

[0005] Also provided herein in certain aspects are trispecific antigen-binding proteins, wherein said proteins comprise (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to a target antigen, wherein the domains are linked in the order H₂N-(A)-(B)-(C)-COOH, H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(B)-(C)-(A)-COOH, H₂N-(C)-(B)-(A)-COOH, or H₂N-(C)-(A)-(B)-COOH by linkers L1 and L2, and wherein the first domain binds to human CD3 with a K_D of greater than 100 nM.

[0006] Also provided herein in certain aspects are trispecific antigen-binding proteins, wherein said proteins comprise (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (C) which specifically binds to a target antigen, wherein the domains are linked in the order H₂N-(A)-(B)-(C)-COOH, H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(B)-(C)-(A)-COOH, H₂N-(C)-(B)-(A)-COOH, or H₂N-(C)-(A)-(B)-COOH by linkers L1 and L2, and wherein the protein has a molecular weight of less than 55 kDa.

[0006a] Also provided herein in certain aspects are trispecific antigen-binding proteins, wherein said protein comprises (a) a first domain (A) which comprises a single chain variable fragment (scFv) that specifically binds to human CD3; (b) a second domain (B) which comprises a single domain antibody (sdAb) that binds human serum albumin, wherein the second domain comprises an amino acid sequence of SEQ ID NO: 47; (c) a third domain (C) which comprises a scFv or sdAb that specifically binds to a target tumor antigen; wherein the domains are linked in the order H₂N-(C)-(B)-(A)-COOH or by linkers L1 and L2 in the order H₂N-(C)-L1-(B)-L2-(A)-COOH.

[0006b] Also provided herein in certain aspects are trispecific antigen-binding protein, wherein said protein comprises (a) a first domain (A) which comprises a single chain variable fragment (scFv) that specifically binds to human CD3; (b) a second domain (B) which comprises a single domain antibody (sdAb) that binds human serum albumin, wherein the second domain comprises an amino acid sequence with a 90% identity to SEQ ID NO: 47; (c) a third domain (C) which comprises a scFv or sdAb that specifically binds to a target tumor antigen; wherein the domains are linked by linkers L1 and L2 in the order HN-(C)-(B)-(A)-COOH.

[0007] Also provided herein in certain aspects are trispecific antigen-binding proteins, wherein said proteins comprise (a) a first domain (A) which specifically binds to human CD3; (b) a second domain (B) which is a half-life extension domain; and (c) a third domain (c) which specifically binds to a target antigen, wherein the domains are linked in the order H₂N-(A)-(B)-(C)-COOH, H₂N-(A)-(C)-(B)-COOH, H₂N-(B)-(A)-(C)-COOH, H₂N-(B)-(C)-(A)-COOH, H₂N-

(C)-(B)-(A)-COOH, or H₂N-(C)-(A)-(B)-COOH by linkers L1 and L2, and wherein B comprises a single domain antibody that binds to serum albumin.

[0008] Various embodiments of trispecific antigen-binding proteins are also provided herein, contemplated for any aspect herein, alone or in combination. In some embodiments, first domain comprises a variable light chain and variable heavy chain each of which is capable of specifically binding to human CD3. In some embodiments, the variable light chain is a λ (lamda) light chain. In some embodiments, the variable light chain is a κ (kappa) light chain. In some embodiments, the first domain comprises a single-chain variable fragment (scFv) specific to human CD3. In some embodiments, the first domain is specific for CD3 ϵ (epsilon). In some embodiments, the first domain is specific for CD3 δ (delta). In some embodiments, the first domain is specific for CD3 γ (gamma). In some embodiments, the first domain comprises complementary determining regions (CDRs) selected from the group consisting of muromonab-CD3 (OKT3), orelizumab (TRX4), teplizumab (MGA031), visilizumab (Nuvion), SP34, X35, VIT3, BMA030 (BW264/56), CLB-T3/3, CRIS7, YTH12.5, F111-409, CLB-T3.4.2, TR-66, WT32, SPv-T3b, 11D8, XIII-141, XIII-46, XIII-87, 12F6, T3/RW2-8C8, T3/RW2-4B6, OKT3D, M-T301, SMC2, F101.01, UCHT-1 and WT-31. In some embodiments, the first

CLAIMS

WHAT IS CLAIMED IS:

1. A trispecific antigen-binding protein, wherein said protein comprises
 - (a) a first domain (A) which comprises a single chain variable fragment (scFv) that specifically binds to human CD3;
 - (b) a second domain (B) which comprises a single domain antibody (sdAb) that binds human serum albumin, wherein the second domain comprises an amino acid sequence of SEQ ID NO: 47;
 - (c) a third domain (C) which comprises a scFv or sdAb that specifically binds to a target tumor antigen;wherein the domains are linked in the order H₂N-(C)-(B)-(A)-COOH or by linkers L1 and L2 in the order H₂N-(C)-L1-(B)-L2-(A)-COOH.
2. The trispecific antigen-binding protein of claim 1, wherein the first domain is humanized or human.
3. The trispecific antigen-binding protein of claim 1, wherein linkers L1 and L2 are each independently selected from (GS)_n (SEQ ID NO: 49), (GGS)_n (SEQ ID NO: 50), (GGGS)_n (SEQ ID NO: 51), (GGSG)_n (SEQ ID NO: 52), (GGSGG)_n (SEQ ID NO: 53), or (GGGGS)_n (SEQ ID NO: 54), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
4. The trispecific antigen-binding protein of claim 1, wherein linkers L1 and L2 are each independently (GGGGS)₄ (SEQ ID NO: 55) or (GGGGS)₃ (SEQ ID NO: 56).
5. The trispecific antigen-binding protein of claim 1, wherein the first domain is specific for CD3 ϵ (epsilon).
6. The trispecific antigen-binding protein of claim 1, wherein the first domain has crossreactivity with cynomolgus CD3.
7. A polynucleotide encoding the polypeptide of the trispecific antigen-binding protein of claim 1.
8. A vector comprising the polynucleotide of claim 7.
9. A pharmaceutical composition comprising (i) the trispecific antigen-binding protein according to claim 1 and (ii) a pharmaceutically acceptable carrier.
10. A pharmaceutical composition comprising (i) the vector according to claim 8 and (ii) a pharmaceutically acceptable carrier.
11. A trispecific antigen-binding protein, wherein said protein comprises

- (a) a first domain (A) which comprises a single chain variable fragment (scFv) that specifically binds to human CD3;
- (b) a second domain (B) which comprises a single domain antibody (sdAb) that binds human serum albumin, wherein the second domain comprises an amino acid sequence with a 90% identity to SEQ ID NO: 47;
- (c) a third domain (C) which comprises a scFv or sdAb that specifically binds to a target tumor antigen;
- wherein the domains are linked by linkers L1 and L2 in the order HN-(C)-(B)-(A)-COOH.
12. The trispecific antigen-binding protein of claim 1, wherein the second domain comprises an amino acid sequence with a 95% identity to SEQ ID NO: 47.
13. The trispecific antigen-binding protein of claim 11, wherein the first domain is humanized or human.
14. The trispecific antigen-binding protein of claim 11, wherein linkers L1 and L2 are each independently selected from (GS)_n (SEQ ID NO: 49), (GGS)_n (SEQ ID NO: 50), (GGGS)_n (SEQ ID NO: 51), (GGSG)_n (SEQ ID NO: 52), (GGSGG)_n (SEQ ID NO: 53), or (GGGGS)_n (SEQ ID NO: 54), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
15. The trispecific antigen-binding protein of claim 11, wherein linkers L1 and L2 are each independently (GGGGS)₄ (SEQ ID NO: 55) or (GGGGS)₃ (SEQ ID NO: 56).
16. The trispecific antigen-binding protein of claim 11, wherein the first domain is specific for CD3ε (epsilon).
17. The trispecific antigen-binding protein of claim 11, wherein the first domain has crossreactivity with cynomolgus CD3.
18. A polynucleotide encoding the polypeptide of the trispecific antigen-binding protein of claim 11.
19. A vector comprising the polynucleotide of claim 18.
20. A pharmaceutical composition comprising (i) the trispecific antigen-binding protein according to claim 11 and (ii) a pharmaceutically acceptable carrier.
21. A pharmaceutical composition comprising (i) the vector according to claim 19 and (ii) a pharmaceutically acceptable carrier.