

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 Fv (scFv) domain directed to a CD-3 antigen, wherein said first polypeptide further comprises a half-life extension domain, and

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

wherein said first scFv domain comprises an first-inactive VH domain and an active ~~first~~ VL domain joined through a first scFv linker moiety comprising a first protease cleavage site between said ~~first inactive~~ VH and said ~~first-active~~ VL domain, said ~~first-inactive~~ VH domain and said ~~first-active~~ 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~~that does not specifically bind said CD-3 antigen;

wherein said second scFv domain comprises an active ~~second~~ VH domain and an inactive ~~second~~ VL domain joined through a second scFv linker moiety comprising a second protease cleavage site between said ~~second-active~~ VH and said ~~second-inactive~~ VL domain, said ~~second-active~~ VH domain and said ~~second-inactive~~ VL domain interacting to form a second VH/VL pair that, ~~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;

wherein upon protease cleavage at said first and second protease cleavage sites, the ~~first-active~~ VL domain of the first scFv domain and the ~~first-active~~ VH domain of the second scFv domain form an active VH/VL pair to specifically bind said CD-3 antigen; and

wherein said first and said second target antigen binding domain bind a tumor 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) said first scFv linker moiety comprising said first protease cleavage site is 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) said second scFv linker moiety comprising said second protease cleavage site is 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
 KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLTVTVSS (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
 KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSVSSQGTLTVTVSSHHHHHH (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 the tumor antigen selected from the group consisting of EpCAM and EGFR.

7. A pair of polypeptides according to any previous claim wherein said first and said second protease cleavage site are cleaved by a human protease selected from the group consisting of MMP2, MMP9, meprin, matrilysin, 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
 EVQLVESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY
 ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLTVTVSS
 (amino acids 137-261 of SEQ ID NO:44) and said aVL domain comprises
 QTVVTQEPSTLVSPGGTVTLTCSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS

GSLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL (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.