

The α and β chains of $\alpha\beta$ TCR's are generally regarded as each having two "domains", namely variable and constant domains. The variable domain consists of a concatenation of variable region and joining region. In the present specification and claims, the term "TCR alpha variable domain" therefore refers to the concatenation of TRAV and TRAJ regions, and the term TCR alpha constant domain refers to the extracellular TRAC region, or to a C-terminal truncated TRAC sequence. Likewise the term "TCR beta variable domain" refers to the concatenation of TRBV and TRBD/TRBJ regions, and the term TCR beta constant domain refers to the extracellular TRBC region, or to a C-terminal truncated TRBC sequence.

The unique sequences defined by the IMGT nomenclature are widely known and accessible to those working in the TCR field. For example, they can be found in the IMGT public database. The "T cell Receptor Factsbook", (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8 also discloses sequences defined by the IMGT nomenclature, but because of its publication date and consequent time-lag, the information therein sometimes needs to be confirmed by reference to the IMGT database.

One TCR in accordance with the invention comprises an alpha chain extracellular domain as shown in SEQ ID No: 3 (**TRAV10 + TRAC**) and a beta chain extracellular domain as shown in SEQ ID No: 4 (**TRBV24-1 + TRBC-2**). The terms "parental TCR", "parental MAGE-A4 TCR", are used synonymously herein to refer to this TCR comprising the extracellular alpha and beta chain of SEQ ID Nos: 3 and 4 respectively. It is desirable to provide TCRs that are mutated or modified relative to the parental TCR that have a higher affinity and/or a slower off-rate for the peptide-HLA complex than the parental TCR.

For the purpose of providing a reference TCR against which the binding profile of such mutated or modified TCRs may be compared, it is convenient to use a soluble TCR in accordance with the invention having the extracellular sequence of the parental MAGE-A4 TCR alpha chain given in SEQ ID No. 3 and the extracellular sequence of the parental MAGE-A4 TCR beta chain given in SEQ ID No: 4. That TCR is referred to herein as the "the reference TCR" or "the reference MAGE-A4 TCR". Note that SEQ ID No: 5 comprises the parental alpha chain extracellular sequence of SEQ ID No: 3 and that C162 has been substituted for T162 (i.e. T48 of TRAC). Likewise SEQ ID No: 6 is the parental beta chain extracellular sequence of SEQ ID No: 4 and that C169 has been substituted for S169 (i.e. S57 of TRBC2), A187 has been substituted for C187 and D201 has been substituted for N201. These cysteine substitutions relative to the parental alpha and beta chain extracellular sequences enable the formation of an interchain disulfide bond which stabilises the refolded soluble TCR, i.e. the TCR formed by refolding extracellular alpha and beta chains. Use of the stable disulfide linked soluble TCR as the reference TCR enables more convenient assessment of binding affinity and binding half life. TCRs of the invention may comprise the mutations described above.

CLAIMS

1. A T cell receptor (TCR) having the property of binding to GGYDGREHTV (SEQ ID No: 1) in complex with HLA-A*0201 with a dissociation constant of from about 0.05 μ M to about 20.0 μ M when measured with surface plasmon resonance at 25°C and at a pH between 7.1 and 7.5 using a soluble form of the TCR, and has at least a ten-fold selectivity of binding to SEQ ID No:1 in complex with HLA-A*0201 over binding to GGYDGEEHSV (SEQ ID No: 2) in complex with HLA-A*0201 in which the TCR comprises a TCR alpha chain variable domain and a TCR beta chain variable domain, and in which the TCR variable domains form contacts with at least residues V2, Y3 and D4 of GGYDGREHTV (SEQ ID No: 1).
2. A TCR according to Claim 1, which is an alpha-beta heterodimer, having an alpha chain TRAV10 + TRAC constant domain sequence and a beta chain TRBV24-1 + TRBC-2 constant domain sequence.
3. A TCR according to Claim 1, which is in single chain format of the type V α -L-V β , V β -L-V α , V α -C α -L-V β , or V α -L-V β -C β , in which V α and V β 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.
4. A TCR according to Claim 1, 2 or 3, which is associated with a detectable label, a therapeutic agent or a PK modifying moiety.
5. A TCR according to any preceding claim, in which the alpha chain variable domain comprises an amino acid sequence that has at least 80% identity to the sequence of amino acid residues 1-105 of SEQ ID No: 3 and has the following CDR mutation:

M4	V or L
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with reference to the numbering of the CDRs shown in SEQ ID No: 3, and/or the beta chain variable domain comprises an amino acid sequence that has at least 80% identity to the sequence of amino acid residues 1-105 of SEQ ID No: 4 and has at least one of the following CDR mutations:

N10	E
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with reference to the numbering of the CDRs shown in SEQ ID No: 4.

6. A TCR according to any preceding claim, in which the alpha chain variable domain is the alpha chain variable domain sequence set forth in SEQ ID No. 3 and the beta chain variable domain is the beta chain variable domain sequence set forth in SEQ ID No. 9; or the alpha chain variable domain is the alpha chain variable domain sequence set forth in SEQ ID No. 7 and the beta chain variable domain is the beta chain variable domain sequence set forth in SEQ ID No. 9; or the alpha chain variable domain is the alpha chain variable domain sequence set forth in SEQ ID No. 8 and the beta chain variable domain is the beta chain variable domain sequence set forth in SEQ ID No: 9.
7. A TCR according to any preceding claim, in which the alpha chain variable domain comprises the amino acid sequence of amino acid residues 1-105 of SEQ ID No: 3 or 5 or 7 to 8 or an amino acid sequence in which amino acid residues 1-27, 34-47, and 54-90 thereof have at least 90% or 95% identity to the sequence of amino acid residues 1-27, 34-47, and 54-90 respectively of SEQ ID No: 3 or 5 or 7 to 8 and in which amino acid residues 28-34, 48-53 and 91- 105 have at least 90% or 95% identity to the sequence of amino acid residues 28-33, 48-53 and 91-105 respectively of SEQ ID No: 3 or 5 or 7 to 8.
8. A TCR according to any preceding claim, in which the alpha chain variable domain comprises the amino acid sequence of amino acid residues 1-105 of SEQ ID No: 7 or 8 or an amino acid sequence in which amino acid residues 1-27, 34-47 and 55-89 thereof have at least 90% or 95% identity to the sequence of amino acid residues 1-27, 34-47, and 55-89 respectively of SEQ ID No: 7 or 8 and in which amino acid residues 28-33, 48-53 and 91-105 have at least 90% or 95% identity to the sequence of amino acid residues 28-33, 48-53 and 91-105 respectively of SEQ ID No: 7 or 8.
9. A TCR according to any preceding claim, in which in the alpha chain variable domain the sequence of:
- (i) amino acid residues 1-27 thereof has:
- (a) at least 90% identity to the sequence of amino acid residues 1-26 of SEQ ID No: 3; or
- (b) has one, two or three amino acid residues inserted or deleted relative to the sequence of (a);
- (ii) amino acid residues 28-33 is VSPFSN;

(iii) amino acid residues 34-47 thereof has (a) at least 90% identity to the sequence of amino acid residues 34-47 of SEQ ID NO: 3 or (b) has one, two or three amino acid residues inserted or deleted relative to the sequence of (a);

(iv) amino acid residues 48-53 is LTIMTF or LTRMTF or LTIVTF or LTILTF

5 (v) amino acid residues 54-90 thereof has at least 90% identity to the sequence of amino acid residues 55-89 of SEQ ID No: 3 or has one, two or three insertions, deletions or substitutions relative thereto; and

(vi) amino acids 91-105 is CVVSGGTDSWGKLQF

10. A TCR according to any preceding claim, in which the beta chain variable domain
10 comprises the amino acid sequence of SEQ ID No: 4 or 6 or 9 or an amino acid sequence in which amino acid residues 1-45, 51-67, and 74-109 thereof have at least 90% or 95% identity to the sequence of amino acid residues 1-45, 51-67, and 74-109 respectively of SEQ ID No: 4 or 6 or 9 and in which amino acid residues 46-50, 68-73 and 109-123 have at least 90% or
15 95% identity to the sequence of amino acid residues 46-50, 68-73 and 109-123 respectively of SEQ ID No: 4 or 6 or 9.

11. A TCR according to any preceding claim, in which in the beta chain variable domain the sequence of:

(i) amino acid residues 1-45 thereof has:

20 (a) at least 90% identity to the amino acid sequence of residues 1-26 of SEQ ID No: 4; or

(b) has one, two or three amino acid residues inserted or deleted relative to the sequence of (a);

(ii) amino acid residues 46-50 is KGHDR;

25 (iii) amino acid residues 51-67 thereof has (a) at least 90% identity to the sequence of amino acid residues 51-67 of SEQ ID NO: 4 or (b) has one, two or three amino acid residues inserted or deleted relative to the sequence of (a);

(iv) amino acid residues 68-73 is SFDVK;

(v) amino acid residues 54-90 thereof has (a) at least 90% identity to the sequence of amino acid residues 54-90 of SEQ ID NO: 4 or (b) has one, two or three amino acid residues inserted or deleted relative to the sequence of (a); and

(vi) amino acids 109-123 is CATSGQGAYNEQFF or CATSGQGAYREQFF

5 12. A nucleic acid encoding a TCR according to any preceding claim.

13. An isolated or non-naturally occurring cell, especially a T-cell, presenting a TCR according to any preceding claim.

14. A cell harbouring:

10 (a) a TCR expression vector which comprises nucleic acid as claimed in claim 13 in a single open reading frame, or two distinct open reading frames encoding the alpha chain and the beta chain respectively; or

15 (b) a first expression vector which comprises nucleic acid encoding the alpha chain of a TCR as claimed in any of claims 1 to 12, and a second expression vector which comprises nucleic acid encoding the beta chain of a TCR as claimed in any of claims 1 to 12.

15. A pharmaceutical composition comprising a TCR according to any of Claims 1 to 11, a nucleic acid according to Claim 12 or a cell according to Claim 13 or 14, together with one or more pharmaceutically acceptable carriers or excipients.

20 16. A TCR according to any of Claims 1 to 13, a nucleic acid according to Claim 14 or a cell according to Claim 15 or 16 for use in medicine.

17. A TCR, nucleic acid or cell for use according to Claim 16, for use in a method of treating cancer.