

CLAIMS

1. A protein comprising, from N- to C-terminal:

- a) a first single domain antigen binding domain (sdABD) that binds to a human tumor target antigen (TTA) (sdABD-TTA);
- b) a first domain linker;
- c) a constrained Fv domain comprising:
 - i) a first variable heavy domain comprising a vhCDR1, vhCDR2 and vhCDR3;
 - ii) a constrained non-cleavable linker (CNCL); and
 - iii) a first variable light domain comprising vlCDR1, vlCDR2 and vlCDR3;
- d) a second domain linker;
- e) a second sdABD-TTA;
- f) a cleavable linker (CL);
- g) a constrained pseudo Fv domain comprising:
 - i) a first pseudo light variable domain;
 - ii) a non-cleavable linker (NCL); and
 - iii) a first pseudo heavy variable domain;
- h) a third domain linker; and
- i) a third sdABD that binds to human serum albumin;

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3; wherein said first variable heavy domain and said first pseudo variable light domain intramolecularly associate to form an inactive Fv; and wherein said first variable light domain and said first pseudo variable heavy domain intramolecularly associate to form an inactive Fv.

2. A protein comprising, from N- to C-terminal:

- a) a first sdABD-TTA;
- b) a first domain linker;
- c) a constrained Fv domain comprising:
 - i) a first variable heavy domain comprising a vhCDR1, vhCDR2 and vhCDR3;
 - ii) a constrained cleavable linker (CCL); and
 - iii) a first variable light domain comprising vlCDR1, vlCDR2 and vlCDR3;
- d) a second domain linker;

- e) a second sdABD-TTA;
- f) a cleavable linker (CL);
- g) a constrained pseudo Fv domain comprising:
 - i) a first pseudo heavy variable domain;
 - ii) a non-cleavable linker (NCL); and
 - iii) a first pseudo light variable domain;
- h) a third domain linker; and
- i) a third sdABD that binds to human serum albumin;

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3; wherein said first variable heavy domain and said first pseudo variable light domain intramolecularly associate to form an inactive Fv; and wherein said first variable light domain and said first pseudo variable heavy domain intramolecularly associate to form an inactive Fv.

3. A protein according to any of claims 1 to 2 wherein said first variable heavy domain is N-terminal to said first variable light domain and said pseudo light variable domain is N-terminal to said pseudo variable heavy domain.
4. A protein according to any of claims 1 to 2 wherein said first variable heavy domain is N-terminal to said first variable light domain and said pseudo variable heavy domain is N-terminal to said pseudo variable light domain.
5. A protein according to any of claims 1 to 2 wherein said first variable light domain is N-terminal to said first variable heavy domain and said pseudo light variable domain is N-terminal to said pseudo variable heavy domain.
6. A protein according to any of claims 1 to 2 wherein said first variable light domain is N-terminal to said first variable heavy domain and said pseudo variable heavy domain is N-terminal to said pseudo variable light domain.
7. A protein according to any of claims 1-6 wherein said first and second TTA is the same.
8. A protein according to any of claims 1-6 wherein said first and second TTA are different.
9. A protein according to any of claims 1-8 wherein said first and second TTA are selected from EGFR, EpCAM, FOLR1 and B7H3.
10. A protein according to any of claims 1-9 wherein said first and second sdABD-TTAs are selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID

NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:29; SEQ ID NO:33; SEQ ID NO:37 and SEQ ID NO:41.

11. A protein according to claim 1 to 10 wherein said half-life extension domain has SEQ ID NO:45.

12. A protein according to claim 1 to 11 wherein said cleavable linker is cleaved by a human protease selected from the group consisting of MMP2, MMP9, Meprin A, Meprin B, Cathepsin S, Cathepsin K, Cathepsin L, GranzymeB, uPA, Kallikrein7, matriptase and thrombin.

13. A protein according to claim 1 comprising a protein selected from the group consisting of Pro186, Pro225, Pro226, Pro233, Pro311, Pro312, Pro313, Pro495, Pro246, Pro254, Pro255, Pro256, Pro420, Pro421, Pro432, Pro479, Pro480, Pro187, Pro221, Pro222, Pro223, Pro224, Pro393, Pro394, Pro395, Pro396, Pro429, Pro430 and Pro431.

14. A nucleic acid encoding a protein according to any of claims 1 to 13.

15. An expression vector comprising the nucleic acid of claim 14.

16. A host cell comprising the expression vector of claim 15.

17. A method of making a protein comprising culturing the host cell of claim 16 under conditions wherein said protein is expressed and recovering said protein.

18. Use of a protein of any of claims 1-13 in the manufacture of a medicament for the treatment of cancer.

19. A composition comprising:

a) a first protein comprising, from N- to C-terminal:

i) a first sdABD-TTA;

ii) a first domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable heavy chain comprising a vhCDR1, vhCDR2 and vhCDR3;

2) a cleavable linker; and

3) a first pseudo variable light domain comprising iVLCDR1, iVLCDR2 and iVLCDR3;

iv) a second domain linker;

v) a first sdABD that binds to human serum albumin;

a) a second protein comprising, from N- to C-terminal:

i) a third sdABD that binds to a human tumor target antigen;

ii) a third domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable light chain comprising a VLCDR1, VLCDR2 and

VLCDR3;

2) a cleavable linker; and

3) a first pseudo variable heavy domain comprising iVHCDR1,

iVHCDR2 and iVHCDR3;

iv) a fourth domain linker;

v) a fourth sdABD that binds to human serum albumin;

wherein

said first variable heavy domain and said first variable light domain are capable of binding human CD3 when associated;

said first variable heavy domain and said first pseudo variable light domain intermolecularly associate to form an inactive Fv;

said first variable light domain and said first pseudo variable heavy domain intermolecularly associate to form an inactive Fv;

wherein said first and third sdABD are selected from the group consisting of SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:25, SEQ ID NO:29; SEQ ID NO:33; SEQ ID NO:37 and SEQ ID NO:41.

20. A composition comprising:

a) a first protein comprising, from N- to C-terminal:

i) a first sdABD-TTA;

ii) a first domain linker;

iii) a second sdABD-TTA;

iv) a second domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable heavy chain comprising a vhCDR1, vhCDR2 and

vhCDR3;

2) a cleavable linker; and

3) a first pseudo variable light domain comprising iVLCDR1, iVLCDR2 and iVLCDR3;

iv) a third domain linker; and

v) a sdABD-HSA;

a) a second protein comprising, from N- to C-terminal:

i) a third sdABD-TTA;

ii) a fourth domain linker;

iii) a fourth sdABD-TTA;

iv) a fifth domain linker;

iii) a pseudo Fv domain comprising, from N- to C-terminal:

1) a variable light chain comprising a VLCDR1, VLCDR2 and VLCDR3;

2) a cleavable linker; and

3) a first pseudo variable heavy domain comprising iVHCDR1, iVHCDR2 and iVHCDR3;

iv) a sixth domain linker;

v) a sdABD-HSA;

wherein

said first variable heavy domain and said first variable light domain are capable of binding human CD3 when associated;

said first variable heavy domain and said first pseudo variable light domain intermolecularly associate to form an inactive Fv;

said first variable light domain and said first pseudo variable heavy domain intermolecularly associate to form an inactive Fv.

21. A protein comprising, from N- to C-terminal:

a) a single domain antigen binding domain (sdABD) that binds to a human tumor target antigen (TTA) (sdABD-TTA);

b) a first domain linker;

c) a constrained Fv domain comprising:

i) a first variable heavy domain comprising a vhCDR1, vhCDR2 and vhCDR3;

ii) a constrained non-cleavable linker (CNCL); and

- iii) a first variable light domain comprising vlCDR1, vlCDR2 and vlCDR3;
- d) a cleavable linker (CL);
- e) a second sdABD that binds to human serum albumin;
- f) a domain linker;
- g) a constrained pseudo Fv domain comprising:
 - i) a first pseudo light variable domain;
 - ii) a non-cleavable linker (NCL); and
 - iii) a first pseudo heavy variable domain;

wherein said first variable heavy domain and said first variable light domain are capable of binding human CD3 but said constrained Fv domain does not bind CD3;

said first variable heavy domain and said first pseudo variable light domain intramolecularly associate to form an inactive Fv; and

said first variable light domain and said first pseudo variable heavy domain intramolecularly associate to form an inactive Fv.