

Claims

1. An antigen binding polypeptide comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein

(1) the VH comprises

(a) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO: 52,

(b) a HCDR2 comprising the amino acid sequence of YINPYNDVTKYX₁X₂KFX₃G (SEQ IDNO: 53), wherein X₁ is A or N;

X₂ is E or Q; and/or

X₃ is Q or K

(c) a HCDR3, and

(d) heavy chain framework regions (HFR)1-4;

(2) the VL comprises

(a) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO: 54,

(b) a LCDR2 comprising the amino acid sequence of SEQ ID NO: 55,

(c) a LCDR3, and

(d) light chain framework regions (LFR)1-4;

wherein

(i) at least one amino acid of HCDR1 comprising the amino acid sequence of SEQ ID NO: 52 and/or at least one amino acid of HCDR2 comprising the amino acid sequence of SEQ ID NO: 53 that is not positively charged is substituted with a positively charged amino acid; and/or

(ii) at least one amino acid of LCDR1 comprising the amino acid of SEQ ID NO: 54 and/or at least one amino acid of LCDR2 comprising the amino acid sequence of SEQ ID NO: 55 that is not positively charged is substituted with a positively charged amino acid; and/or

(iii) position 30 in HFR1 according to Kabat numbering is substituted with a positively charged amino acid, and/or

(iv) position 90 in HFR3 according to Kabat numbering is substituted with a tyrosine (Y) residue,

and wherein the antigen binding polypeptide specifically binds to an α/β T cell receptor (TCR)/CD3 complex.

2. The antigen binding polypeptide according to claim 1, wherein the positively charged amino acids are:
 - (i) at one or more of the following positions of the heavy chain: 31, 53, and 54; and/or
 - (ii) at one or more of the following positions of the light chain: 31 and 56, and wherein the positions are according to Kabat numbering.
3. The antigen binding polypeptide according to claim 1 or 2, wherein
 - (a) the positively charged amino acid in the heavy chain:
 - (i) at position 30 is R, K or H;
 - (ii) at position 31 is R, K or H;
 - (iii) at position 53 is R, K or H; and/or
 - (iv) at position 54 is R or K; and/or
 - (b) the positively charged amino acid in the light chain
 - (i) at position 31 is R or K; and/or
 - (ii) at position 56 is R or K.
4. The antigen binding polypeptide according to any one of claims 1 to 3, wherein the VH and VL form a first binding site, and wherein the antigen binding polypeptide comprises a second antigen binding site.
5. The antigen binding polypeptide according to claim 4, wherein the second antigen binding site comprises at least the variable region of:
 - (i) an α (V_α) and/or β (V_β) chain of a TCR; or
 - (ii) a γ (V_γ) and/or δ (V_δ) chain of a TCR, or
 - (iii) a light chain different from the VL as defined in claim 1 and/or heavy chain different from the VH as defined in claim 1.
6. The antigen binding polypeptide according to any one of claims 1-5, wherein the positively charged amino acid is selected from the group consisting of arginine (R), histidine (H), and lysine (K), preferably wherein the positively charged amino acid is R or K.

7. The antigen binding polypeptide according to any one of claims 1-6, wherein
 - (i) threonine (T) at position 30 in the heavy chain is substituted with asparagine (N) or serine (S);
 - (ii) S at position 31 in the heavy chain is substituted with N;
 - 5 (iii) valine (V) at position 56 in the heavy chain is substituted with isoleucine (I);
 - (iv) glutamic acid (E) at position 100a in the heavy chain is substituted with aspartic acid (D); and/or
 - (v) S at position 31 and/or position 93 in the light chain is/are substituted with N.
- 10 8. A nucleic acid or set of nucleic acids coding for an antigen binding polypeptide according to any one of claims 1 to 7 or a nucleic acid vector comprising said nucleic acid.
9. A recombinant host cell comprising an antigen binding polypeptide according to any one of claims 1 to 7, or a nucleic acid or set of nucleic acids or a vector according to claim 8,
 - 15 wherein said host cell is
 - (i) a lymphocyte, preferably a T lymphocyte or T lymphocyte progenitor cell, for example a CD4 or CD8 positive T cell; or
 - (ii) a cell for recombinant expression, such as a Chinese Hamster Ovary (CHO) cell or a yeast cell.
- 20 10. A pharmaceutical composition comprising the antigen polypeptide according to any one of claims 1 to 7, the nucleic acid or set of nucleic acids or vector according to claim 8, or the host cell according to claim 9, and a pharmaceutically acceptable carrier, diluent stabilizer, and/or excipient.
- 25 11. A method of producing the antigen binding polypeptide according to any one of claims 1 to 7, comprising
 - (i) providing a suitable host cell,
 - (ii) providing a genetic construct comprising a coding sequence encoding the antigen;
 - 30 (iii) binding polypeptide according to any one of claims 1 to 7,
 - (iv) introducing said genetic construct into said suitable host cell, and
 - (iv) expressing said genetic construct by said suitable host cell.

12. The antigen binding polypeptide according to any one of claims 1 to 7, the nucleic acid or set of nucleic acids or vector according to claim 8, the host cell according to claim 9 or the pharmaceutical composition according to claim 10 for use in medicine.
- 5 13. Use of the antigen binding polypeptide according to any one of claims 1 to 7, the nucleic acid or vector according to claim 8, the host cell according to claim 9 or the pharmaceutical composition according to claim 10 for the manufacture of a medicament for diagnosing, preventing, and/or treating a proliferative disease, preferably cancer.
- 10 14. A method for improving or maintaining the binding and/or improving the stability of the antigen binding polypeptide comprising a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein
 - (1) the VH comprises
 - 15 (a) a heavy chain complementarity determining region 1 (HCDR1) comprising the amino acid sequence of SEQ ID NO: 52 (SYVMH),
 - (b) a HCDR2 comprising the amino acid sequence of YINPYNDVTKYX₁X₂KFX₃G (SEQ ID NO: 53), wherein
 - 20 X₁ is A or N;
 - X₂ is E or Q; and/or
 - X₃ is Q or K
 - (c) a HCDR3, and
 - (d) heavy chain framework regions (HFR)1-4;
 - (2) the VL comprises
 - 25 (a) a light chain complementarity determining region 1 (LCDR1) comprising the amino acid sequence of SEQ ID NO: 54 (SATSSVSYMH),
 - (b) a LCDR2 comprising the amino acid sequence of SEQ ID NO: 55 (DTSKLAS),
 - (c) a LCDR3, and
 - (d) light chain frameworks (LCR)1-4;
- 30 wherein
 - (i) at least one amino acid of HCDR1 comprising the amino acid sequence of SEQ ID NO: 52 and/or at least one amino acid of HCDR2 comprising the amino acid sequence of SEQ ID NO: 53 that is not positively charged is substituted with a positively charged amino acid; and/or

- (ii) at least one amino acid of LCDR1 comprising the amino acid of SEQ ID NO: 54 and/or at least one amino acid of LCDR2 comprising the amino acid sequence of SEQ ID NO: 55 that is not positively charged is substituted with a positively charged amino acid; and/or
- 5 (iii) position 30 in HFR1 according to Kabat numbering is substituted with a positively charged amino acid, and/or
- (iv) position 90 in HFR3 according to Kabat numbering is substituted with a tyrosine (Y) residue,
- wherein
- 10 (1) the binding of the antigen binding polypeptide to an α/β T cell receptor (TCR)/CD3 complex is increased compared to the parental antigen binding polypeptide;
- (2) the binding of the antigen binding polypeptide to an α/β T cell receptor (TCR)/CD3 complex is maintained or increased and the stability of the antigen binding polypeptide is increased compared to the parental antigen binding polypeptide; or
- 15 (3) the stability of the antigen binding polypeptide is increased compared to the parental antigen binding polypeptide.
15. An *in vitro* or *ex vivo* method for detecting, determining or enriching T cells expressing the α/β TCR/CD3 complex, comprising the step of contacting cells with the antigen
- 20 binding polypeptide according to any one of claims 1-7.