

Claims:

1. A T cell receptor (TCR) having the property of binding to GGYDGREHTV (SEQ ID NO: 1) HLA-A*02 complex and comprising a TCR alpha chain variable domain and a TCR beta chain variable domain, wherein:

(a) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:17; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:28;

(b) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:25;

(c) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:28;

(d) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:20; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:28;

(e) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(f) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:27;

(g) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(h) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:23; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(i) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:46; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(j) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:47; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(k) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:48; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(l) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:49; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(m) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:50; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(n) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:51; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:29;

(o) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:20; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:65;

(p) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:66;

(q) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:67;

(r) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:68;

(s) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:69;

(t) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:24; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:70;

(u) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:52; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(v) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:53; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(w) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:54; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(x) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:55; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(y) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:56; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(z) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:57; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(aa) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:58; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(bb) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:59; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(cc) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:60; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:26;

(dd) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:71;

(ee) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:72;

(ff) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:73;

(gg) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:74;

(hh) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:75;

(ii) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:76;

(jj) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:77;

(kk) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:78;

(ll) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:22; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:79;

(mm) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:62; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:5;

(nn) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:63; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:5;

(oo) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:64; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:5;

(pp) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:61; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:80; or

(qq) the TCR alpha chain variable domain comprises the amino acid sequence of SEQ ID NO:61; and the TCR beta chain variable domain comprises the amino acid sequence of SEQ ID NO:81.

2. A TCR that binds to a GVDGREHTV (SEQ ID NO: 1) HLA-A*02 complex with an affinity greater than 200 μ M, wherein the alpha chain variable domain comprises:

(a) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of MTFSENT, and a CDR3 comprising the amino acid sequence of VVNSANGLYIPTF;

(b) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of MTFSENT, and a CDR3 comprising the amino acid sequence of VVNRADGLYIPTF;

(c) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of LDY AINT, and a CDR3 comprising the amino acid sequence of VVNHS GGSYIPTF;

(d) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of LDY AINT, and a CDR3 comprising the amino acid sequence of VVNRADGLYIPTF;

(e) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of LTFSENT, and a CDR3 comprising the amino acid sequence of VVNSASGLYIPTF; or

(f) a CDR1 comprising the amino acid sequence of VSPFSN, a CDR2 comprising the amino acid sequence of LTFSENT, and a CDR3 comprising the amino acid sequence of VVNSAQGLYIPTF;

and wherein the beta chain variable domain comprises:

(g) a CDR1 comprising the amino acid sequence of MDHEN, a CDR2 comprising the amino acid sequence of SRFATG, and a CDR3 comprising the amino acid sequence of ASSSDQNSGDPYEQYF;

(h) a CDR1 comprising the amino acid sequence of MDHEN, a CDR2 comprising the amino acid sequence of SYDVKM, and a CDR3 comprising the amino acid sequence of ASSSDQNSGDPYEQYF;

(i) a CDR1 comprising the amino acid sequence of LDHEN, a CDR2 comprising the amino acid sequence of SRFATG, and a CDR3 comprising the amino acid sequence of ASSSDQNSGDPYEQYF;

(j) a CDR1 comprising the amino acid sequence of APLSK, a CDR2 comprising the amino acid sequence of SYDVKM, and a CDR3 comprising the amino acid sequence of ASSSDQNSGDPYEQYF; or

(k) a CDR1 comprising the amino acid sequence of APLSK, a CDR2 comprising the amino acid sequence of SYDVKL, and a CDR3 comprising the amino acid sequence of ASSSDQNSGDPYEQYF.

3. A TCR as claimed in claim 2, wherein the alpha chain variable domain comprises the following set of CDR1, CDR2, and CDR3 sequences; and the beta chain variable domain comprises the following set of CDR1, CDR2, and CDR3 sequences:

(a) alpha chain variable domain CDR1: VSPFSN, CDR2: LDYAIN, and CDR3: VVNRADGLYIPTF, and beta chain variable domain CDR1: APLSK, CDR2: SYDVKM, and CDR3: ASSSDQNSGDPYEQYF;

(b) alpha chain variable domain CDR1: VSPFSN, CDR2: LDY AINT, and CDR3: VVNRADGLYIPTF, and beta chain variable domain CDR1: APLSK, CDR2: SYDVKL, and CDR3: ASSSDQNSGDPYEQYF;

(c) alpha chain variable domain CDR1: VSPFSN, CDR2: MTFSENT, and CDR3: VVNSANGLYIPTF, and beta chain variable domain CDR1: MDHEN, CDR2: SRFATG, and CDR3: ASSSDQNSGDPYEQYF;

(d) alpha chain variable domain CDR1: VSPFSN, CDR2: LTFSENT, and CDR3: VVNSAQGLYIPTF, and beta chain variable domain CDR1: LDHEN, CDR2: SRFATG, and CDR3: ASSSDQNSGDPYEQYF; or

(e) alpha chain variable domain CDR1: VSPFSN, CDR2: LTFSENT, and CDR3: VVNSASGLYIPTF, and beta chain variable domain CDR1: LDHEN, CDR2: SRFATG, and CDR3: ASSSDQNSGDPYEQYF.

4. A TCR claimed in any one of claims 2-3, comprising:

(a) an alpha chain variable domain sequence corresponding to SEQ ID NO:22, and a beta chain variable domain sequence corresponding to SEQ ID NO:26;

(b) an alpha chain variable domain sequence corresponding to SEQ ID NO:22, and a beta chain variable domain sequence corresponding to SEQ ID NO:27;

(c) an alpha chain variable domain sequence corresponding to SEQ ID NO:20, and a beta chain variable domain sequence corresponding to SEQ ID NO:28;

(d) an alpha chain variable domain sequence corresponding to SEQ ID NO:24, and a beta chain variable domain sequence corresponding to SEQ ID NO:29; or

(e) an alpha chain variable domain sequence corresponding to SEQ ID NO:23, and a beta chain variable domain sequence corresponding to SEQ ID NO:29.

5. A TCR as claimed in any preceding claim, which is an alpha-beta heterodimer, having an alpha chain TRAC constant domain sequence and a beta chain TRBC1 or TRBC2 constant domain sequence.

6. A TCR as claimed in claim 5, wherein the alpha and beta chain constant domain sequences are modified by truncation or substitution to delete a native disulphide bond between Cys4 of exon 2 of TRAC and Cys2 of exon 2 of TRBC1 or TRBC2.
7. A TCR as claimed in claim 5 or claim 6, wherein the alpha and/or beta chain constant domain sequence(s) are modified by substitution of cysteine residues for Thr 48 of TRAC and Ser 57 of TRBC1 or TRBC2, the said cysteines forming a non-native disulphide bond between the alpha and beta constant domains of the TCR.
8. A TCR as claimed in any preceding claim, which is in single chain format of the type $V\alpha$ -L- $V\beta$, $V\beta$ -L- $V\alpha$, $V\alpha$ -C α -L- $V\beta$, $V\alpha$ -L- $V\beta$ -C β , wherein $V\alpha$ and $V\beta$ are TCR α and β variable regions respectively, C α and C β are TCR α and β constant regions respectively, and L is a linker sequence.
9. A TCR as claimed in any preceding claim, wherein the TCR is associated with a detectable label, a therapeutic agent or a PK modifying moiety.
10. A TCR as claimed in claim 9, wherein an anti-CD3 antibody is covalently linked to the C- or N-terminus of the alpha or beta chain of the TCR.
11. A TCR as claimed in claim 10, wherein the anti-CD3 antibody is covalently linked to the C- or N-terminus of the beta chain of the TCR via a linker sequence.
12. A TCR as claimed in claim 11, wherein the linker sequence is selected from the group consisting of GGGGS (SEQ ID NO: 30), GGGSG (SEQ ID NO: 31), GGSGG (SEQ ID NO: 32), GSGGG (SEQ ID NO: 33), GSGGGP (SEQ ID NO: 34), GGEPS (SEQ ID NO: 35), GGEGGGP (SEQ ID NO: 36), and GGEGGGSEGGS (SEQ ID NO: 37).
13. A TCR-anti-CD3 fusion molecule wherein the alpha chain variable domain comprises an amino acid sequence selected from SEQ ID NOs: 16-24 or 46-64 and the beta chain variable domain comprises an amino acid sequence selected from SEQ ID NO: 25-29 or 65-81, and

wherein the anti-CD3 antibody is covalently linked to the N-terminus or C-terminus of the TCR beta chain via a linker sequence selected from SEQ ID NOs: 30-37.

14. A TCR-anti-CD3 fusion molecule as claimed in claim 13, comprising
an alpha chain amino acid sequence selected from SEQ ID NOs: 38-41,
and a beta chain amino acid sequence selected from SEQ ID NOs: 42-45.
15. A TCR-anti CD3 fusion molecule as claimed in claim 14, comprising
 - (a) an alpha chain amino acid sequence corresponding to SEQ ID NO: 38 and beta chain amino acid sequence corresponding to SEQ ID NO: 42;
 - (b) an alpha chain amino acid sequence corresponding to SEQ ID NO: 38 and beta chain amino acid sequence corresponding to SEQ ID NO: 43;
 - (c) an alpha chain amino acid sequence corresponding to SEQ ID NO: 39 and beta chain amino acid sequence corresponding to SEQ ID NO: 44;
 - (d) an alpha chain amino acid sequence corresponding to SEQ ID NO: 40 and beta chain amino acid sequence corresponding to SEQ ID NO: 45; or
 - (e) an alpha chain amino acid sequence corresponding to SEQ ID NO: 41 and beta chain amino acid sequence corresponding to SEQ ID NO: 45
16. A nucleic acid encoding a TCR alpha chain and/or a TCR beta chain as claimed in any one of the preceding claims.
17. An expression vector comprising the nucleic acid of claim 16.
18. A cell harbouring
 - (a) an expression vector as claimed in claim 17 encoding TCR alpha and beta chains as claimed in any one of claims 1-15, in a single open reading frame, or two distinct open reading frames; or
 - (b) a first expression vector which comprises nucleic acid encoding the alpha chain of a TCR as claimed in any one of claims 1-15, and a second expression vector which comprises nucleic acid encoding the beta chain of a TCR as claimed in any one of claims 1-15.

19. A non-naturally occurring and/or purified and/or engineered cell, especially a T-cell, presenting a TCR as claimed in any one of claims 1-12.
20. A pharmaceutical composition comprising a TCR as claimed in any one of claims 1-12, a TCR-anti CD3 fusion molecule as claimed in any one of claims 13-15, a nucleic acid as claimed in claim 16, and/or a cell as claimed in claim 18 or 19, together with one or more pharmaceutically acceptable carriers or excipients.
21. The TCR of any one of claims 1-12, TCR-anti-CD3 fusion molecule of any one of claims 13-15, nucleic acid of claim 16, pharmaceutical composition of claim 20 and/or cell of claim 18 or 19, for use in medicine, preferably in a human subject.
22. The TCR of any one of claims 1-12, TCR-anti-CD3 fusion molecule of any one of claims 13-15, nucleic acid of claim 16, pharmaceutical composition of claim 20 and/or cell of claim 18 or 19, for use in a method of treating cancer or a tumour, preferably in a human subject.
23. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to claim 22, wherein the human subject has a tumour that expresses MAGE A4.
24. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to claim 22 or 23, wherein the tumour is a solid tumour.
25. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to any one of claims 22-24, wherein the human subject is of HLA-A*02 subtype.
26. The TCR, TCR-anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to any one of claims 22-25, which is administered by injection, such as intravenous or direct intratumoral injection.

27. An injectable formulation for administering to a human subject comprising a TCR according to any one of claims 1-12 or a TCR-anti-CD3 fusion molecule of any one of claims 13-15.

28. A method of producing a TCR according to any one of claims 1-12, or a TCR-anti-CD3 fusion molecule according to any one of claims 13-15, comprising a) maintaining a cell according to claim 18 under optimal conditions for expression of the TCR chains acid and b) isolating the TCR chains.