

[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 in the order HN-(C)-(B)-(A)-COOH or by linkers L1 and L2.

[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

protein is about 20 to about 40 kDa. In some embodiments, the protein has an elimination half-time of at least about 50 hours. In some embodiments, the protein has an elimination half-time of at least about 100 hours. In some embodiments, the protein has increased tissue penetration as compared to an IgG to the same target antigen.

[0014] Also provided herein, in another aspect are polynucleotides encoding trispecific antigen-binding proteins according to any one of the above embodiments. In another aspect provided herein are vectors comprising the described polynucleotides. In another aspect, provided herein are host cells transformed with the described vectors

[0015] In yet another aspect, provided herein are pharmaceutical compositions comprising a trispecific antigen-binding protein of any of the above embodiments, a polynucleotide encoding a trispecific antigen-binding protein of any of the above embodiments, a vector comprising the described polynucleotides, or a host cell transformed with a vector of any of the above embodiments and a pharmaceutically acceptable carrier.

[0015a] Also provided herein, are pharmaceutical compositions comprising (i) the trispecific antigen-binding protein as described herein and (ii) a pharmaceutically acceptable carrier.

[0015b] Also provided herein, are pharmaceutical compositions comprising (i) the vector as described herein and (ii) a pharmaceutically acceptable carrier.

[0016] Also provided herein, are processes for the production of trispecific antigen-binding proteins according to any of the aspects and embodiments herein, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding any trispecific antigen-binding protein herein under conditions allowing the expression of the protein and recovering and purifying the produced protein from the culture.

[0017] Also provided herein are methods for the treatment amelioration of a proliferative disease, a tumorous disease, an inflammatory disease, an immunological disorder, an autoimmune disease, an infectious disease, viral disease, allergic reactions, parasitic reactions, graft-versus-host diseases or host-versus-graft diseases comprising the administration of a trispecific antigen-binding protein of any of the above embodiments to a subject in need of such a treatment or amelioration. In some embodiments, the subject is a human. In some embodiments, the method further comprises administration of an agent in combination with the trispecific antigen-binding protein described herein.

INCORPORATION BY REFERENCE

[0018] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative

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 in the order HN-(C)-(B)-(A)-COOH or by linkers L1 and L2.
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.