

Claims

1. A multispecific antigen-binding protein comprising antigen-binding sites for EGFR and CD16A, wherein the antigen-binding sites are fused to a polypeptide comprising heavy chain constant domains CH2 and CH3 and wherein the antigen-binding site for EGFR comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) wherein:

(i) VH comprises a heavy chain CDR1 having the amino acid sequence set forth in SEQ ID NO:21; a heavy chain CDR2 having the amino acid sequence set forth in SEQ ID NO:22; a heavy chain CDR3 having the amino acid sequence set forth in SEQ ID NO:23 and VL comprises a light chain CDR1 having an amino acid sequence set forth in SEQ ID NO:24; a light chain CDR2 having an amino acid sequence set forth in SEQ ID NO:25; and a light chain CDR3 having an amino acid sequence set forth in SEQ ID NOs:26; or

(ii) VH has the amino acid sequence set forth in SEQ ID NOs:1 and VL has the amino acid sequence set forth in SEQ ID NO:2.

2. The multispecific antigen binding protein of claim 1, wherein the antigen binding site for CD16A comprises

(i) a variable heavy chain domain (VH) comprising a heavy chain CDR1 having the amino acid sequence set forth in SEQ ID NO:5; a heavy chain CDR2 having the amino acid sequence set forth in SEQ ID NO:6 or 11; a heavy chain CDR3 having the amino acid sequence set forth in SEQ ID NO:7, and a variable light chain domain (VL) comprising a light chain CDR1 having an amino acid sequence set forth in SEQ ID NO:8; a light chain CDR2 having an amino acid sequence set forth in SEQ ID NO:9; and a light chain CDR3 having an amino acid sequence set forth in SEQ ID NO:10; or

(ii) a VH comprising the amino acid sequence set forth in SEQ ID NO:12 or 14 and a VL comprising the amino acid sequence set forth in SEQ ID NO:13.

3. The multispecific antigen-binding protein of any one of the claims 1 or 2, wherein the antigen-binding protein comprises at least two antigen-binding sites for EGFR and at least two antigen-binding sites for CD16A.

4. The multispecific antigen-binding protein of claim 3, wherein the antigen-binding protein consists of two polypeptide chains and each polypeptide chain comprises a first single-chain Fv unit (scFv1) comprising a VL linked by a peptide linker to a VH of a first antigen-binding site, said first single-chain Fv unit (scFv1) being fused by a hinge region N-terminally to a CH2 domain of the CH2-CH3 polypeptide and a second single-chain Fv unit (scFv2) comprising a VL linked by a peptide linker to a VH of a second antigen-binding site, said second single-chain Fv unit (scFv2) being fused by a peptide linker C-terminally to the CH3 domain of the CH2-CH3 polypeptide.

5. The multispecific antigen-binding protein of claim 4, wherein the scFv1 is an antigen-binding site for CD16A and the scFv2 is an antigen-binding site for EGFR.

6. The multispecific antigen-binding protein of claim 3, wherein the antibody comprises a F(ab')₂ fragment and a Fc-portion, wherein:

(i) the F(ab')₂ fragment comprises two Fv antigen-binding sites for CD16A and two single-chain Fv (scFv) comprising antigen-binding sites for EGFR are fused to the Fc-portion, wherein each of the scFv is fused C-terminally to the CH3 domain of the Fc-portion; or

(ii) the F(ab')₂ fragment comprises two Fv antigen-binding sites for EGFR and two single-chain Fv (scFv) comprising antigen-binding sites for CD16A are fused to the Fc-portion, wherein each of the scFv is fused C-terminally to the CH3 domain of the Fc-portion.

7. The multispecific antigen-binding protein of claim 6 comprising a heavy chain and a light chain, wherein

(i) the heavy chain has the structure VH(CD16A)-CH1-CH2-CH3-VH(EGFR)-VL(EGFR) and the light chain has the structure VL(CD16A)-CL

or

(ii) the heavy chain has the structure VH(EGFR)-CH1-CH2-CH3-VL(CD16A)-VH(CD16A) and the light chain has the structure VL(EGFR)-CL.

8. The multispecific antigen-binding protein of anyone of claims 1 to 7, wherein the antigen-binding protein does not bind to Fc-gamma receptor, but binds to neonatal Fc-receptor.

9. The multispecific antigen-binding protein of anyone of claims 1 to 8, wherein the protein comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO:28 and a light chain having the amino acid sequence set forth in SEQ ID NO:29; a heavy chain having the amino acid sequence set forth in SEQ ID NO:43 and a light chain having the amino acid sequence set forth in SEQ ID NO:44; or a heavy chain having the amino acid sequence set forth in SEQ ID NO:45 and a light chain having the amino acid sequence set forth in SEQ ID NO:44.

10. The multispecific antigen-binding protein of any one of the claims 1 or 9 further comprising

- (i) an antigen-binding site for serum albumin, or
- (ii) serum albumin fused to the antigen-binding protein.

11. The multispecific antigen-binding protein of any one of the claims 1 to 10 for use as a therapeutic compound.

12. The multispecific antigen-binding protein of claim 11, for use in the treatment of a cancer characterized by EGFR-positive or EGFRvIII-positive cells.

13. The multispecific antigen-binding protein for the use according to claim 12, wherein the cancer is selected from the group consisting of colorectal cancer, head and neck cancer, lung cancer and glioblastoma.

14. Use of the multispecific antigen-binding protein of any one of the claims 1 to 10 in the manufacture of a medicament~~A method~~ for the treatment or amelioration of a proliferative disease or a tumorous disease,~~the method comprising the step of administering to a subject in need thereof the multispecific antigen binding protein of any one of the claims 1 to 10.~~

15. The ~~use~~method of claim 14, wherein the proliferative disease or tumorous disease is characterized by EGFR-positive or EGFRvIII-positive cells.

16. The ~~method~~use of claim 15, wherein the the proliferative disease or tumorous disease is selected from the group consisting of colorectal cancer, head and neck cancer, lung cancer and glioblastoma.