC1, CD138, CD70, CD16, uPAR, Siglec-15, CD47, CD38, , NKp46, PD-L2, CD160, LOX-1, SIRP α , CD27 and wherein the Fc domain comprises a human IgG Fc domain.

7. The multi-specific antibody-like protein of Claim 1, wherein D1 has a binding specificity to CD3, CD20, CEA, HER2, EGFR, or NKG2D ligand.

8. The multi-specific antibody-like protein of Claim 1, wherein D2 has a binding specificity to HER3, EGFR, CD3, or CD19.

9. The multi-specific antibody-like protein of Claim 1, wherein D3 has a binding specificity to HER3, EGFR, CD3, or NKG2D ligands.

10. The multi-specific antibody-like protein of Claim 1, wherein D4 has a binding specificity to 4-1BB, or EGFR.

11. The multi-specific antibody-like protein of Claim 1, wherein D5 has a binding specificity to PD-L1 or HER3.

12. The multi-specific antibody-like protein of Claim 1, wherein the antibody is a tri-specific antibody having the binding specificity to EGFR, HER3, and CD3.

13. The multi-specific antibody-like protein of Claim 1, wherein the antibody is a tetra-specific antibody having the binding specificity to EGFR, HER3, CD3, and 4-1BB.

14. The multi-specific antibody-like protein of Claim 1, wherein the antibody is a tetra-specific antibody having the binding specificity to EGFR, HER3, CD3, and PD-L1.

15. The multi-specific antibody-like protein of Claim 1, wherein the antibody is a penta-specific antibody having the binding specificity to EGFR, HER3, CD3, 4-1BB and PD-L1.

16. A complementary determining region (CDR) having an affinity to CEA, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 301, 302, 303, 304, 305, or 306.

17. A protein having an affinity to CEA, comprising the CDR of Claim 16.

18. A multi-specific antibody-like protein having a binding affinity to CEA, wherein the antibody-like protein comprises an amino acid sequence having at least 98% sequence identity to an amino acid sequence selected from SEQ ID NO. 279, 280, 281 or 282.

19. A complimentary determining region (CDR) having a binding affinity to CD3, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO. 307, 308, 309, 310, 311 or 312.
20. A protein having an affinity to CD3, comprising CDR of Claim 19.
21. A multi-specific antibody-like protein having a binding affinity to CD3, wherein the antibody-like protein comprises an amino acid sequence having at least 98% sequence identity to an amino acid sequence selected from SEQ ID NO. 227-230, 231-234, 235-238, 239-242 and 291-294.
22. An isolated nucleic acid sequence encoding the multi-specific antibody-like protein of Claim 1.
23. An expression vector comprising the isolated nucleic acid sequences of Claim 22.
24. A host cell comprising the isolated nucleic acid sequence of Claim 22.
25. An immunoconjugate comprising the multi-specific antibody-like protein of Claim 1 and a cytotoxic agent.
26. A pharmaceutical composition, comprising the multi-specific antibody-like protein of Claim 1 and a pharmaceutically acceptable carrier.
27. A pharmaceutical composition, comprising the immunoconjugate of Claim 25 and a pharmaceutically acceptable carrier.
28. A method for treating or preventing a cancer, an autoimmune disease, or an infectious disease in a subject, said method comprising administering to the subject a pharmaceutical composition comprising a purified multi-specific antibody-like protein of Claim 1.
29. A method for producing the multi-specific antibody-like protein of Claim 1, comprising
culturing a host cell such that the DNA sequence encoding the multi-specific antibody-like protein of Claim 1 is expressed, and
purifying said multi-specific antibody-like protein.
30. A solution comprising an effective concentration of the multi-specific antibody-like protein of Claim 1, wherein the solution is blood plasma in a subject.