

What is claimed is:

1. A multispecific antigen-binding molecule, comprising:
 - (a) a first polypeptide comprising, from N-terminus to C-terminus (i) a first antigen-binding domain that specifically binds a T cell antigen, (ii) a first multimerizing domain, and (iii) a second antigen-binding domain that specifically binds a T cell antigen; and
 - (b) a second polypeptide comprising, from N-terminus to C-terminus (i) a third antigen-binding domain that specifically binds a target antigen, and (ii) a second multimerizing domain,wherein the first and the second multimerizing domains associate with one another to form the molecule.
2. The molecule of claim 1, wherein the second polypeptide further comprises a fourth antigen-binding domain at the C-terminus of the second multimerizing domain.
3. The molecule of claim 2, wherein the fourth antigen-binding domain specifically binds a target antigen.
4. The molecule of claim 2, wherein the fourth antigen-binding domain specifically binds a T cell antigen.
5. The molecule of claim 3, wherein the third antigen-binding domain and the fourth antigen-binding domain specifically bind distinct target antigens.
6. The molecule of claim 5, wherein the distinct target antigens are expressed on the surface of the same cell.
7. The molecule of claim 3, wherein the third antigen-binding domain and the fourth antigen-binding domain specifically bind the same target antigen.
8. The molecule of any one of claims 1 to 7, wherein the first antigen-binding domain and the second antigen-binding domain specifically bind the same T-cell antigen.
9. The molecule of any one of claims 1 to 7, wherein the first antigen-binding domain and the second antigen-binding domain specifically bind distinct T-cell antigens.

10. The molecule of claim 9, wherein the first antigen-binding domain specifically binds a first T-cell antigen that is a co-stimulatory molecule, and the second antigen-binding domain specifically binds a second T-cell antigen that is a check-point inhibitor.

11. The molecule of claim 10, wherein the co-stimulatory molecule is CD28 and the check-point inhibitor is PD-1.

12. The molecule of claim 4, wherein the first, second and fourth antigen-binding domains specifically bind the same T-cell antigen.

13. The molecule of claim 4, wherein the first, second and fourth antigen-binding domains bind distinct T-cell antigens.

14. The molecule of claim 4, wherein the first and fourth antigen-binding domains specifically bind the same T-cell antigen.

15. The molecule of claim 4, wherein the second and fourth antigen-binding domains specifically bind the same T-cell antigen.

16. The molecule of any one of claims 1 to 15, wherein one or more of the antigen-binding domains is a Fab domain.

17. The molecule of any one of claims 1 to 16, wherein one or more of the antigen-binding domains is an scFv domain.

18. The molecule of claim 17, wherein the scFv domain comprises a heavy chain variable region (HCVR) comprising a cysteine mutation at residue 44, and a light chain variable region comprising a cysteine mutation at residue 100 (Kabat numbering).

19. The molecule of claim 17 or 18, wherein the scFv comprises a HCVR and a LCVR joined together via a polypeptide linker of from 10 to 30 amino acids, optionally a (G4S)₄ linker.

20. The molecule of claim 17 or 18, wherein the scFv is connected to the C-terminus of the first and/or second multimerizing domain via a linker of from 5 to 25 amino acids, optionally a (G4S)₃ linker.

21. The molecule of any one of claims 1 to 20, wherein the first antigen-binding domain and the third antigen-binding domain are Fab domains.

22. The molecule of any one of claims 1 to 21, wherein the second antigen-binding domain is an scFv domain.

23. The molecule of any one of claims 2 to 22, wherein the fourth antigen-binding domain is an scFv domain.

24. The molecule of claim 1, wherein the first, second and third antigen-binding domains are Fab domains.

25. The molecule of claim 1, wherein the first and third antigen-binding domains are Fab domains, and the second antigen-binding domain is an scFv domain.

26. The molecule of claim 2, wherein the first, second, third and fourth antigen-binding domains are Fab domains.

27. The molecule of claim 3, wherein the first, second, third and fourth antigen-binding domains are Fab domains.

28. The molecule of claim 5, wherein the first and third antigen-binding domains are Fab domains, and the second and fourth antigen-binding domains are scFv domains.

29. The molecule of claim 5, wherein the first, second, third and fourth antigen-binding domains are Fab domains.

30. The molecule of claim 4, wherein the first and third antigen-binding domains are Fab domains, and the second and fourth antigen-binding domains are scFv domains.

31. The molecule of claim 4, wherein the first, second, third and fourth antigen-binding domains are Fab domains.

32. The molecule of any one of claims 9 to 11, wherein the first and third antigen-binding domains are Fab domains, and the second and fourth antigen-binding domains are scFv domains.

33. The molecule of any one of claims 9 to 11, wherein the first, second, third and fourth antigen-binding domains are Fab domains.

34. The molecule of any one of claims 1 to 33, wherein the T cell antigen is a T cell receptor complex antigen.

35. The molecule of claim 34, wherein the T cell antigen is CD3.
36. The molecule of any one of claims 1 to 33, wherein the T cell antigen is a co-stimulatory molecule or a check-point inhibitor on a T cell.
37. The molecule of any one of claims 1 to 33, wherein the T cell antigen is selected from the group consisting of CD27, CD28, 4-1BB and PD-1.
38. The molecule of any one of claims 1 to 37, wherein the target antigen is a tumor-associated antigen.
39. The molecule of any one of claims 1 to 38, wherein the first and second multimerizing domains are immunoglobulin Fc domains.
40. The molecule of claim 39, wherein the first and second multimerizing domains associate with one another via disulfide bonding.
41. The molecule of any one of claims 1 to 40, wherein the first multimerizing domain and the second multimerizing domain are human IgG1 or human IgG4 Fc domains.
42. The molecule of any one of claims 39 to 41, wherein the first multimerizing domain or the second multimerizing domain comprises an amino acid substitution that reduces affinity for Protein A binding compared to a wild-type Fc domain of the same isotype.
43. The molecule of claim 42, wherein the amino acid substitution comprises an H435R modification, or H435R and Y436F modifications (EU numbering).
44. The molecule of claim 43, wherein the first multimerizing domain comprises the H435R and Y436F modifications.
45. The molecule of any one of claims 1 to 44, wherein the first polypeptide, the second polypeptide, or both the first and the second polypeptides comprise a modified hinge domain that reduces binding affinity for an Fcγ receptor relative to a wild-type hinge domain of the same isotype.
46. A multispecific antigen-binding molecule, comprising:
- (a) a first polypeptide comprising, from N-terminus to C-terminus (i) a first Fab that specifically binds a T cell antigen, (ii) a first immunoglobulin Fc domain, and (iii) a first scFv that specifically binds a T cell antigen; and

(b) a second polypeptide comprising, from N-terminus to C-terminus (i) a second Fab that specifically binds a target antigen, (ii) a second immunoglobulin Fc domain, and (iii) a second scFv that specifically binds a target antigen,

wherein the first and the second immunoglobulin domains associate with one another via disulfide bonding to form the molecule.

47. The molecule of claim 46, wherein the second Fab and the second scFv specifically bind distinct target antigens.

48. The molecule of claim 47, wherein the distinct target antigens are expressed on the surface of the same cell.

49. The molecule of claim 46, wherein the second Fab and the second scFv specifically bind the same target antigen.

50. A multispecific antigen-binding molecule, comprising:

(a) a first polypeptide comprising, from N-terminus to C-terminus (i) a first Fab that specifically binds a T cell antigen, (ii) a first immunoglobulin Fc domain, and (iii) a second Fab that specifically binds a T cell antigen; and

(b) a second polypeptide comprising, from N-terminus to C-terminus (i) a third Fab that specifically binds a target antigen, (ii) a second immunoglobulin Fc domain, and (iii) a fourth Fab that specifically binds a target antigen,

wherein the first and the second immunoglobulin domains associate with one another via disulfide bonding to form the molecule.

51. The molecule of claim 50, wherein the third Fab and the fourth Fab specifically bind distinct target antigens.

52. The molecule of claim 51, wherein the distinct target antigens are expressed on the surface of the same cell.

53. The molecule of claim 50, wherein the third Fab and the fourth Fab specifically bind the same target antigen.

54. A multispecific antigen-binding molecule, comprising:

(a) a first polypeptide comprising, from N-terminus to C-terminus (i) a first Fab that specifically binds a T cell antigen, (ii) a first immunoglobulin Fc domain, and (iii) a first scFv that specifically binds a T cell antigen; and

(b) a second polypeptide comprising, from N-terminus to C-terminus (i) a second Fab that specifically binds a target antigen, (ii) a second immunoglobulin Fc domain, and (iii) a second scFv that specifically binds a T cell antigen,

wherein the first and the second immunoglobulin domains associate with one another via disulfide bonding to form the molecule.

55. A multispecific antigen-binding molecule, comprising:

(a) a first polypeptide comprising, from N-terminus to C-terminus (i) a first Fab that specifically binds a T cell antigen, (ii) a first immunoglobulin Fc domain, and (iii) a second Fab that specifically binds a T cell antigen; and

(b) a second polypeptide comprising, from N-terminus to C-terminus (i) a second Fab that specifically binds a target antigen, and (ii) a second immunoglobulin Fc domain,

wherein the first and the second immunoglobulin domains associate with one another via disulfide bonding to form the molecule.

56. The molecule of any one of claims 46 to 55, wherein the T cell antigen is a T cell receptor complex antigen.

57. The molecule of any one of claims 46 to 55, wherein the T cell antigen is CD3.

58. The molecule of any one of claims 46 to 55, wherein the T cell antigen is a co-stimulatory molecule or a check-point inhibitor on a T cell.

59. The molecule of any one of claims 46 to 55, wherein the T cell antigen is selected from the group consisting of CD27, CD28, 4-1BB and PD-1.

60. The molecule of any one of claims 46 to 59, wherein the target antigen is a tumor-associated antigen.

61. The molecule of any one of claims 46 to 60, wherein the first immunoglobulin Fc domain and the second immunoglobulin Fc domain are human IgG1 or human IgG4 Fc domains.

62. The molecule of claim 61, wherein the first immunoglobulin Fc domain or the second immunoglobulin Fc domain comprises an amino acid substitution that reduces affinity for Protein A binding compared to a wild-type Fc domain of the same isotype.

63. The molecule of claim 62, wherein the amino acid substitution comprises an H435R modification, or H435R and Y436F modifications (EU numbering).

64. The molecule of claim 63, wherein the first immunoglobulin Fc domain comprises the H435R and Y436F modifications.

65. The molecule of any one of claims 46 to 64, wherein the first polypeptide, the second polypeptide, or both the first and the second polypeptides comprise a modified hinge domain that reduces binding affinity for an Fcγ receptor relative to a wild-type hinge domain of the same isotype.

66. The molecule of any one of claims 1 to 65, wherein the target antigen is a peptide in the context of the groove of a major histocompatibility complex (MHC) protein.

67. A pharmaceutical composition comprising the molecule of any one of claims 1 to 66 and a pharmaceutically acceptable carrier or diluent.

68. A method of treating cancer, comprising administering the molecule of any one of claims 1 to 66 to a subject in need thereof.

69. A method of treating an infection, comprising administering the molecule of any one of claims 1 to 66 to a subject in need thereof.

70. The method of claim 69, wherein the infection is a bacterial infection.

71. The method of claim 69, wherein the infection is a viral infection.

72. The method of claim 69, wherein the infection is a fungal infection.

73. The method of claim 69, wherein the infection is a parasite infection.

74. The method of any one of claims 68 to 73, wherein the target antigen is present at a density of from 10 to 10,000,000 copies per target cell, from 100 to 1,000,000 copies per target cell, from 100 to 20,000 copies per target cell, or from 100 to 5000 copies per target cell.

75. The method of any one of claims 68 to 73, wherein the target antigen is present at a density of from 1000 to 20,000 copies per target cell, or greater than 20,000 copies per target cell.

76. The method of any one of claims 68 to 75, wherein the molecule is administered in combination with a second therapeutic agent.

77. The method of claim 76, wherein the second therapeutic agent comprises a bispecific antigen-binding molecule comprising a first antigen-binding domain that binds a target antigen (TA) and a second antigen-binding domain that binds a T-cell antigen.

78. The method of claim 77, wherein the target antigen is a tumor-cell antigen.

79. The method of any one of claims 76 to 78, wherein the second therapeutic agent comprises a bispecific anti-TA x anti-CD28 antibody.

80. The method of claim 79, wherein the second therapeutic agent comprises a bispecific anti-EGFR x anti-CD28 antibody.

81. The method of claim 76, wherein the second therapeutic agent comprises an antibody that binds a check-point inhibitor on a T cell.

82. The method of claim 81, wherein the second therapeutic agent comprises an anti-PD-1 antibody.

83. The method of any one of claims 76 to 82, wherein the molecule is administered in combination with two or more second therapeutic agents.

84. Use of the molecule of any one of claims 1 to 66 in the manufacture of a medicament for treating cancer in a subject in need thereof.

85. Use of the molecule of any one of claims 1 to 66 in the manufacture of a medicament for treating an infection in a subject in need thereof.

86. The use of claim 85, wherein the infection is a bacterial infection, a viral infection, a fungal infection, or a parasite infection.

87. Use of the molecule of any one of claims 1 to 66 in the treatment of cancer.

88. Use of the molecule of any one of claims 1 to 66 in the treatment of an infection.

89. The use of claim 88, wherein the infection is a bacterial infection, a viral infection, a fungal infection, or a parasite infection.

90. The molecule of any one of claims 1 to 66 for use in medicine.

91. The molecule of any one of claims 1 to 66 for use in treating cancer.

92. The molecule of any one of claims 1 to 66 for use in treating an infection.

93. The molecule of claim 92, wherein the infection is a bacterial infection, a viral infection, a fungal infection, or a parasite infection.