

monoclonal antibodies having two heavy chains and two light chains, sdAbs can bind antigens with similar affinity and specificity as mAbs. Used as building blocks, the sdAbs can be fused to IgG Fc domains to create IgG-like antibodies, including bivalent and bispecific antibodies (see, for example, Hmila I. *et al. Mol. Immunol.* 2008; 45: 3847-3856).

[0007] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[0008] The present application provides a multispecific antigen binding protein (MABP) comprising one or more single-domain antibodies (sdAbs) fused to a full-length four-chain antibody or an antigen binding fragment derived therefrom.

[0009] Accordingly, disclosed is a MABP comprising: (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds a first epitope, and (b) a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds a second epitope, wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some examples, the first epitope and the second epitope are from the same antigen. In some examples, the first epitope and the second epitope are from different antigens. In some examples, the MABP is bispecific.

[0009a] In one aspect, the present disclosure refers to a multispecific antigen binding protein comprising: (a) a first antigen binding portion comprising a single-chain variable fragment (scFv) comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the scFv specifically binds a first epitope, (b) a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds a second epitope, and (c) an Fc region, wherein the first antigen binding portion and the second antigen binding portion are fused to each other, and wherein the N-terminus of the sdAb is fused to the C-terminus of the scFv and the C-terminus of the sdAb is fused to the N-terminus of the Fc region.

[0010] In some examples according to any one of the MABPs described above, the first antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains. In some examples, the first antigen binding portion is an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L . In some examples, the second antigen binding portion comprises a single polypeptide chain. In some examples, the C terminus of the second antigen binding portion is fused to the N-terminus of at least one heavy chain of the first antigen binding portion. In some examples, the C terminus of the second antigen binding portion is fused to the N-terminus of at least one light chain of the first antigen binding portion. In some examples, the N terminus of the second antigen binding portion is fused to the C-terminus of at least one heavy chain of the first antigen binding portion. In some examples, the N terminus of the second antigen binding portion is fused to the C-terminus of at least one light chain of the first antigen binding portion. In some

examples, the second antigen binding portion is a Fab-like domain comprising a first polypeptide chain comprising a first sdAb fused to a C_H1 domain, and a second polypeptide chain comprising a second sdAb fused to a C_L domain.

[0011] In some examples according to any one of the MABPs described above, the first antigen binding portion comprises a human, humanized or chimeric antibody or antigen binding fragment thereof.

[0012] In some examples according to any one of the MABPs described above, the first antigen binding portion comprises an Fc region. In some examples, the second antigen binding portion is fused to the N-terminus of the Fc region. In some examples, the Fc region is an IgG1 Fc. In some examples, the Fc region is an IgG4 Fc, such as an IgG4 Fc having an S228P mutation.

[0013] In some examples according to any one of the MABPs described above, the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker. In some examples, the peptide linker is no more than about 30 (such as no more than about any one of 25, 20 or 15) amino acids long. In some examples, the peptide linker comprises the amino acid sequence of SEQ ID NO: 1, 8 or 13. In some examples, the first antigen binding portion and the second antigen binding portion are fused to each other chemically.

[0014] In some examples according to any one of the MABPs described above, the sdAb is a camelid, humanized, or human sdAb.

[0015] In some examples according to any one of the MABPs described above, the first epitope is from an immune checkpoint molecule. In some examples, the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40. In some examples, the first antigen binding portion is an anti-PD-1 antibody or antigen binding fragment thereof. In some examples, the anti-PD-1 antibody is selected from the group consisting of pembrolizumab (*e.g.*, KEYTRUDA[®]) and nivolumab (*e.g.*, OPVIDO[®]). In some examples, the first antigen binding portion is an anti-PD-L1 antibody or antigen binding fragment thereof. In some examples, the anti-PD-L1 antibody is duravalumab or atezolizumab. In some examples, the sdAb specifically binds an immune checkpoint molecule, such as an immune checkpoint molecule selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3,

LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40. In some examples, the second antigen binding portion comprises an anti-CTLA-4 sdAb.

[0016] In some examples according to any one of the MABPs described above, the first epitope is from a tumor antigen. In some examples, the tumor antigen is selected from the group consisting of HER2, BRAF, EGFR, VEGFR2, CD20, RANKL, CD38, and CD52. In some examples, the first antigen binding portion is an anti-HER2 antibody or antigen binding fragment thereof. In some examples, the anti-HER2 antibody is trastuzumab. In some examples, the second antigen binding portion comprises an anti-CD3 sdAb.

[0017] In some examples according to any one of the MABPs described above, the first epitope is from an angiogenic factor. In some examples, the first antigen binding portion is an anti-Ang2 antibody or antigen binding fragment thereof, such as LC10. In some examples, the second epitope is from a second angiogenic factor. In some examples, the second antigen binding portion is an anti-VEGF sdAb.

[0018] In some examples according to any one of the MABPs described above, the first epitope is from a pro-inflammatory molecule. In some examples, the pro-inflammatory molecule is selected from the group consisting of IL-1 α , TNF- α , IL-5, IL-6, IL-6R, and eotaxin-1. In some examples, the first antigen binding portion is an anti-TNF- α antibody or antigen binding fragment thereof. In some examples, the anti-TNF- α antibody is adalimumab. In some examples, the second antigen binding portion comprises an anti-IL-1 α sdAb. In some examples, the first antigen binding portion is an anti-IL-5 antibody or antigen binding fragment thereof. In some examples, the anti-IL-5 antibody is mepolizumab. In some examples, the second antigen binding portion comprises an anti-eotaxin-1 sdAb.

[0019] In some examples according to any one of the MABPs described above, the MABP can be produced recombinantly, such as in mammalian cells (*e.g.*, CHO cells), at an expression level of at least about 10 mg/L, such as at least about 10 mg/L, 15 mg/L, 50 mg/mL, or higher. In some examples, the MABP has a solubility of at least about 100 mg/mL, such as at least about 150 mg/mL, 200 mg/mL or higher. In some examples, the MABP has an aggregation onset temperature (T_{agg}) of at least about 65°C, such as about 65 °C to about 75 °C. In some examples, the MABP has an unfolding midpoint temperature (T_m) of at least about 65°C, such as about 65 °C to about 75 °C. In some examples, the MABP is stable for at least about one week at 25 °C at a concentration of at least about 50 mg/mL. In some examples, the

MABP is stable for at least about one week at 37 °C at a concentration of at least about 50 mg/mL. In some examples, the MABP is stable after at least about 5 freeze-thaw cycles at a concentration of at least 50 mg/mL.

[0020] Disclosed is a pharmaceutical composition comprising any one of the MABPs described above and a pharmaceutically acceptable carrier. In some examples, the concentration of the MABP is at least about 100 mg/mL, such as at least about 150 mg/mL, 200 mg/mL or higher.

[0020a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the multispecific antigen binding protein as described herein, and a pharmaceutically acceptable carrier.

[0021] Disclosed is a method of treating a disease in an individual, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some examples, the disease is a cancer. In some examples, the cancer is selected from the group consisting of breast cancer, renal cancer, melanoma, lung cancer, glioblastoma, head and neck cancer, prostate cancer, ovarian carcinoma, bladder carcinoma, and lymphoma. In some examples, the disease is an inflammatory or autoimmune disease. In some examples, the inflammatory or autoimmune disease is selected from the group consisting of arthritis (such as rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, and arthritic ulcerative colitis), colitis, psoriasis, severe asthma, and moderate to severe Crohn's disease.

[0021a] In another aspect, the present disclosure refers to use of an effective amount of the multispecific antigen binding protein as described herein in the manufacture of a medicament for treating a disease in an individual, wherein the disease is a cancer, an inflammatory disease, or an autoimmune disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 depicts a schematic structure of an exemplary bispecific antigen binding protein (also referred herein as "BABP") comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and an sdAb, wherein the N-terminus of the sdAb is fused to the C-terminus of one heavy chain via an optional peptide linker. The full-length antibody has two antigen binding sites that specifically bind the first epitope. The sdAb specifically binds the second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L ; (2) V_H - C_H1 - C_H2 - C_H3 - V_HH ; (3) V_H - C_H1 - C_H2 - C_H3 ; and (4) V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the first epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the first epitope, and V_HH specifically binds the second epitope.

[0023] FIG. 2 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two

CLAIMS

What is claimed is:

1. A multispecific antigen binding protein comprising:
 - (a) a first antigen binding portion comprising a single-chain variable fragment (scFv) comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the scFv specifically binds a first epitope,
 - (b) a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds a second epitope, and
 - (c) an Fc region,wherein the first antigen binding portion and the second antigen binding portion are fused to each other, and wherein the N-terminus of the sdAb is fused to the C-terminus of the scFv and the C-terminus of the sdAb is fused to the N-terminus of the Fc region.
2. The multispecific antigen binding protein of claim 1, wherein the first epitope and the second epitope are from the same antigen.
3. The multispecific antigen binding protein of claim 1, wherein the first epitope and the second epitope are from different antigens.
4. The multispecific antigen binding protein of any one of claims 1-3, wherein the Fc region is an IgG1 Fc, or an IgG4 Fc comprising an S228P mutation.
5. The multispecific antigen binding protein of any one of claims 1-4, wherein the sdAb is a camelid, humanized, or human sdAb.
6. The multispecific antigen binding protein of any one of claims 1-5, wherein the scFv is a human, humanized, or chimeric scFv.
7. The multispecific antigen binding protein of any one of claims 1-6, wherein the multispecific antigen binding protein is bispecific.
8. The multispecific antigen binding protein of any one of claims 1-7, wherein the multispecific antigen binding protein comprises two identical polypeptides each comprising from the N-terminus to the C-terminus: scFv-an optional peptide linker-sdAb-CH2-CH3.
9. The multispecific antigen binding protein of any one of claims 1-8, wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker.

10. The multispecific antigen binding protein of claim 8 or 9, wherein the peptide linker is no more than 30 amino acids long.
11. The multispecific antigen binding protein of any one of claims 8-10, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 1, 8, or 13.
12. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from an immune checkpoint molecule.
13. The multispecific antigen binding protein of claim 12, wherein the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40.
14. The multispecific antigen binding protein of claim 12 or 13, wherein the scFv is an anti-PD-1 scFv.
15. The multispecific antigen binding protein of claim 14, wherein the anti-PD-1 scFv is derived from pembrolizumab or nivolumab.
16. The multispecific antigen binding protein of claim 12 or 13, wherein the scFv is an anti-PD-L1 scFv.
17. The multispecific antigen binding protein of claim 16, wherein the anti-PD-L1 scFv is derived from durvalumab or atezolizumab.
18. The multispecific antigen binding protein of any one of claims 1-17, wherein the sdAb specifically binds an immune checkpoint molecule.
19. The multispecific antigen binding protein of claim 18, wherein the immune checkpoint molecule is selected from the group consisting of PD-1, PD-L1, PD-L2, CTLA-4, B7-H3, TIM-3, LAG-3, VISTA, ICOS, 4-1BB, OX40, GITR, and CD40.
20. The multispecific antigen binding protein of claim 19, wherein the sdAb is an anti-CTLA-4 sdAb.
21. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a tumor antigen, such as any of HER2, BRAF, EGFR, VEGFR2, CD20, RANKL, CD38, or CD52.
22. The multispecific antigen binding protein of any one of claims 1-11, wherein the scFv is an anti-Ang2 scFv.
23. The multispecific antigen binding protein of any one of claims 1-11, 21, and 22, wherein the sdAb is an anti-VEGF sdAb.

24. The multispecific antigen binding protein of any one of claims 1-11, wherein the first epitope is from a pro-inflammatory molecule, such as any of IL-1 β , TNF- α , IL-5, IL-6, IL-6R, or eotaxin-1.
25. The multispecific antigen binding protein of any one of claims 1-24, wherein the multispecific antigen binding protein can be produced recombinantly in mammalian cells at an expression level of at least 10 mg/L.
26. The multispecific antigen binding protein of any one of claims 1-25, wherein the multispecific antigen binding protein has an aggregation onset temperature of at least 65°C measured by Capillary Differential Scanning Calorimetry (DSC) or Dynamic Light Scattering (DLS).
27. A pharmaceutical composition comprising the multispecific antigen binding protein of any one of claims 1-26, and a pharmaceutically acceptable carrier.
28. Use of an effective amount of the multispecific antigen binding protein of any one of claims 1-26 in the manufacture of a medicament for treating a disease in an individual, wherein the disease is a cancer, an inflammatory disease, or an autoimmune disease.
29. The use of claim 28, wherein:
- (i) the cancer is selected from the group consisting of breast cancer, renal cancer, melanoma, lung cancer, glioblastoma, head and neck cancer, prostate cancer, ovarian carcinoma, bladder carcinoma, and lymphoma; or
 - (ii) the inflammatory or autoimmune disease is selected from the group consisting of arthritis, colitis, psoriasis, severe asthma, and moderate to severe Crohn's disease.