

CLAIMS

1. A pair of polypeptide constructs comprising:

a) a first polypeptide comprising a first target antigen binding domain linked using a first domain linker to a first single chain scFv polypeptide directed to a CD-3 antigen, wherein said first polypeptide further comprises a half-life extension domain, and

b) a second polypeptide comprising a first target antigen binding domain linked using a second domain linker to a second single chain scFv polypeptide directed to a CD-3 antigen, wherein said second polypeptide further comprises a half-life extension domain;

wherein said first scFv domain comprises a first VH domain and a first VL domain joined through a first scFv linker moiety comprising a first protease cleavage site between said first VH and said first VL domain, said first VH domain and said first VL domain interacting to form a first VH/VL pair, wherein said first VH domain is inactive (iVH) and said first VL domain is active (aVL), such that said first scFv domain does not specifically bind said CD-3 antigen;

wherein said second scFv domain comprises a second VH domain and a second VL domain joined through a second scFv linker moiety comprising a second protease cleavage site between said second VH and said second VL domain, said second VH domain and said second VL domain interacting to form a second VH/VL pair, wherein said second VL domain is inactive (iVL) and said second VH is active (aVH), such that said second scFv domain does not specifically bind said CD-3 antigen.

2. A pair of polypeptide constructs according to claim 1:

a) wherein said first polypeptide comprises, from N- to C-terminal:

i) said first target antigen binding domain;

ii) said first domain linker;

iii) said first scFv domain comprising:

1) said first active VL domain;

2) a cleavable linker; and

3) said first inactive VH domain;

v) a third domain linker; and

vi) said half-life extension domain; and

b) wherein said second polypeptide comprises, from N- to C-terminal:

i) said second target antigen binding domain;

ii) said second domain linker;

iii) said second scFv domain comprising:

1) said first active VH domain;

2) a cleavable linker; and

3) said first inactive VL domain;

iv) a fourth domain linker; and

v) said half-life extension domain.

3. A pair of polypeptides according to claim 1 or 2, wherein said half-life extension domain binds to human HSA.

4. A pair of polypeptides according to any previous claim wherein said half-life extension domain has the sequence

EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESV
KGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSS (amino acids 397 to 511 of SEQ ID NO:76).

5. A pair of polypeptides according to any previous claim wherein said half-life extension domain has the sequence

EVQLVESGGGLVQPGNSLRRLSCAASGFTFSKFGMSWVRQAPGKGLEWVSSISGSGRDTLYAESV
KGRFTISRDNAAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLVTVSSHHHHHH (amino acids 397 to 517 of SEQ ID NO:76).

6. A pair of polypeptides according to any previous claim wherein said first and said second target antigen binding domains bind a target antigen selected from the group consisting of EpCAM, EGFR and FOLR1.

7. A pair of polypeptides according to any previous claim wherein said first and said second protease cleavage site is cleaved by a human protease selected from the group consisting of MMP2, MMP9, meprin, matriptase, thrombin, uPA and cathepsin.

8. A pair of polypeptides according to any previous claim wherein said first and said second polypeptide each comprise an additional target antigen binding domain.

9. A pair of polypeptides according to any previous claim wherein said first and second target antigen binding domains are the same.

10. A pair of polypeptides according to any of claims 1 to 8 wherein said first and second target antigen binding domains are different.

11. A pair of polypeptides according to any previous claim wherein said aVH domain comprises the sequence

EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY
ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSS
(amino acids 137-261 of SEQ ID NO:44) and said aVL domain comprises

QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIGGTKFLAPGTPARFS
GSLGGAALTLGSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL (amino acids 1-109 of SEQ
ID NO:46).

12. An expression vector comprising a first nucleic acid encoding said first polypeptide of any of claims 1 to 11 and a second nucleic acid encoding said second polypeptide of any of claims 1 to 11.

13. A host cell comprising the expression vector of claim 12.

14. Use of a pair of polypeptides according to any of claims 1 to 11 in the preparation of a medicament for the treatment of cancer.

15. A single chain scFv polypeptide directed to a CD-3 antigen, said scFv polypeptide comprising:

a) a first target antigen binding domain;

b) a domain linker;

c) a first scFv domain comprising a first VH domain and a first VL domain joined through a first scFv linker moiety comprising a first protease cleavage site between said first VH and said first VL domain, said first VH domain and said first VL domain interacting to form a first VH/VL pair, one of said first VH domain and said first VL domain is an inactive first VH domain or inactive first VL domain, such that said first scFv domain does not specifically bind said CD-3 antigen;

d) a domain linker optionally comprising a second protease cleavage site;

e) a second target antigen binding domain;

f) a domain linker;

d) a second scFv domain comprising a second VH domain and a second VL domain joined via a second scFv linker moiety comprising a second protease cleavage site between said second VH domain and said second VL domain, said second VH domain and said second VL domain interacting to form a second VH/VL pair, one of said second VH domain and said second VL is an inactive second VH or inactive second VL domain, such that said second scFv domain does not specifically bind said CD-3 antigen;

wherein said active VH and said active VL will form an active single chain scFv capable of binding said CD-3 antigen upon exposure of said single chain scFv polypeptide to a protease that will cleave said first and said second protease cleavage sites.