

CLAIMS

1. A multispecific polypeptide comprising a first single chain variable fragment (scFv) domain and a second scFv domain linked together by a binding domain linker and an immunoglobulin Fc domain,

wherein the immunoglobulin Fc domain comprises a hinge region and an immunoglobulin constant region;

wherein the multispecific polypeptide is capable of forming a homodimer by association with a second identical multispecific polypeptide;

wherein the first scFv domain specifically binds to human 5T4 and comprises:

(i) an immunoglobulin heavy chain variable region comprising a heavy chain complementarity determining region (HCDR)-1 amino acid sequence of SEQ ID NO: 30, an HCDR2 amino acid sequence of SEQ ID NO: 32, and an HCDR3 amino acid sequence of SEQ ID NO: 34; and

(ii) an immunoglobulin light chain variable region comprising a light chain complementarity determining region (LCDR)-1 amino acid sequence of SEQ ID NO: 42, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NOs: 36; and

wherein the second scFv domain specifically binds to human 4-1BB and comprises:

(i) an immunoglobulin heavy chain variable region comprising an HCDR1 amino acid sequence of SEQ ID NO: 2, an HCDR2 amino acid sequence of SEQ ID NO: 4, and an HCDR3 amino acid sequence of the SEQ ID NO: 6; and

(ii) an immunoglobulin light chain variable region comprising an LCDR1 amino acid sequence of SEQ ID NO: 8, an LCDR2 amino acid sequence of SEQ ID NO: 10, and an LCDR3 amino acid sequence of SEQ ID NO: 12.

2. The multispecific polypeptide of claim 1, wherein the multispecific polypeptide comprises,

from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii)

the immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain.

3. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises a mutation in the framework region compared to the framework region of SEQ ID NOs: 130 or 170.

5 4. The multispecific polypeptide of claim 3, wherein the mutation introduces a stabilizing disulfide bond.

5. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44.

10 6. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48.

7. The multispecific polypeptide of claim 1, wherein the second scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light
15 chain variable region of SEQ ID NO: 16.

8. The multispecific polypeptide of claim 1, wherein the first scFv domain comprises the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin light chain variable region of SEQ ID NO: 48, and
wherein the second scFv domain comprises the immunoglobulin heavy chain variable region of
20 SEQ ID NO: 14 and the immunoglobulin light chain variable region of SEQ ID NO: 16.

9. The multispecific polypeptide of claim 1, wherein the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 120, and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110.

10. The multispecific polypeptide of claim 9, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 172 or comprises an amino acid sequence identical to SEQ ID NO: 172.

11. The multispecific polypeptide of claim 1, wherein the amino acid sequence of the first scFv domain is at least 97% identical to SEQ ID NO: 122 and the amino acid sequence of the second scFv domain is at least 97% identical to SEQ ID NO: 110.

12. The multispecific polypeptide of claim 11, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 174 or comprises an amino acid sequence of SEQ ID NO: 174.

13. The multispecific polypeptide of claim 1, wherein binding of the multispecific polypeptide to an effector cell results in increased effector cell activation, increased effector cell proliferation, or wherein binding of the multispecific polypeptide to an effector cell and a 5T4-expressing cell results in enhanced effector cell-dependent lysis of the 5T4-expressing cell.

14. Use of a therapeutically effective amount of the multispecific polypeptide of claim 1, in the manufacture of a medicament for treating cancer, said cancer is characterised by expression of 5T4.

15. The use as claimed in claim 14, wherein the cancer is breast cancer, pancreatic cancer, ovarian cancer, non-small cell lung cancer, mesothelioma, chronic lymphocytic leukemia (CLL), mantle cell leukemia (MCL), acute lymphoblastic leukemia (ALL), squamous cell carcinoma, melanoma, adrenal cancer, bladder cancer, cervical cancer, renal cancer, gastric cancer, prostate cancer, thyroid cancer, liver cancer, uterine cancer, neurofibroma, sarcoma, carcinoma, or head and neck cancer.

16. The use as claimed in claim 14, wherein the multispecific polypeptide comprises, from amino-terminus to carboxyl-terminus: (i) the first scFv domain, (ii) the hinge region, (iii) the

immunoglobulin constant region, (iv) the binding domain linker, and (v) the second scFv domain.

17. The use as claimed in claim 14, wherein the second scFv domain comprises immunoglobulin heavy chain variable region of SEQ ID NO: 14 and the immunoglobulin light chain variable

5 region of SEQ ID NO: 16, and

wherein the first scFv domain comprises

(i) the immunoglobulin heavy chain variable region of SEQ ID NO: 38 and the immunoglobulin light chain variable region of SEQ ID NO: 44; or

(ii) the immunoglobulin heavy chain variable region of SEQ ID NO: 46 and the immunoglobulin

10 light chain variable region of SEQ ID NO: 48.

18. The use as claimed in claim 14, wherein the amino acid sequence of the second scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 110, and

wherein the amino acid sequence of the first scFv domain of the multispecific polypeptide is at least 97% identical to SEQ ID NO: 120 or SEQ ID NO: 122.

15 19. The multispecific polypeptide of claim 1, wherein said polypeptide exhibits statistically significant enhanced effector cell activation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB antibody comprising a heavy chain variable region comprising SEQ ID NO: 28 and a light chain variable region comprising SEQ ID NO: 16 and an anti-5T4 scFv comprising a heavy chain

20 variable region comprising SEQ ID NO: 46 and a light chain variable region comprising SEQ ID NO: 66.

20. The multispecific polypeptide of claim 1, wherein said polypeptide induces statistically significant increased effector cell proliferation compared to a second multispecific polypeptide, wherein the second multispecific polypeptide is an IgG-scFv structure comprising an anti-4-1BB

25 antibody comprising a heavy chain variable region comprising SEQ ID NO: 28 and a light chain

variable region comprising SEQ ID NO:16 and an anti-5T4 scFv comprising a heavy chain variable region comprising SEQ ID NO:46 and a light chain variable region comprising SEQ ID NO:66.