

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.

[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.

[0017a] Also provided herein, in another aspect is 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; and (c) a third domain (C) which comprises a sdAb that specifically binds to a target tumor antigen that is EGFR, PSMA, HER2 or MSLN, wherein the domains are linked in the order H₂N-(C)-(B)-(A)-COOH, or by linkers L1 and L2; and wherein the protein is less than about 60 kDa.

[0017b] Also provided herein, in another aspect is a pharmaceutical composition comprising (i) the trispecific antigen-binding protein as described and (ii) a pharmaceutically acceptable carrier.

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; and
 - (c) a third domain (C) which comprises a sdAb that specifically binds to a target tumor antigen that is EGFR, PSMA, HER2 or MSLN,wherein the domains are linked in the order H₂N-(C)-(B)-(A)-COOH, or by linkers L1 and L2; and wherein the protein is less than about 60 kDa.
2. The trispecific antigen-binding protein of claim 1, wherein 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.
3. The trispecific antigen-binding protein of claim 1, wherein the first domain is humanized or human.
4. 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.
5. 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).
6. A pharmaceutical composition comprising (i) the trispecific antigen-binding protein according to claim 1 and (ii) a pharmaceutically acceptable carrier.
7. The trispecific antigen-binding protein of claim 1, wherein the first domain is specific for CD3ε (epsilon).

8. The trispecific antigen-binding protein of claim 1, wherein the first domain has crossreactivity with cynomolgus CD3.
9. The trispecific antigen-binding protein of claim 1, wherein the third domain specifically binds to EGFR.
10. The trispecific antigen-binding protein of claim 1, wherein the third domain specifically binds to PSMA.
11. The trispecific antigen-binding protein of claim 1, wherein the third domain specifically binds to HER2.
12. The trispecific antigen-binding protein of claim 1, wherein the third domain specifically binds to MSLN.