

Claims

1. A T cell receptor (TCR), which has an activity of binding to SLLMWITQC-HLA A2 complex and comprises a TCR α chain variable domain and a TCR β chain variable domain, the TCR α chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR α chain variable domain are listed as follows,

CDR1 α : TSINN

CDR2 α : IRSNERE

CDR3 α : ATDANGKII and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
T at position 1 of CDR1 α	G
S at position 2 of CDR1 α	W
I at position 3 of CDR1 α	A or V
N at position 4 of CDR1 α	Q
S at position 3 of CDR2 α	T
N at position 4 of CDR2 α	G
E at position 5 of CDR2 α	Q
R at position 6 of CDR2 α	A or Q
A at position 1 of CDR3 α	M
T at position 2 of CDR3 α	Y
A at position 4 of CDR3 α	E or Q
N at position 5 of CDR3 α	F or Y or W
G at position 6 of CDR3 α	A or Q
K at position 7 of CDR3 α	W or R or T or S or M or L
I at position 8 of CDR3 α	W or V or Y or P, and
I at position 9 of CDR3 α	N or E or D or Q or V or K or G or M or S

and/or, the TCR β chain variable domain comprises three CDR regions, and the reference sequences of the three CDR regions of the TCR β chain variable domain are listed as follows,

CDR1 β : SGHDY

CDR2 β : FNNNVP

CDR3 β : ASSLGSENEY and contains at least one of the following mutations:

Residue before mutation	Residue after mutation
N at position 3 of CDR2 β	H
N at position 4 of CDR2 β	G
V at position 5 of CDR2 β	A
P at position 6 of CDR2 β	V
S at position 3 of CDR3 β	Q or M
L at position 4 of CDR3 β	R or K or M, and
S at position 6 of CDR3 β	A or G or P.

2. The TCR of claim 1, wherein the TCR has the activity of binding to SLLMWITQC-HLA A2 complex and comprises a TCR α chain variable domain and a TCR β chain variable domain, there is a mutation in the α chain variable domain of the TCR as shown in SEQ ID NO: 1, and the mutated amino acid residue site includes one or more of 27T, 28S, 29I, 30N, 51S, 52N, 53E, 54R,

90A, 91T, 93A, 94N, 95G, 96K, 97I and 98I, wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or there is a mutation in the β chain variable domain of the TCR as shown in SEQ ID NO: 2, and the mutated amino acid residue site includes one or more of 51N, 52N, 53V, 54P, 95S, 96L and 98S, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2;

preferably, upon mutation, the TCR α chain variable domain comprises one or more amino acid residues selected from the group consisting of: 27G, 28W, 29A or 29V, 30Q, 51T, 52G, 53Q, 54Q or 54A, 90M, 91Y, 93E or 93Q, 94F or 94Y or 94W, 95A or 95Q, 96W or 96R or 96T or 96S or 96M or 96L, 97W or 97V or 97Y or 97P, and 98E or 98N or 98D or 98Q or 98K or 98V or 98G or 98M or 98S, wherein the amino acid residue is numbered as shown in SEQ ID NO: 1; and/or upon mutation, the TCR β chain variable domain comprises one or more amino acid residues selected from the group consisting of: 51H, 52G, 53A, 54V, 95Q or 95M, 96R or 96K or 96M, and 98A or 98G or 98P, wherein the amino acid residue is numbered as shown in SEQ ID NO: 2.

3. The TCR of claim 1, wherein the affinity of the TCR for SLLMWITQC-HLA A2 complex is at least 2 times of that of the wild type TCR.

4. The TCR of claim 1, wherein the α chain variable domain of the TCR comprises an amino acid sequence having at least 90% of sequence homology with the amino acid sequence shown in SEQ ID NO: 1; and/or the β chain variable domain of the TCR comprises an amino acid sequence having at least 90% of sequence homology with the amino acid sequence shown in SEQ ID NO: 2.

5. The TCR of claim 1, wherein the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and the TCR β chain variable domain comprises CDR1 β , CDR2 β and CDR3 β , wherein the CDR1 β comprises a sequence: SGHDY;

or

the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and the TCR β chain variable domain comprises CDR1 β , CDR2 β and CDR3 β , and the CDR2 β comprises a sequence: FN[2 β X1][2 β X2][2 β X3][2 β X4], wherein each of [2 β X1], [2 β X2], [2 β X3] and [2 β X4] is independently selected from any natural amino acid residue; preferably,

the [2 β X1] is N or H;

or

the [2 β X2] is N or G;

or

the [2 β X3] is V or A;

or

the [2 β X4] is P or V;

or

the CDR2 β comprises a sequence selected from the group consisting of:
FNHGAV and FNNNP;

or

the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and
5 the TCR β chain variable domain comprises CDR1 β , CDR2 β and CDR3 β , and

the CDR3 β comprises a sequence: AS[3 β X1][3 β X2]G[3 β X3]NEQY, wherein [3 β X1],
[3 β X2], and [3 β X3] are independently selected from any natural amino acid residue;
preferably, the [3 β X1] is S, Q or M;

or

10 the [3 β X2] is L, R, K, or M;

or

the [3 β X3] is S, A, G or P;

or

the CDR3 β comprises a sequence selected from the group consisting of:

15 ASQRGSNEQY, ASQKGANEQY, ASQLGSNEQY, ASQLGANEQY, ASQLGGNEQY,
ASQLGPNEQY, ASQRGANEQY, ASQMGSNEQY, ASQMGANEQY and ASMLGSNEQY;

or

the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and
the TCR α chain variable domain comprising CDR1 α , CDR2 α and CDR3 α , and the CDR1 α
20 comprises a sequence: [1 α X1][1 α X2][1 α X3][1 α X4]N, wherein [1 α X1], [1 α X2], [1 α X3], and
[1 α X4] are independently selected from any natural amino acid residue; preferably,

the [1 α X1] is T or G;

or

the [1 α X2] is S or W;

25 or

the [1 α X3] is I or A or V;

or

the [1 α X4] is N or Q;

or

30 the CDR1 α comprises a sequence selected from the group consisting of: GSIQN,
GWAQN, GWVQN and TSINN;

or

the TCR comprises a TCR α chain variable domain and a TCR β chain variable domain, and
the TCR α chain variable domain comprising CDR1 α , CDR2 α and CDR3 α , and the CDR2 α
35 comprises a sequence: IR [2 α X1][2 α X2][2 α X3][2 α X4]E, wherein [2 α X1], [2 α X2], [2 α X3], and

[2 α X4] are independently selected from any natural amino acid residue; preferably,

the [2 α X1] is S or T;

or

the [2 α X2] is N or G;

or

the [2 α X3] is E or Q;

or

the [2 α X4] is R, A or Q;

or

the CDR2 α comprises a sequence selected from the group consisting of: IRTGEQE, IRTGQAE and IRSNERE.

6. The TCR of claim 1, wherein the TCR has a CDR selected from the group consisting of:

TCR No.	CDR1 α	CDR2 α	CDR3 α	CDR1 β	CDR2 β	CDR3 β
1	TSINN	IRSNERE	ATDAFAWWI	SGHDY	FNNNVP	ASSLGSNEQY
2	GWAQN	IRTGEQE	ATDANARWN	SGHDY	FNHGAV	ASSLGSNEQY
3	GSIQN	IRTGEQE	ATDANARWE	SGHDY	FNHGAV	ASSLGSNEQY
4	GSIQN	IRTGEQE	ATDANARWE	SGHDY	FNHGAV	ASQKGSNEQY
5	GSIQN	IRTGEQE	ATDANARWE	SGHDY	FNHGAV	ASQKGSNEQY
6	GSIQN	IRTGEQE	ATDANARWD	SGHDY	FNHGAV	ASQLGSNEQY
7	GWAQN	IRSNERE	ATDANARWN	SGHDY	FNHGAV	ASQLGSNEQY
8	GWVQN	IRTGEQE	ATDANARWD	SGHDY	FNHGAV	ASSLGSNEQY
9	GSIQN	IRSNERE	ATDANARWD	SGHDY	FNHGAV	ASQLGSNEQY
10	GWAQN	IRSNERE	ATDANARWD	SGHDY	FNHGAV	ASQLGSNEQY
11	GSIQN	IRSNERE	ATDANARWE	SGHDY	FNHGAV	ASQKGSNEQY
12	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQLGGNEQY
13	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
14	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQLGPNEQY
15	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
16	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
17	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
18	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
19	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQKGSNEQY
20	TSINN	IRSNERE	MYDENGKII	SGHDY	FNNNVP	ASSLGSNEQY
21	TSINN	IRSNERE	MYDQNGKII	SGHDY	FNNNVP	ASSLGSNEQY
22	TSINN	IRSNERE	ATDAYGTVQ	SGHDY	FNNNVP	ASSLGSNEQY
23	TSINN	IRSNERE	ATDANGTVQ	SGHDY	FNNNVP	ASSLGSNEQY
24	TSINN	IRSNERE	ATDANGSVQ	SGHDY	FNNNVP	ASSLGSNEQY
25	TSINN	IRSNERE	ATDAYGRYI	SGHDY	FNNNVP	ASSLGSNEQY
26	TSINN	IRSNERE	ATDANQMWK	SGHDY	FNNNVP	ASSLGSNEQY
27	TSINN	IRSNERE	ATDAYGLPI	SGHDY	FNNNVP	ASSLGSNEQY
28	TSINN	IRSNERE	ATDANGLPV	SGHDY	FNNNVP	ASSLGSNEQY
29	TSINN	IRSNERE	ATDANGRWE	SGHDY	FNNNVP	ASSLGSNEQY
30	TSINN	IRSNERE	ATDANGTVG	SGHDY	FNNNVP	ASSLGSNEQY
31	TSINN	IRSNERE	ATDANGRWM	SGHDY	FNNNVP	ASSLGSNEQY
32	GWAQN	IRTGEQE	ATDANARWD	SGHDY	FNNNVP	ASSLGSNEQY
33	GWAQN	IRTGQAE	ATDANARWE	SGHDY	FNNNVP	ASSLGSNEQY
34	GSIQN	IRSNERE	ATDAFAWWI	SGHDY	FNNNVP	ASSLGSNEQY
35	GWVQN	IRTGEQE	ATDANARWN	SGHDY	FNNNVP	ASSLGSNEQY

36	GWAQN	IRTGEQE	ATDANARWN	SGHDY	FNNNVP	ASSLGSNEQY
37	GWVQN	IRTGEQE	ATDANARWD	SGHDY	FNNNVP	ASSLGSNEQY
38	GSIQN	IRTGEQE	ATDAWARWI	SGHDY	FNNNVP	ASSLGSNEQY
39	GWVQN	IRTGQAE	ATDANARWE	SGHDY	FNNNVP	ASSLGSNEQY
40	GSIQN	IRTGEQE	ATDANARWN	SGHDY	FNNNVP	ASSLGSNEQY
41	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQMGSNEQY
42	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQMGANEQY
43	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASQMGANEQY
44	TSINN	IRSNERE	ATDANGKII	SGHDY	FNNNVP	ASMLGSNEQY.

7. The TCR of claim 1, wherein the TCR is soluble.

8. The TCR of claim 1, wherein the TCR is an $\alpha\beta$ heterodimeric TCR.

9. The TCR of claim 31, wherein the TCR comprises (i) all or part of the TCR α chain other than its transmembrane domain, and (ii) all or part of the TCR β chain other than its transmembrane domain, wherein both of (i) and (ii) comprise the variable domain and at least a portion of the constant domain of the TCR chain;

preferably,

an artificial interchain disulfide bond is contained between the α chain constant region and the β chain constant region of the TCR;

more preferably,

cysteine residues forming an artificial interchain disulfide bond between the TCR α chain constant region and β chain constant region are substituted for one or more groups of amino acids selected from the following:

Thr48 of TRAC*01 exon 1 and Ser57 of TRBC1*01 or TRBC2*01 exon 1;

Thr45 of TRAC*01 exon 1 and Ser77 of TRBC1*01 or TRBC2*01 exon 1;

Tyr10 of TRAC*01 exon 1 and Ser17 of TRBC1*01 or TRBC2*01 exon 1;

Thr45 of TRAC*01 exon 1 and Asp59 of TRBC1*01 or TRBC2*01 exon 1;

Ser15 of TRAC*01 exon 1 and Glu15 of TRBC1*01 or TRBC2*01 exon 1;

Arg53 of TRAC*01 exon 1 and Ser54 of TRBC1*01 or TRBC2*01 exon 1;

Pro89 of TRAC*01 exon 1 and Ala19 of TRBC1*01 or TRBC2*01 exon 1; and

Tyr10 of TRAC*01 exon 1 and Glu20 of TRBC1*01 or TRBC2*01 exon 1.

10. The TCR of claim 1, wherein the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NOs: 58-86; and/or the amino acid sequence of the β chain variable domain of the TCR is selected from the group consisting of: SEQ ID NOs: 87-102.

11. The TCR of claim 1, wherein the TCR is selected from the group consisting of:

TCR number	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
1	58	2
2	59	87

3	60	87
4	60	88
5	60	89
6	61	90
7	62	90
8	63	87
9	64	91
10	65	90
11	66	89
12	1	92
13	1	93
14	1	94
15	1	95
16	1	96
17	1	97
18	1	98
19	67	2
20	68	2
21	69	2
22	70	2
23	71	2
24	72	2
25	73	2
26	74	2
27	75	2
28	76	2
29	77	2
30	78	2
31	79	2
32	80	2
33	81	2
34	82	2
35	83	2
36	59	2
37	63	2
38	84	2
39	85	2
40	86	2
41	1	99
42	1	100
43	1	101
44	1	102.

12. The TCR of claim 1, wherein the TCR is a single chain TCR;

or

the TCR is a single-chain TCR consisting of an α chain variable domain and a β chain variable domain, and the α chain variable domain and the β chain variable domain are connected

5 by a flexible short peptide sequence (linker); preferably,

the hydrophobic core of the TCR α chain variable domain and/or β chain variable domain is mutated;

or

- 5 the amino acid sequence of the α chain variable domain of the TCR is selected from the group consisting of: SEQ ID NOs: 9-37; and/or the amino acid sequence of the β chain variable domain of the TCR is selected from the group consisting of: SEQ ID NOs: 38-53;

or

the TCR is selected from the group consisting of:

TCR Number	Sequence of α chain variable domain SEQ ID NO:	Sequence of β chain variable domain SEQ ID NO:
s-1	9	4
s-2	10	38
s-3	11	38
s-4	11	39
s-5	11	40
s-6	12	41
s-7	13	41
s-8	14	38
s-9	15	42
s-10	16	41
s-11	17	40
s-12	3	43
s-13	3	44
s-14	3	45
s-15	3	46
s-16	3	47
s-17	3	48
s-18	3	49
s-19	18	4
s-20	19	4
s-21	20	4
s-22	21	4
s-23	22	4
s-24	23	4
s-25	24	4
s-26	25	4
s-27	26	4
s-28	27	4
s-29	28	4
s-30	29	4
s-31	30	4
s-32	31	4
s-33	32	4
s-34	33	4
s-35	34	4

s-36	10	4
s-37	14	4
s-38	35	4
s-39	36	4
s-40	37	4
s-41	3	50
s-42	3	51
s-43	3	52
s-44	3	53.

13. The TCR of any one of preceding claims, wherein a conjugate binds to the α chain and/or β chain of the TCR at C- or N-terminus;

preferably,

the conjugate that binds to the TCR is a detectable label, a therapeutic agent, a PK modified

5 moiety, or a combination of any of these;

more preferably,

the therapeutic agent that binds to the TCR is an anti-CD3 antibody linked to the α or β chain of the TCR at C- or N-terminus.

10 14. A multivalent TCR complex comprising at least two TCR molecules, and at least one TCR molecule is the TCR of any one of preceding claims.

15. A nucleic acid molecule comprising a nucleic acid sequence encoding the TCR of any one of claims 1-13, or a complement sequence thereof.

16. A vector comprising the nucleic acid molecule of claim 15.

15 17. A host cell comprising the vector of claim 16 or having the exogenous nucleic acid molecule of claim 15 integrated into its genome.

18. An isolated cell expressing the TCR of any one of claims 1-13.

19. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, and a TCR of any one of claims 1-13, or a TCR complex of claim 14, or the cell of claim 18.

20 20. Use of the TCR of any one of claims 1-13, or the TCR complex of claim 14, or the cell of claim 18 for preparing a medicament for treating tumor.

21. A method for preparing the TCR of any one of claims 1-13, comprising the steps of:

(i) culturing the host cell of claim 17 to express the TCR of any one of claims 1-13;

(ii) isolating or purifying the TCR.