

Claims:

1. A T cell receptor (TCR) having the property of binding to GVDGREHTV (SEQ ID NO: 1) HLA-A*02 complex and comprising a TCR alpha chain variable domain and/or a TCR beta chain variable domain,
- the alpha chain variable domain comprises an amino acid sequence that has at least 90% identity to amino acids 1-113 of SEQ ID NO: 2, and/or
- the beta chain variable domain comprises an amino acid sequence that has at least 90% identity to amino acids 1-116 of SEQ ID NO: 3.
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 CDRs 1, 2 and 3 comprise SEQ ID NOs 6, 7 and 8 respectively, and/or the beta chain CDRs 1, 2 and 3 comprise SEQ ID NOs 11, 12 and 13 respectively; and/or at least one of the CDRs contains one or more conservative substitutions with respect to SEQ ID NO: 6-8 and 11-13 and/or at least one of the CDRs contains up to three tolerated substitutions with respect to SEQ ID NO: 6-8 and 11-13 and/or one or more of the mutations set out in tables 1, 2 and 3.
3. A TCR as claimed in claim 1 or claim 2, wherein the alpha chain variable domain has at least one of the following CDR mutations with reference to the numbering of SEQ ID NO: 2:

Residue no.	
M50	L
T51	D
F52	Y
S53	A
E54	I
H94	S, R
S95	A
G96	N, D, S, Q
S98	L

(Table 1)

and/or the beta chain variable domain has at least one of the following CDR mutations with reference to the numbering of SEQ ID NO: 3:

Residue no.	
M27	A, L
D28	P
H29	L
E30	S

Residue no.	
N31	K
Y50	R
D51	F
V52	A
K53	T
M54	G, L
F95	S
L96	D
M97	Q
T98	N

(Table 2)

4. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain has 1, 2, 3, 4, 5, 6, 7, 8 or 9 of the mutations shown in Table 1 and/or the beta chain variable domain has 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 of the mutations shown in Table 2
5. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain has at least one of the following groups of mutations
- Group 1: M50L, T51D, F52Y, S53A, E54I
- Group 2: H94S, S95A, G96N, S98L
- Group 3: H94R S95A, G96D, S98L
- Group 4: M50L, T51D, F52Y, S53A, E54I, H94R S95A, G96D, S98L
- Group 5: M50L, H94S, S95A, G96S, S98L
- Group 6: M50L, H94S, S95A, G96Q, S98L
- and/or the beta chain variable domain has at least one of the following groups of mutations
- Group 1: M27A, D28P, H29L, E30S, N31K F95S, L96D, M97Q, T98N
- Group 2: Y50R, D51F, V52A, K53T, M54G, F95S, L96D, M97Q, T98N
- Group 3: F95S, L96D, M97Q, T98N
- Group 4: M27L, Y50R, D51F, V52A, K53T, M54G, F95S, L96D, M97Q, T98N
- Group 5: M27A, D28P, H29L, E30S, N31K, M54L, F95S, L96D, M97Q, T98N
6. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain and beta chain variable domain have the following groups of mutations, respectively:

Alpha Chain		Beta Chain	
Group 4	M50L, T51D, F52Y, S53A, E54I, H94R S95A, G96D, S98L	Group 1	M27A, D28P, H29L, E30S, N31K F95S, L96D, M97Q, T98N
Group 4	M50L, T51D, F52Y, S53A, E54I, H94R S95A, G96D, S98L	Group 5	M27A, D28P, H29L, E30S, N31K, M54L, F95S, L96D, M97Q, T98N
Group 2	H94S, S95A, G96N, S98L	Group 2	Y50R, D51F, V52A, K53T, M54G, F95S, L96D, M97Q, T98N
Group 6	M50L, H94S, S95A, G96Q, S98L	Group 4	M27L, Y50R, D51F, V52A, K53T, M54G, F95S, L96D, M97Q, T98N
Group 5	M50L, H94S, S95A, G96S, S98L	Group 4	M27L, Y50R, D51F, V52A, K53T, M54G, F95S, L96D, M97Q, T98N

- 5 7. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain has at least one of the following mutations with reference to the numbering of SEQ ID NO: 2:

Residue no.	
C19	V
K1	A

(Table 3)

- 10 8. A TCR as claimed in any preceding claim, wherein in the alpha chain variable domain the sequence of amino acid residues 27 - 32, 50 - 56 and 91 - 103 is selected from the following:

Residues 27 – 32 (CDR1)	Residues 50 – 56 (CDR2)	Residues 91 – 103 (CDR3)
VSPFSN	MTFSENT	VVNHSGGSYIPTF
VSPFSN	MTFSENT	VVNSANGLYIPTF
VSPFSN	MTFSENT	VVNRADGLYIPTF
VSPFSN	LDYAIN	VVNHSGGSYIPTF
VSPFSN	LDYAIN	VVNRADGLYIPTF
VSPFSN	LTFSENT	VVNSASGLYIPTF
VSPFSN	LTFSENT	VVNSAQGLYIPTF

9. A TCR as claimed in any one of claims 1-4 and 7, wherein the alpha chain variable domain comprises an amino acid sequence that has at least 90% identity, such as 90%, 91%, 92%, 93%,
5 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to SEQ ID NOs: 16-24 or 46 – 64.

10. A TCR as claimed in any preceding claim, wherein in the beta chain variable domain the sequence of amino acid residues 27 - 31, 49 - 54 and 92 - 107 is selected from the following:

Residues 27 – 31 (CDR1)	Residues 49 – 54 (CDR2)	Residues 92 – 107 (CDR3)
MDHEN	SYDVKM	ASSFLMTSGDPYEQYF
MDHEN	SRFATG	ASSSDQNSGDPYEQYF
MDHEN	SYDVKM	ASSSDQNSGDPYEQYF
LDHEN	SRFATG	ASSSDQNSGDPYEQYF
APLSK	SYDVKM	ASSSDQNSGDPYEQYF
APLSK	SYDVKL	ASSSDQNSGDPYEQYF

(Table 5)

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11. A TCR as claimed in anyone of claims 1-4, 7 and 9, wherein the beta chain variable domain comprises an amino acid sequence that has at least 90% identity, such as 90%, 91%, 92%, 93%,
94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to SEQ ID NOs: 25-29 or 65 - 81.

- 15 12. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain sequence of amino acid residues 27-32, 50-56 and 91-103 and the beta chain variable domain sequence of amino acid residues 27-31, 49-54 and 92-107 is selected from the following:

Alpha chain			Beta chain		
27-32	50-56	91.103	27-31	49-54	92-107
VSPFSN	LDYAIN	VVNRADGLYIPTF	APLSK	SYDVKM	ASSSDQNSGDPYEQYF
VSPFSN	LDYAIN	VVNRADGLYIPTF	APLSK	SYDVKL	ASSSDQNSGDPYEQYF
VSPFSN	MTFSENT	VVNSANGLYIPTF	MDHEN	SRFATG	ASSSDQNSGDPYEQYF
VSPFSN	LTFSENT	VVNSAQGLYIPTF	LDHEN	SRFATG	ASSSDQNSGDPYEQYF
VSPFSN	LTFSENT	VVNSASGLYIPTF	LDHEN	SRFATG	ASSSDQNSGDPYEQYF

(Table 6)

- 20 13. A TCR as claimed in any preceding claim, wherein the alpha chain variable domain comprises any one of the amino acid sequences of SEQ ID NOs: 16 to 24 or 46 to 64 and the beta

chain variable domain comprises any one of the amino acid sequences of SEQ ID NOs: 25 to 29 or 65 to 81.

14. A TCR claimed in any preceding claim wherein the alpha chain variable domain and the
5 beta chain variable domain are selected from the amino acid sequences of:

Alpha chain variable domain	Beta chain variable domain
SEQ ID NO: 22	SEQ ID NO: 26
SEQ ID NO: 22	SEQ ID NO: 27
SEQ ID NO: 20	SEQ ID NO: 28
SEQ ID NO: 24	SEQ ID NO: 29
SEQ ID NO: 23	SEQ ID NO: 29

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

16. A TCR as claimed in claim 15, 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.

17. A TCR as claimed in claim 15 or claim 16, 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.

18. 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.

19. A TCR as claimed in any preceding claim associated with a detectable label, a therapeutic
agent or a PK modifying moiety.

20. A TCR as claimed in claim 19, wherein an anti-CD3 antibody is covalently linked to the C-
30 or N-terminus of the alpha or beta chain of the TCR.

21. A TCR as claimed in claim 20, 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.
- 5 22. A TCR as claimed in claim 21, 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 GGEGGGSEGGGS (SEQ ID NO: 37).
- 10 23. 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.
- 15 24. A TCR-anti-CD3 fusion molecule as claimed in claim 23, comprising
an alpha chain amino acid sequence selected from SEQ ID NOs: 38-41, or an alpha chain amino acid sequence that has at least 90% identity, such as 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to the amino acid sequences as set forth in SEQ ID NOs:
20 38 to 41,
and a beta chain amino acid sequence selected from SEQ ID NOs: 42-45 sequence or a beta chain amino acid sequence that has at least 90% identity, such as 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity, to the amino acid sequences as set forth in SEQ ID No: 42 to 45.
- 25 25. A TCR-anti CD3 fusion molecule as claimed in claim 24, 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
30 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
35 (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

26. A nucleic acid encoding a TCR alpha chain and/or a TCR beta chain as claimed in any one of the preceding claims.
27. An expression vector comprising the nucleic acid of claim 26.
- 5 28. A cell harbouring
- (a) an expression vector as claimed in claim 27 encoding TCR alpha and beta chains as claimed in any one of claims 1 to 25, in a single open reading frame, or two distinct open reading frames; or
- 10 (b) a first expression vector which comprises nucleic acid encoding the alpha chain of a TCR as claimed in any one of claims 1 to 25, and a second expression vector which comprises nucleic acid encoding the beta chain of a TCR as claimed in any one of claims 1 to 25.
29. A non-naturally occurring and/or purified and/or engineered cell, especially a T-cell,
- 15 presenting a TCR as claimed in any one of claims 1 to 22.
30. A pharmaceutical composition comprising a TCR as claimed in any one of claims 1-22, a TCR-anti CD3 fusion molecule as claimed in any one of claims 23-25, a nucleic acid as claimed in claim 26, and/or a cell as claimed in claim 28 or 29, together with one or more pharmaceutically acceptable carriers or excipients.
- 20 31. The TCR of any one of claims 1 to 22, TCR-anti-CD3 fusion molecule of any one of claims 23-25, nucleic acid of claim 26, pharmaceutical composition of claim 30 and/or cell of claim 28 or 29, for use in medicine, preferably in a human subject.
- 25 32. The TCR of any one of claims 1 to 22, TCR-anti-CD3 fusion molecule of any one of claims 23-25, nucleic acid of claim 26, pharmaceutical composition of claim 30 and/or cell of claim 28 or 29, for use in a method of treating cancer or a tumour, preferably in a human subject.
- 30 33. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to claim 32, wherein the human subject has a tumour that expresses MAGE A4.
34. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to claims 32 or 33, wherein the tumour is a solid tumour.
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35. The TCR, TCR anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to any one of claims 32 to 34, wherein the human subject is of HLA-A*02 subtype.
- 5 36. The TCR, TCR-anti-CD3 fusion molecule, nucleic acid, pharmaceutical composition and/or cell for use according to any one of claims 32 to 35, which is administered by injection, such as intravenous or direct intratumoral injection
- 10 37. A method of treating a human subject in need thereof comprising administering to said subject a pharmaceutically effective dose of a pharmaceutical composition according to claim 30.
38. A method according to claim 37, which further comprises administering separately, in combination or sequentially an anti-neoplastic agent.
- 15 39. An injectable formulation for administering to a human subject comprising a TCR according to any one of claims 1 to 22 or a TCR-anti-CD3 fusion molecule of any one of claims 23-25.
- 20 40. A method of producing a TCR according to any one of claims 1 to 22, or a TCR-anti-CD3 fusion molecule according to any one of claims 23-25, comprising a) maintaining a cell according to claim 28 under optimal conditions for expression of the TCR chains acid and b) isolating the TCR chains.