

sequence as set forth in wt anti-CD3 (SEQ ID NO. 22). In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein comprises a sequence wherein amino acid position 28 is mutated to glutamic acid, position 59 is mutated to glutamic acid, position 108 is mutated to leucine, position 166 is mutated to serine, and position 196 is mutated to glycine, wherein the hKd and the cKd are between about 125 nM and about 140 nM. In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein comprises a sequence wherein amino acid position 34 is mutated to leucine, amino acid position 61 is mutated to glutamic acid, amino acid position 108 is mutated to proline, amino acid position 194 is mutated to asparagine, amino acid position 239 is mutated to alanine, and wherein the hKd and the cKd are about 20-fold to about 50-fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO. 22). In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein comprises a sequence wherein amino acid position 34 is mutated to leucine, amino acid position 61 is mutated to glutamic acid, amino acid position 108 is mutated to proline, amino acid position 194 is mutated to asparagine, amino acid position 239 is mutated to alanine, wherein the hKd and the cKd are between about 100 nM and about 120 nM. In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein comprises a sequence wherein amino acid position 31 is mutated to glutamic acid, amino acid position 65 is mutated to aspartic acid, amino acid position 108 is mutated to glycine, amino acid position 171 is mutated to valine, and amino acid position 194 is mutated to glutamic acid, and wherein the hKd and the cKd are about 20-fold to about 50-fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO. 22). In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein comprises a sequence wherein amino acid position 31 is mutated to glutamic acid, amino acid position 65 is mutated to aspartic acid, amino acid position 108 is mutated to glycine, amino acid position 171 is mutated to valine, and amino acid position 194 is mutated to glutamic acid, and wherein the hKd and the cKd are between about 100 nM and about 120 nM.

[0014a] In some [embodimentexamples](#), there is provided a single chain variable fragment CD3 binding protein, that comprises a variable heavy chain region (VH), a variable light chain region (VL), and a linker, wherein the VH comprises complementarity determining regions HC CDR1, HC CDR2, and HC CDR3, wherein the VL comprises complementarity determining regions LC CDR1, LC CDR2, and LC CDR3, and wherein

(a) the amino acid sequence of HC CDR1 is GFTFNKYAIN (SEQ ID NO. 39);

(b) the amino acid sequence of HC CDR2 is RIRSKYNNYATYYADQVK (SEQ ID NO. 49);

(c) the amino acid sequence of HC CDR3 is HANFGNSYISYWAY (SEQ ID NO. 51);

(d) the amino acid sequence of LC CDR1_{is} ASSTGAVTSGNYPN (SEQ ID NO. 61);

(e) the amino acid sequence of LC CDR2_{is} GTKFLVP (SEQ ID NO. 86); and

(f) the amino acid sequence of LC CDR3_{is} TLWYSNRWV (SEQ ID NO. 87).

[0015] In some [embodimentexamples](#), the single chain variable fragment CD3 binding protein does not bind to mouse CD3.

[0016] In one [embodimentexample](#) is provided a polynucleotide encoding a single chain variable fragment CD3 binding protein according to the present disclosure. In one [embodimentexample](#) is provided a vector comprising the polynucleotide described herein. In one [embodimentexample](#) is provided a host cell transformed with the vector described herein. In a further [embodimentexample](#) is provided a pharmaceutical composition comprising (i) a single chain variable fragment CD3

CLAIMS

WHAT IS CLAIMED IS

1. A single chain variable fragment CD3 binding protein that comprises a variable heavy chain region (VH), a variable light chain region (VL), and a linker, wherein the VH comprises complementarity determining regions HC CDR1, HC CDR2, and HC CDR3, wherein the VL comprises complementarity determining regions LC CDR1, LC CDR2, and LC CDR3, and wherein

- (a) the amino acid sequence of HC CDR1 is GFTFNKYAIN (SEQ ID NO. 39);
- (b) the amino acid sequence of HC CDR2 is RIRSKYNNYATYYADQVK (SEQ ID NO. 49);
- (c) the amino acid sequence of HC CDR3 is HANFGNSYISYWAY (SEQ ID NO. 51);
- (d) the amino acid sequence of LC CDR1 is ASSTGAVTSGNYPN (SEQ ID NO. 61);
- (e) the amino acid sequence of LC CDR2 is GTKFLVP (SEQ ID NO. 86); and
- (f) the amino acid sequence of LC CDR3 is TLWYSNRWV (SEQ ID NO. 87).

2. The single chain variable fragment CD3 binding protein of claim 1, wherein said protein comprises the following formula:

f1-r1-f2-r2-f3-r3-f4-r4-f5-r5-f6-r6-f7

wherein, r1 is SEQ ID NO: 39; r2 is SEQ ID NO: 49; r3 is SEQ ID NO: 51; r4 is SEQ ID NO: 61; r5 is SEQ ID NO: 86; and r6 is SEQ ID NO: 87; and wherein f₁, f₂, f₃, f₄, and f₅ are framework residues selected so that said protein is at least eighty percent identical to the amino acid sequence set forth in SEQ ID NO: 22.

3. The single chain variable fragment CD3 binding protein of claim 1, wherein said protein has an amino acid sequence set forth as SEQ ID NO. 8.

4. The single chain variable fragment CD3 binding protein of claim 1, wherein said linker comprises the amino acid sequence GGGSGGGSGGGGS (SEQ ID NO: 1).

5. The single chain variable fragment CD3 binding protein of any one of claims 1-4, wherein said protein binds to CD3 selected from human CD3 and cynomolgus CD3.

6. The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 and cynomolgus CD3 with comparable binding affinity (Kd).

7. The single chain variable fragment CD3 binding protein of any one of claims 1-6, wherein said protein binds to human CD3 with a human Kd (hKd) between 1 nM and 200 nM and to cynomolgus CD3 with a cynomolgus Kd (cKd) between 1 nM and 300 nM.

8. The single chain variable fragment CD3 binding protein any one of claims 1-7, wherein the hKd and the cKd are between 3 nM to 5 nM, 6 nM to 10 nM, 11 nM to 20 nM, 25 nM to 40 nM, 40 nM to 60 nM, 70 nM to 90 nM, 100 nM to 120 nM, 125 nM to 140 nM, 145 nM to 160 nM, 170 nM and to 200 nM, 210 nM to 250 nM, 260 nM to 300 nM.

9. The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 with a human Kd (hKd), binds to cynomolgus CD3 with a cynomolgus Kd (cKd), and wherein the hKd and the cKd are better than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO. 22).

~~10. The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 with a human Kd (hKd), binds to cynomolgus CD3 with a cynomolgus Kd (cKd), and wherein the hKd and the cKd are 1.5 fold to 2 fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO. 22).~~

~~11. The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 with a human Kd (hKd), binds to cynomolgus CD3 with a cynomolgus Kd (cKd), and wherein the hKd and the cKd are between about 3 fold to 5 fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO.22).~~

~~12. The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 with a human Kd (hKd), binds to cynomolgus CD3 with a cynomolgus Kd (cKd), and wherein the hKd and the cKd are between 6 fold to 15 fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO.22).~~

~~13. — The single chain variable fragment CD3 binding protein of any one of claims 1-5, wherein said protein binds to human CD3 with a human Kd (hKd), binds to cynomolgus CD3 with a cynomolgus Kd (cKd), and wherein the hKd and the cKd are between about 20 fold to about 50 fold higher than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO.22).~~

14.10. The single chain variable fragment CD3 binding protein of any one of claims 1-5 and ~~9-13~~, wherein said protein has the amino acid sequence set forth as SEQ ID NO. 8, and wherein the hKd and cKd are better than the binding affinity towards CD3 of a protein which has the sequence as set forth in wt anti-CD3 (SEQ ID NO. 22).

15.11. The single chain variable fragment CD3 binding protein of any one of claims 1-8, wherein said protein has the amino acid sequence set forth as SEQ ID NO. 8, wherein the hKd and the cKd are between 3 nM and 5 nM.

16.12. A polynucleotide that encodes a single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~15.

17.13. A vector that comprises the polynucleotide of claim ~~12~~16.

18.14. A host cell transformed with the vector according to claim ~~13~~17.

19.15. A pharmaceutical composition that comprises (i) a single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~15, the polynucleotide according to claim ~~12~~16, the vector according to claim ~~13~~17 or the host cell according to claim ~~14~~17, and (ii) a pharmaceutically acceptable carrier.

20.16. A process for the production of a single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~15, said process comprising culturing a host transformed or transfected with a vector comprising a nucleic acid sequence encoding single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~15 under conditions allowing the expression of the single chain variable fragment CD3 binding protein and recovering and purifying the produced protein from the culture.

21.17. The single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~~~15~~ for use in therapy.

22.18. Use of the single chain variable fragment CD3 binding protein according to any one of claims 1-~~11~~~~15~~ in the manufacture of a medicament for the treatment or amelioration of a CD3-associated proliferative disease, a CD3-associated tumorous disease, a CD3-associated inflammatory disease, a CD3-associated immunological disorder, a CD3-associated autoimmune disease, a CD3-associated infectious disease, a CD3-associated viral disease, a CD3-associated allergic reaction, a CD3-associated parasitic reaction, a CD3-associated graft-versus-host disease, or a CD3-associated host-versus-graft disease, wherein the medicament is to be administered to a subject in need thereof.

23.19. The use of claim ~~18~~~~22~~, wherein the subject is human.

24.20. The use of claim ~~19~~~~23~~, wherein the medicament is to be administered in combination with an agent.

25.21. A multispecific binding protein that comprises the single chain variable fragment CD3 binding protein according to claim 1.

26.22. An antibody that comprises the single chain variable fragment CD3 binding protein according to claim 1.

27.23. A multispecific antibody, a bispecific antibody, a single domain antibody, a variable heavy domain, a peptide, or a ligand, that comprises the single chain variable fragment CD3 binding protein according to claim 1.

28.24. An antibody that comprises the single chain variable fragment CD3 binding protein according to claim 1, wherein said antibody is a scFv antibody.

29.25. A multispecific binding protein or antibody that comprises the single chain variable fragment CD3 binding protein according to claim 1 and a serum albumin binding domain.