

T CELL RECEPTOR

FIELD OF THE INVENTION

5 The present invention relates to a T cell receptor (TCR). In particular, the present invention relates to an engineered TCR which has a high level of cell surface expression when expressed as an exogenous TCR compared to the corresponding germline TCR sequence. The present invention also relates to methods for increasing the cell surface expression of a TCR.

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BACKGROUND TO THE INVENTION

T cell receptor (TCR) gene therapy is the transfer of antigen-specific TCR chains into recipient T-cells, thus redirecting the specificity of T-lymphocytes to target antigens of
15 interest.

Introduced TCRs differ greatly in their ability to be expressed on the cell surface. Introduced, strongly expressed TCRs are co-expressed with the endogenous TCR or can even out-compete the endogenous TCR for cell surface expression. Introduced,
20 weakly expressed TCRs are absent from the cell surface when co-expressed with an endogenous strong TCR (Figure 1).

High levels of expression of an antigen specific TCR on the T cell surface results in a greater avidity of the T cell, which is beneficial for the efficacy of therapeutic TCR
25 therapy.

There is thus a need for methods and approaches which increase the cell surface expression of weakly expressed TCR.

30 SUMMARY

The present inventors have determined a number of key amino acid positions and residues within the TCR framework regions that enhance TCR cell surface expression.

35 Thus in an embodiment the present invention provides an engineered TCR comprising at least one of the following amino acid residues:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
5 L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;
S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;
R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;
V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain;
10 F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α
chain;

wherein the at least one amino acid residue is not present in the corresponding
germline TCR amino acid sequence.

15 Disclosed herein is an engineered T cell receptor (TCR) comprising at least one of
the following amino acid residues, numbered according to the International
ImMunoGeneTics information system (IMGT):

L96 of the α chain; R9 of the β chain; T24 of the α chain; T20 of the α chain;
20 M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain;
F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain;
L21 of the β chain; L103 of the β chain; T3 of the α chain; P9 of the α chain;
A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain;
D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain;
25 Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain;
T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain;
N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain;
S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein the at least one amino acid residue is not present in the corresponding
30 germline TCR amino acid sequence, preferably wherein the at least one amino acid
residue is selected from:

L96 of the α chain;
R9 of the β chain;
T24 of the α chain;
35 T20 of the α chain;
M50 of the α chain;
T5 of the α chain;

Q8 of the α chain;
S86 of the α chain;
F39 of the α chain;
D55 of the α chain; or
5 R43 of the β chain.

Disclosed herein is an engineered cell surface expressed T cell receptor (TCR) comprising at least one of the following amino acid residues, numbered according to the IMGT system:

10 L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
15 M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;
S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;
R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;
V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain;
F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
20 K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α
chain;

wherein the cell is human, and the at least one amino acid residue is not present in the corresponding germline TCR amino acid sequence, preferably wherein the at least one amino acid residue is selected from:

25 L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
30 T20 of the α chain;
M50 of the α chain;
T5 of the α chain;
Q8 of the α chain;
S86 of the α chain;
35 F39 of the α chain;
D55 of the α chain; or
R43 of the β chain.

The at least one amino acid residue may be selected from a list of:

- 5 L96 of the α chain;
- R9 of the β chain;
- Y10 of the β chain;
- T24 of the α chain;
- V19 of the α chain;
- T20 of the α chain;
- M50 of the α chain;
- 10 T5 of the α chain;
- Q8 of the α chain;
- S86 of the α chain;
- F39 of the α chain;
- D55 of the α chain; and
- 15 R43 of the β chain.

The engineered TCR may comprise L96 of the α chain.

The engineered TCR may comprise R9 of the β chain.

The engineered TCR may comprise Y10 of the β chain.

The engineered TCR may comprise T24 of the α chain.

The engineered TCR may comprise a plurality of amino acid residues as defined herein.

The engineered TCR may comprise L96 of the α chain; R9 of the β chain and Y10 of the β chain.

The engineered TCR may comprise each of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;

R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

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In one embodiment the present invention provides a nucleic acid sequence encoding a TCR α chain and/or a β chain as defined herein.

Disclosed herein is a nucleic acid sequence encoding a TCR α chain and/or a β chain, wherein the α chain and/or a β chain is altered to comprise one or more amino acid residues as defined herein.

The nucleic acid sequence may comprise a nucleic acid sequence encoding an α chain and a nucleic acid sequence encoding a β chain linked by an internal self-cleaving sequence or an internal ribosome entry site.

Disclosed herein is a vector comprising a nucleic acid sequence as defined herein.

The vector may be a retrovirus vector, lentivirus vector or a transposon.

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Disclosed herein is a cell comprising a nucleic acid sequence or a vector as defined herein.

Disclosed herein is a cell, isolated from a human subject and transduced with a nucleic acid sequence or vector encoding an engineered TCR comprising at least one of the following residues, numbered according to the IMGT system:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The cell may be a T-cell, natural killer cell or a stem cell.

5 The cell may be derived from a T-cell, natural killer cell or stem cell isolated from a subject.

In one embodiment the present invention provides a pharmaceutical composition comprising a cell which expresses a TCR comprising at least one of the following amino acid residues:

10 L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
15 M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
20 K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

Disclosed herein is a pharmaceutical composition comprising a vector as defined herein or a cell which expresses a TCR as defined herein.

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The cell may express a TCR comprising a plurality of amino acid residues as defined herein.

The cell may express an engineered TCR as defined herein.

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Disclosed herein is a pharmaceutical composition as defined herein for use in treating and/or preventing a disease.

Disclosed herein is a vector as defined herein or a cell as defined herein for use in the
35 manufacture of a medicament for treating and/or preventing a disease.

In an embodiment the present invention relates to method for treating a disease which comprises the step of administering a cell as defined herein to a subject.

Disclosed herein is a method for producing a cell as defined herein which comprises
5 the step of transducing a cell *in vitro* or *ex vivo* with a vector as defined herein.

Disclosed herein is the use of an engineered TCR comprising at least one of the following amino acid residues, numbered according to the IMGT system:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of
10 the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α
chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain;
A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of
the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α
chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain;
15 F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of
the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α
chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain;
A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and
M101 of the α chain;
20 wherein the at least one amino acid residue is not present in the corresponding
germline TCR amino acid sequence or an engineered TCR according to any of claims
1 to 7; a nucleic acid sequence encoding the α chain and/or a β chain of said
engineered TCR or a vector comprising said nucleic acid sequence encoding the α
chain and/or a β chain of said engineered TCR to increase the cell surface
25 expression of the TCR.

In an embodiment the present invention provides a method for increasing the cell surface expression of a TCR which comprises the steps of:

(i) providing a TCR α chain and/or β chain sequence;
30 (ii) determining an amino acid residue of the TCR α chain and/or β chain
sequence at one or more positions selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
35 R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;

S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain; and

(iii) altering the amino acid residue at one or more positions listed in step

(ii) to an amino acid residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The method may comprise the step of altering a TCR amino acid sequence such that it comprises a plurality of amino acid residues as defined herein.

The TCR amino acid sequence may be altered by mutagenesis of a nucleic acid sequence encoding the TCR.

In an embodiment the present invention relates to a method for selecting a high expression TCR which comprises the steps of:

(i) providing a group of TCR amino acid sequences; and

(ii) selecting a TCR amino acid sequence comprising at least one amino acid residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;

M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

the method may comprise the step of selecting a TCR amino acid sequence comprising a plurality of amino acid residues as defined herein.

In an embodiment the present invention provides a method for determining the strength of a TCR which comprises the steps of:

- (i) providing a TCR α chain and/or β chain sequence; and
- (ii) determining if the TCR sequence comprises at least one amino acid

residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein a sequence which comprises at least one of the recited amino acid residues is determined to be a high expression TCR and a sequence which does not comprise at least one of the recited amino acid sequences is determined to be a low expression TCR.

Disclosed herein is a method for determining the strength of a TCR which comprises the steps of:

- (i) providing a TCR α chain and/or β chain sequence; and
- (ii) determining if the TCR sequence comprises at least one amino acid

residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein the amino acid residues are numbered according to the IMGT system, further wherein a sequence which comprises at least one of the recited amino acid residues is determined to be a high expression TCR and a sequence which does not comprise at least one of the recited amino acid sequences is determined to be a low expression TCR.

In an embodiment the present invention provides a computer program product in which a computer program is stored in a non-transient fashion, which when executed on a processing device causes the processing device to carry out the method as defined herein.

In an embodiment the present invention provides the use of an engineered TCR, a nucleic acid sequence or a vector of the present invention to increase the cell surface expression of the TCR.

It has been demonstrated that substitution with key amino acid residues in a weakly expressed TCR results in a greatly improved expression. It has also been demonstrated that single amino acid substitutions of individual key residues to replace 'weak' residues with 'strong' amino acid residues as defined by the present invention, as well as a range of amino-acid substitution combinations, increased TCR expression.

The changing of an amino acid can be achieved economically, quickly and efficiently by mutagenesis, for example PCR mutagenesis. This method of increasing TCR cell surface expression does not interfere with peptide / MHC recognition by the TCR and

does not affect the TCR folding or structure. Thus TCR expression is increased with a minimal impact on TCR structure.

DESCRIPTION OF THE FIGURES

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Figure 1 – A) Depiction of a weak exogenous TCR and a strong exogenous TCR in a cell which also expresses an endogenous TCR. B) Schematic Drawing of the TCR α and TCR β chains – SP: Signal peptide; FR: Framework Region; CDR: Complementarity Determining Region; IG: Immunoglobulin like domain of the C-region; CP: Connecting Peptide; TM: Transmembrane domain; IC: Intracellular domain

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Figure 2 – Primary T cells transduced with a modified WT1 TCR that shows TCR dominance

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Figure 3 – TCR clonotyping of strong and weak endogenous TCR

Displayed in the table is (i) the total number of strong and weak alpha and beta variable segments that were sequenced and (ii) the alpha variable segments and beta variable segments that show dominance in either the strong or weak endogenous TCR population, alongside the total number of each of the variable segments within each population. The alpha and beta segments have been classified using the IMGT nomenclature.

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Figure 4 – Illustration of constructs used to assess the characteristics of generic strong and weak expression TCRs

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Figure 5 – Expression of strong and weak TCRs in a non-competitive environment (Jurkat cells without TCR)

Figure 6 – Expression of strong and weak TCRs in a competitive environment (Jurkat cells expressing the WT1 hybrid TCR)

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Figure 7 – Expression of strong and weak TCRs in Jurkat cells expressing low, medium and high expression of the modified WT1 TCR

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Figure 8 – Bioinformatics analyses of strong and weak expression TCRs.

A) The sequencing information from the clonotyping was used to compare all the residues at each position for all the strong and weak TCRs that had been sequenced. After TCR alignment, residues that had a high occurrence in particular positions in the strong TCR, compared to the weak TCR are indicated (*) in the figure using TRAV38-1 as an example.

B) Analysis of the predicted 3D structure of strong and weak TCR variable segments

Figure 9 – Mutation of all identified key residues to reverse strong and weak expression TCRs in both a non-competitive environment (Jurkat cells not expressing TCR) and a competitive environment (Jurkat cells expressing the WT1 hybrid TCR)

Figure 10 - Alteration of individual key residues impacts the expression level of an exogenous TCR in a non-competitive environment (Jurkat cells not expressing TCR)

Figure 11 – Alteration of individual key residues impacts the expression level of an exogenous TCR in a competitive environment (Jurkat cells expressing the WT1 hybrid TCR)

Figure 12 - A general purpose computing device which is used to provide or support some embodiments

Figure 13 - Jurkat cells without TCR (A) and Jurkat cells expressing the modified WT1 TCR (B) where transduced with the pMP71 retrovirus encoding the indicated TCRs. 72h after transduction, cells were stained with anti-CD19, V5 Tag, Myc Tag and murine constant beta TCR antibodies. Cells were gated on high CD19 expression and the V5 Tag, Myc Tag and murine constant beta TCR expression was determined. The MFI of V5 Tag, Myc Tag and murine constant beta TCR expression were also determined for the CD19+ high expressing cells. (C) MFI of V5 Tag and Myc Tag for Jurkat cells without TCR. (D) MFI of V5 Tag, Myc Tag and murine constant beta TCR for Jurkat cells expressing the modified WT1 TCR

Figure 14 – Change of variable domain residues to L96 α , R9 β and Y10 β improves CMV and WT1 TCR expression in Jurkat cells

Figure 15 – Change of variable domain residues to L96 α , R9 β and Y10 β improves antigen specific cytokine production

Figure 16 – Change of variable domain residues to L96 α , R9 β and Y10 β improves antigen specific cytokine production

Figure 17 – Cells transduced with well expressed and poorly expressed TCRs contain similar amounts of TCR mRNA

Figure 18 – Contribution of TCR alpha vs TCR beta to TCR expression

DETAILED DESCRIPTION

T CELL RECEPTOR

During antigen processing, antigens are degraded inside cells and then carried to the cell surface by major histocompatibility complex (MHC) molecules. T cells are able to recognise this peptide:MHC complex at the surface of the antigen presenting cell. There are two different classes of MHC molecules, MHC I and MHC II, that deliver peptides from different cellular compartments to the cell surface.

The T cell receptor or TCR is the molecule found on the surface of T cells that is responsible for recognizing antigens bound to MHC molecules. The TCR heterodimer consists of an α and β chain in 95% of T cells, whereas 5% of T cells have TCRs consisting of γ and δ chains.

Engagement of the TCR with antigen and MHC results in activation of its lymphocyte through a series of biochemical events mediated by associated enzymes, co-receptors, and specialized accessory molecules.

Each chain of the TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin (Ig)-variable (V) domain, one Ig-constant (C) domain, a transmembrane/cell membrane-spanning region, and a short cytoplasmic tail at the C-terminal end (Figure 1B).

The variable domain of both the TCR α -chain and β -chain have three hypervariable or complementarity determining regions (CDRs). CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the beta chain interacts with the C-terminal part of the peptide. CDR2 is thought to

recognize the MHC molecule. Framework regions (FRs) are positioned between the CDRs. These regions provide the structure of the TCR variable region.

The repertoire of TCR variable regions is generated by combinatorial joining of variable (V), joining (J) and diversity (D) genes; and by N region diversification (nucleotides inserted by the enzyme deoxynucleotidyl-transferase).

α and γ chains are formed from recombination events between the V and J segments. β and δ chains are formed from recombination events involving the V, D and J segments.

The human TCR α locus, which also includes the TCR δ locus, is located on chromosome 14 (14q11.2). The TCR β locus is located on chromosome 7 (7q34). The variable region of the TCR α chain is formed by recombination between one of 46 different V α (variable) segments and one of 58 J α (joining) segments (Koop et al.; 1994; Genomics; 19: 478-493). The variable region of a TCR β chain is formed from recombination between 54 V β , 14 J β and 2 D β (diversity) segments (Rowen et al.; 1996; Science; 272:1755-1762).

The V and J (and D as appropriate) gene segments for each TCR chain locus have been identified and the germline sequence of each gene is known and annotated (for example see Scaviner & Lefranc; 2000; Exp Clin Immunogenet; 17:83-96 and Folch & Lefranc; 2000; Exp Clin Immunogenet; 17:42-54).

FR1, CDR1, FR2, CDR2, FR3 and CDR3 of the α chain are encoded by the V α gene. FR4 is encoded by the J α gene (Figure 1B).

FR1, CRD1, FR2, CDR2 and FR3 of the β chain are encoded by the V β gene. CDR3 is encoded by the D β gene and FR4 is encoded by the J β gene (Figure 1B).

As the germline sequence of each variable gene is known in the art (see Scaviner & Lefranc; as above and Folch & Lefranc; as above) the V α and/or V β of a particular TCR can be sequenced and the germline V segment which is utilised in the TCR can be identified (see, for example, Hodges *et al.*; 2003; J Clin Pathol; 56:1-11, Zhou *et al.*; 2006; Laboratory Investigation; 86; 314-321)

The constant domain of the TCR domain consists of short connecting sequences in which a cysteine residue forms a disulfide bond, making a link between the two

chains. The TCR of the present invention may have an additional cysteine residue in each of the α and β chains such that the TCR comprises two disulphide bonds in the constant domains (see below).

5 The structure allows the TCR to associate with other molecules like CD3 which possess three distinct chains (γ , δ , and ϵ) in mammals and the ζ -chain. These accessory molecules have negatively charged transmembrane regions and are vital to propagating the signal from the TCR into the cell. The CD3- and ζ -chains, together with the TCR, form what is known as the T cell receptor complex.

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The signal from the T cell complex is enhanced by simultaneous binding of the MHC molecules by a specific co-receptor. On helper T cells, this co-receptor is CD4 (specific for class II MHC); whereas on cytotoxic T cells, this co-receptor is CD8 (specific for class I MHC). The co-receptor not only ensures the specificity of the
15 TCR for an antigen, but also allows prolonged engagement between the antigen presenting cell and the T cell and recruits essential molecules (e.g., LCK) inside the cell involved in the signaling of the activated T lymphocyte.

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The term "T-cell receptor" is thus used in the conventional sense to mean a molecule capable of recognising a peptide when presented by an MHC molecule. The molecule may be a heterodimer of two chains α and β (or optionally γ and δ) or it may be a single chain TCR construct.

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The present invention also provides the α chain or β chain from such a T cell receptor.

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The TCR of the present invention may be a hybrid TCR comprising sequences derived from more than one species. For example, it has surprisingly been found that murine TCRs have been found to be more efficiently expressed in human T cells than human TCRs. The TCR may therefore comprise human variable regions and murine constant regions. A disadvantage of this approach is that the murine constant sequences may trigger an immune response, leading to rejection of the transferred T cells. However, the conditioning regimens used to prepare patients for adoptive T-cell therapy may result in sufficient immunosuppression to allow the engraftment of T
35 cells expressing murine sequences.

The engineered TCR according to the first aspect of the present invention comprises at least one amino acid residue as defined herein which is not encoded by the germline V α or V β gene. In other words, the engineered TCR of the present invention comprises an α chain and/or β chain which comprises an altered amino acid residue at one or more of the positions described herein, wherein the altered amino acid residue amino acid is a residue as defined herein, compared to the corresponding α chain and/or β chain as encoded by the unaltered germline V α or V β gene.

The amino acid residues identified herein are numbered according to the International ImMunoGeneTics information system' (IMGT). This system is well known in the art (Lefrance et al.; 2003; Dev Comp Immunol; 27: 55-77) and is based on the high conservation of the structure of the variable region. The numbering takes into account and combines the definition of the FR and CDRs, structural data from X-ray diffraction studies and the characterization of the hypervariable loops.

The delimitations of the FR and CDR regions are defined within the IGMT numbering system. The FR1 region comprises positions 1–26 (25–26 amino acids, depending on the V-GENE group or subgroup) with 1st-CYS at position 23. The FR2 region comprises positions 39–55 (16–17 amino acids) with a conserved TRP at position 41. The FR3 region comprises positions 66–104 (36–39 amino acids, depending on the VGENE group or subgroup) with a conserved hydrophobic amino acid at position 89 and the 2nd-CYS at position 104. Residue 1 of the IGMT numbering system is the first residue in FR1. Residue 104 of the IGMT numbering system is the last residue in FR3.

As such, the numbering system used herein refers to the position of the amino acid within the entire α chain or the entire β chain, as appropriate.

The IGMT numbering therefore allows a standardized description of amino acid positions within TCR variable regions and comparisons with the germline encoded sequences to be performed.

Methods suitable for generating an engineered TCR according to the first aspect of the present invention are known in the art.

For example mutagenesis may be performed to alter specific nucleotides in a nucleic acid sequence encoding the TCR. Such mutagenesis will alter the amino acid

sequence of the TCR so that it comprises at least one of the amino acid residues according to the present invention.

An example of a mutagenesis method is the Quikchange method (Papworth *et al.*; 1996; Strategies; 9(3); 3-4). This method involves the use of a pair of complementary mutagenic primers to amplify a template nucleic acid sequence in a thermocycling reaction using a high-fidelity non-strand-displacing DNA polymerase, such as *pfu* polymerase.

The present inventors have determined that the presence of particular amino acid residues in specific positions of the TCR framework regions alters the cell surface expression of the TCR.

As used herein, cell surface expression is synonymous with expression strength. As such, 'strong' or 'high' expression is equivalent to high levels of cell surface expression of the TCR. 'Weak' or 'low' expression is equivalent to low levels of cell surface expression of the TCR.

Increasing the cell surface expression of a TCR means that a TCR comprising at least one amino acid residue according to the present invention has a higher level of cell surface expression relative to an equivalent TCR comprising the amino acid sequence encoded by the germline sequence. An equivalent TCR comprising the amino acid sequence encoded by the germline sequence refers to a TCR which has not been altered to comprise an amino acid residue according to the present invention – i.e. the unaltered TCR has the wild-type amino acid residue at the specific position.

In one embodiment the present engineered TCR may have a cell surface expression which is at least 1.5-, 2-, 2.5-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater than the corresponding TCR comprising the unmodified germline TCR sequence.

Cell surface expression of a TCR may be determined by methods which are known in the art. For example, the cell surface expression of a TCR may be determined using conventional flow cytometry methods known in the art (see, for example, Shapiro; Practical Flow Cytometry; John 2005; Science).

For example, the cell surface expression of a TCR may be expressed as the mean fluorescent intensity (MFI) of TCR expression in a population of cells (see Shapiro; as above). In one embodiment the MFI of a population of cells expressing the present engineered TCR may be at least 1.5-, 2-, 2.5-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, or 10-fold greater than the MFI of an corresponding population of cells expressing the corresponding TCR comprising the unmodified germline TCR sequence.

The cell surface expression of a TCR may be expressed as the percentage of cells within a population which express the TCR at the cell surface. Such a percentage may be determined using conventional flow cytometry methods as is well known in the art (see, for example, Shapiro; as above and Henel *et al.*; 2007; Lab Medicine; 38; 7; 428-436).

In one embodiment, the present engineered TCR may be expressed by at least 2, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60 or at least 70% more cells in a population compared to the corresponding TCR comprising the unmodified germline TCR sequence.

In one embodiment, the cell surface expression of the engineered TCR is increased compared to the corresponding TCR comprising the unmodified germline TCR sequence but the relative level of the mRNA encoding the engineered TCR or the unmodified germline TCR are essentially the same. As used herein, "essentially the same" may mean that mRNA levels differ by, for example, less than 1.5 fold. Relative mRNA levels may be determined using methods which are known in the art; for example RT-qPCR, northern blotting and flow cytometry RNA assays (for example as demonstrated in Figure 17).

Thus in a first aspect the present invention provides an engineered TCR comprising at least one of the following amino acid residues:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;

V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

- 5 wherein the at least one amino acid residue is not present in the corresponding germline TCR amino acid sequence.

The at least one amino acid residue may be selected from a list of:

- 10 L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
T20 of the α chain;
15 M50 of the α chain;
T5 of the α chain;
Q8 of the α chain;
S86 of the α chain;
F39 of the α chain;
20 D55 of the α chain; and
R43 of the β chain.

In one embodiment, the at least one amino acid residue may be selected from a list of:

- 25 L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
30 T20 of the α chain;
M50 of the α chain;
T5 of the α chain;
Q8 of the α chain; and
S86 of the α chain.

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The engineered TCR may comprise L96 of the α chain. In one embodiment the engineered TCR may comprise L96 of the α chain and at least one of R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise L96 of the α chain; R9 of the β chain and Y10 of the β chain.

The engineered TCR may comprise V19 of the α chain; R9 of the β chain and Y10 of the β chain.

The engineered TCR may comprise R9 of the β chain. In one embodiment the engineered TCR may comprise R9 of the β chain and at least one of L96 of the α chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain

The engineered TCR may comprise Y10 of the β chain. In one embodiment the engineered TCR may comprise Y10 of the β chain and at least one of L96 of the α chain; R9 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β

chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise T24 of the α chain. In one embodiment the engineered TCR may comprise T24 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise V19 of the α chain. In one embodiment the engineered TCR may comprise V19 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise T20 of the α chain. In one embodiment the engineered TCR may comprise T20 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain;

M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise M50 of the α chain. In one embodiment the engineered TCR may comprise M50 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise T5 of the α chain. In one embodiment the engineered TCR may comprise T5 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise Q8 of the α chain. In one embodiment the engineered TCR may comprise Q8 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise S86 of the α chain. In one embodiment the engineered TCR may comprise S86 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise F39 of the α chain. In one embodiment the engineered TCR may comprise F39 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α

chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise D55 of the α chain. In one embodiment the engineered TCR may comprise D55 of the α chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The engineered TCR may comprise R43 of the β chain. In one embodiment the engineered TCR may comprise R43 of the β chain and at least one of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The term "at least one" as used herein may include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty or more amino acid residues as described herein.

The engineered TCR may comprise a plurality of the amino acid residues recited above. In other words, the TCR may comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15 or at least 20 of the amino acid residues recited above.

The engineered TCR may comprise R9 and Y10 in the β chain.

In one embodiment, the engineered TCR may comprise each of L96 of the α chain;
5 R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of
the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α
chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain;
V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of
the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α
10 chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain;
S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of
the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α
chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

15 In one embodiment, the engineered TCR may comprise each of L96 of the α chain;
R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of
the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α
chain; F39 of the α chain; D55 of the α chain; and R43 of the β chain.

20 In one embodiment, the engineered TCR may comprise each of L96 of the α chain;
R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of
the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; and S86 of the α
chain.

25 CONSERVATIVE SUBSTITUTION

The present invention also encompasses an engineered TCR comprising a
conservative substitution of an amino acid residue selected from:

30 L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
35 M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;
S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;
R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;

V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

5

The at least one amino acid residue may be selected from:

L96 of the α chain;

R9 of the β chain;

Y10 of the β chain;

10

T24 of the α chain;

V19 of the α chain;

T20 of the α chain;

M50 of the α chain;

T5 of the α chain;

15

Q8 of the α chain;

S86 of the α chain;

F39 of the α chain;

D55 of the α chain; or

R43 of the β chain.

20

The at least one amino acid residue may be any amino acid residue or plurality of amino acid residues as described herein.

Conservative amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as high expression of the TCR is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

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Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

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ALIPHATIC	Non-polar	G A P
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		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
AROMATIC		K R
		H F W Y

The present invention also encompasses homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc.

NUCLEIC ACID SEQUENCE

The present invention further provides a nucleotide sequence encoding an engineered TCR receptor according to the first aspect of the invention or a part thereof, for example the variable sequence of the α chain or the β chain; the α chain and/or the β chain.

As used herein, the terms “polynucleotide”, “nucleotide”, and “nucleic acid” are intended to be synonymous with each other.

It will be understood by a skilled person that numerous different polynucleotides and nucleic acids can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described here to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed.

Nucleic acids according to the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the use as described herein, it is to be understood that the polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or life span of polynucleotides of interest.

The nucleotide sequence may be double or single stranded, and may be RNA or DNA.

- 5 The nucleotide sequence may be codon optimised. Different cells differ in their usage of particular codons. This codon bias corresponds to a bias in the relative abundance of particular tRNAs in the cell type. By altering the codons in the sequence so that they are tailored to match with the relative abundance of corresponding tRNAs, it is possible to increase expression.

10

Many viruses, including HIV and other lentiviruses, use a large number of rare codons and by changing these to correspond to commonly used mammalian codons, increased expression of the packaging components in mammalian producer cells can be achieved. Codon usage tables are known in the art for mammalian cells, as well as for a variety of other organisms.

15

Codon optimisation may also involve the removal of mRNA instability motifs and cryptic splice sites.

- 20 The nucleic acid sequence may comprise a nucleic acid sequence which enables both the nucleic acid sequence encoding an α chain and a nucleic acid sequence a β chain to be expressed from the same mRNA transcript.

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For example, the nucleic acid sequence may comprise an internal ribosome entry site (IRES) between the nucleic acid sequences which encode the α chain and the β chain. An IRES is a nucleotide sequence that allows for translation initiation in the middle of a mRNA sequence.

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The nucleic acid sequence may comprise a nucleic acid sequence encoding an α chain and a nucleic acid sequence a β chain linked by an internal self-cleaving sequence.

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The internal self-cleaving sequence may be any sequence which enables the polypeptide comprising the α chain and the polypeptide comprising the β chain to become separated.

The cleavage site may be self-cleaving, such that when the polypeptide is produced, it is immediately cleaved into individual peptides without the need for any external cleavage activity.

5 The term "cleavage" is used herein for convenience, but the cleavage site may cause the peptides to separate into individual entities by a mechanism other than classical cleavage. For example, for the Foot-and-Mouth disease virus (FMDV) 2A self-cleaving peptide (see below), various models have been proposed for to account for the "cleavage" activity: proteolysis by a host-cell proteinase, autoproteolysis or a
10 translational effect (Donnelly et al (2001) J. Gen. Virol. 82:1027-1041). The exact mechanism of such "cleavage" is not important for the purposes of the present invention, as long as the cleavage site, when positioned between nucleic acid sequences which encode proteins, causes the proteins to be expressed as separate entities.

15 The self-cleaving peptide may be a 2A self-cleaving peptide from an aphtho- or a cardiovirus.

VECTOR

20 The present invention also provides a vector comprising a nucleotide sequence as described herein.

The term "vector" includes an expression vector i.e. a construct capable of *in vivo* or
25 *in vitro/ex vivo* expression.

Viral delivery systems include but are not limited to adenovirus vector, an adeno-associated viral (AAV) vector, a herpes viral vector, retroviral vector, lentiviral vector, baculoviral vector.

30 Retroviruses are RNA viruses with a life cycle different to that of lytic viruses. In this regard, a retrovirus is an infectious entity that replicates through a DNA intermediate. When a retrovirus infects a cell, its genome is converted to a DNA form by a reverse transcriptase enzyme. The DNA copy serves as a template for the production of new
35 RNA genomes and virally encoded proteins necessary for the assembly of infectious viral particles.

There are many retroviruses, for example murine leukemia virus (MLV), human immunodeficiency virus (HIV), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), Rous sarcoma virus (RSV), Fujinami sarcoma virus (FuSV), Moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), Moloney murine sarcoma virus (Mo-MSV), Abelson murine leukemia virus (A-MLV), Avian myelocytomatosis virus-29 (MC29), and Avian erythroblastosis virus (AEV) and all other retroviridae including lentiviruses.

A detailed list of retroviruses may be found in Coffin *et al* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763).

Lentiviruses also belong to the retrovirus family, but they can infect both dividing and non-dividing cells (Lewis et al (1992) EMBO J. 3053-3058).

The vector may be capable of transferring a nucleotide according to the second aspect of the invention to a cell, such as a T-cell. The vector should ideally be capable of sustained high-level expression in T cells, so that the introduced TCR may compete successfully with the endogenous TCR for a limited pool of CD3 molecules.

The vector may be a retroviral vector. The vector may be based on or derivable from the MP71 vector backbone. The vector may lack a full-length or truncated version of the Woodchuck Hepatitis Response Element (WPRE).

For efficient infection of human cells, viral particles may be packaged with amphotropic envelopes or gibbon ape leukemia virus envelopes.

Increasing the supply of CD3 molecules may increase TCR expression in gene modified cells. The vector may therefore also comprise the genes for CD3-gamma, CD3-delta, CD3-epsilon and/or CD3-zeta. The vector may just comprise the gene for CD3-zeta. The genes may be linked by self-cleaving sequences, such as the 2A self-cleaving sequence. Alternatively one or more separate vectors may be provided encoding CD3 gene for co-transfer with the TCR-encoding vector(s).

CELL

The present invention further relates to a cell which comprises a nucleotide sequence according to the present invention. The cell may express a T-cell receptor of the first aspect of the invention.

- 5 The cell may be a T-cell. The T-cell may be any T-cell subset, including for example, $\alpha\beta$ T-cell, $\gamma\delta$ T-cell or regulatory T cells.

The cell may be a natural killer cell.

- 10 The cell may be derived from a cell isolated from a subject. The cell may be part of a mixed cell population isolated from the subject, such as a population of peripheral blood lymphocytes (PBL).

- T cells within the PBL population may be activated by methods known in the art, such
15 as using anti-CD3 and CD28 antibodies.

- The T-cell may be a CD4⁺ helper T cell or a CD8⁺ cytotoxic T cell. The cell may be in a mixed population of CD4⁺ helper T cell/CD8⁺ cytotoxic T cells. Polyclonal activation, for example using anti-CD3 antibodies optionally in combination with anti-
20 CD28 antibodies will trigger the proliferation of CD4⁺ and CD8⁺ T cells, but may also trigger the proliferation of CD4⁺25⁺ regulatory T-cells.

In one embodiment the T cell is a CD8⁺ cytotoxic T cell.

- 25 In one embodiment a cell which expresses an engineered TCR according to the present invention may have increased functional activity compared to a cell which expresses the corresponding TCR comprising the unmodified germline TCR sequence.

- 30 Increased functional activity may refer, for example, to increased cytokine production by the cell following binding of antigen to the TCR. The cytokine may be selected from IFN γ , IL-2, GM-CSF, TNF α , IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13.

The cytokine may be IFN γ and/or IL-2.

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As used herein "increased cytokine production" means that – upon binding of antigen to the TCR - the amount of cytokine produced by a cell which expresses an

engineered TCR according to the present invention in greater than the amount produced by an equivalent cell which expresses the corresponding TCR comprising the unmodified germline TCR sequence.

- 5 The amount of cytokine produced by a cell which expresses an engineered TCR according to the present invention may be at least 1.5-, 2-, 2.5-, 3-, 4-, 5-, 10-, 20- or 50-fold greater than that produced by an equivalent cell which expresses the corresponding TCR comprising the unmodified germline TCR sequence.
- 10 The amount of cytokine produced by a cell may be determined using methods which are known in the art – for example flow cytometry or ELISA.

The present invention also provides a method of producing a cell according to the invention which comprises the step of transfecting or transducing a cell *in vitro* or *ex vivo* with a vector according to the invention.

15

The cell may be isolated from the subject to which the genetically modified cell is to be adoptively transferred. In this respect, the cell may be made by isolating a T-cell from a subject, optionally activating the T-cell, TCR gene transfer *ex vivo* and subsequent immunotherapy of the subject by adoptive transfer of the TCR-transduced cells.

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Alternatively the cell may be isolated from a different subject, such that it is allogeneic. The cell may be isolated from a donor subject. For example, if the subject is undergoing allogeneic haematopoietic stem cell transplantation (Allo-HSCT), the cell may be derived from the donor, from which the HSCs are derived. If the subject is undergoing or has undergone solid organ transplant, the cell may be derived from the subject from whom the solid organ was derived.

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Alternatively the cell may be, or be derived from, a stem cell, such as a haemopoietic stem cell (HSC). Gene transfer into HSCs does not lead to TCR expression at the cell surface as stem cells do not express the CD3 molecules. However, when stem cells differentiate into lymphoid precursors that migrate to the thymus, the initiation of CD3 expression leads to the surface expression of the introduced TCR in thymocytes.

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35 An advantage of this approach is that the mature T cells, once produced, express only the introduced TCR and little or no endogenous TCR chains, because the

expression of the introduced TCR chains suppresses rearrangement of endogenous TCR gene segments to form functional TCR alpha and beta genes.

5 A further benefit is that the gene-modified stem cells are a continuous source of mature T-cells with the desired antigen specificity. The cell may therefore be a gene-modified stem cell, which, upon differentiation, produces a T-cell expressing a TCR of the first aspect of the invention. The present invention also provides a method of producing a T-cell expressing a TCR of the first aspect of the invention by inducing the differentiation of a stem cell which comprises a nucleotide sequence according to
10 the invention.

A disadvantage of the stem cell approach is that TCRs with the desired specificity may get deleted during T-cell development in the thymus or may induce tolerance when expressed in peripheral T-cells. Another possible issue is the risk of insertional
15 mutagenesis in stem cells.

PHARMACEUTICAL COMPOSITION

The present invention further provides a pharmaceutical composition comprising a
20 vector or a cell according to the present invention.

The pharmaceutical composition may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical composition may optionally comprise one or more further pharmaceutically active polypeptides and/or
25 compounds. Such a formulation may, for example, be in a form suitable for intravenous infusion.

USE

30 The present invention further relates to a pharmaceutical composition comprising a cell according to the present invention for use in treating and/or preventing a disease.

'Treating' relates to the therapeutic use of the cells of the present invention. Herein the cells may be administered to a subject having an existing disease or condition in
35 order to lessen, reduce or improve at least one symptom associated with the disease and/or to slow down, reduce or block the progression of the disease.

'Preventing' relates to the prophylactic use of the cells of the present invention. Herein such cells may be administered to a subject who has not yet contracted the disease and/or who is not showing any symptoms of the disease to prevent or impair the cause of the disease or to reduce or prevent development of at least one symptom associated with the disease. The subject may have a predisposition for, or
5 be thought to be at risk of developing, the disease.

The present invention also provides the use of an engineered TCR; a nucleic acid sequence or a vector according to the present invention to increase the cell surface
10 expression of the TCR.

The present invention also provides the use of an engineered TCR; a nucleic acid sequence or a vector according to the present invention to increase the functional activity of a cell. The functional activity may be any functional activity as described
15 herein.

METHOD OF TREATMENT

The present invention provides a method for treating and/or preventing a disease which comprises the step of administering the cells of the present invention (for
20 example in a pharmaceutical composition as described above) to a subject.

The method may involve the steps of:

- (i) isolated a cell-containing sample;
- 25 (ii) transducing or transfecting such cells with a nucleic acid sequence or vector provided by the present invention;
- (iii) administering the cells from (ii) to a subject.

The present invention also provides a cell of the present invention for use in treating
30 and/or preventing a disease.

In a preferred embodiment of the present invention, the subject of any of the methods described herein is a mammal, preferably a cat, dog, horse, donkey, sheep, pig, goat, cow, mouse, rat, rabbit or guinea pig, but most preferably the subject is a human.
35

For administration of cells as described herein, any mode of administration of the cell population which is common or standard in the art may be used, e.g. injection or

infusion, by an appropriate route. In one aspect up to 5×10^8 cells are administered per kg in humans. Thus, for example, a human with a body weight of 100 kg may receive a dose of up to 5×10^{10} cells per treatment. The dose can be repeated at later times if necessary.

5

The invention also relates to the use of a vector or a cell according to the present invention in the manufacture of a medicament for treating and/or preventing a disease.

10 DISEASE

The disease to be treated and/or prevented by the methods and uses of the present invention may be any disease which induces a T cell mediated immune response.

15 For example, the disease to be treated and/or prevented by the methods and uses of the present invention may be cancerous disease, such as bladder cancer, breast cancer, colon cancer, endometrial cancer, kidney cancer (renal cell), leukaemia, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer and thyroid cancer.

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The disease to be treated and/or prevented by the methods and uses of the present invention may be an infection, for example a bacterial or viral infection.

METHOD

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The present invention further provides a method for increasing the cell surface expression of a TCR which comprises the steps of:

- (i) providing a TCR α chain and/or β chain sequence;
 - (ii) determining an amino acid residue of the TCR α chain and/or β chain
- 30 at one or more positions selected from:

30

96 of the α chain; 9 of the β chain; 10 of the β chain; 24 of the α chain; 19 of the α chain; 20 of the α chain; 50 of the α chain; 5 of the α chain; 8 of the α chain; 86 of the α chain; 39 of the α chain; 55 of the α chain; 43 of the β chain; 66 of the α chain; 19 of the β chain; 21 of the β chain; 103 of the β chain; 3 of the α chain; 7 of the α chain; 9 of the α chain; 11 of the α chain; 16 of the α chain; 18 of the α chain; 21 of the α chain; 22 of the α chain; 26 of the α chain; 40 of the α chain; 47 of the α chain; 48 of the α chain; 49 of the α chain; 51 of

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the α chain; 52 of the α chain; 53 of the α chain; 67 of the α chain; 68 of the α chain; 74 of the α chain; 76 of the α chain; 79 of the α chain; 81 of the α chain; 83 of the α chain; 90 of the α chain; 92 of the α chain; 93 of the α chain; and 101 of the α chain; and

- 5 (iii) altering the amino acid residue at one or more of the positions listed in step (ii) to an amino acid residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; 10 R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; 15 V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

- 20 As described herein, the germline sequence of each variable gene is known in the art (see Scaviner & Lefranc; as above and Folch & Lefranc; as above) and the $V\alpha$ and/or $V\beta$ germline V segment utilised in a TCR can therefore be determined by sequencing and comparing to the known germline sequences (see, for example, Hodges et al.; as above, Zhou et al.; 2006; as above). The present step of determining an amino acid residue of the TCR α chain and/or β chain at one or more positions therefore 25 specifically relates to determining the amino acid residue that is present at one or more of the particular positions described herein.

- The method may comprise determining the amino acid residue present 30 at any position or plurality of positions as described herein.

The amino acid sequence of the TCR may be altered such that it comprises any amino acid residue or any plurality of amino acid residues as described herein.

- 35 In one embodiment the invention provides a method for increasing the cell surface expression of a TCR which comprises the step of altering a TCR amino acid sequence such that it comprises at least one residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
 V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
 Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
 R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
 5 L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
 M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;
 S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;
 R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;
 V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain;
 10 F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
 K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α
 chain.

The at least one amino acid residue may be selected from:

15 L96 of the α chain;
 R9 of the β chain;
 Y10 of the β chain;
 T24 of the α chain;
 V19 of the α chain;
 20 T20 of the α chain;
 M50 of the α chain;
 T5 of the α chain;
 Q8 of the α chain;
 S86 of the α chain;
 25 F39 of the α chain;
 D55 of the α chain; or
 R43 of the β chain.

30 The amino acid sequence of the TCR may be altered such that it comprises a plurality
 of amino acid residues as defined above.

The amino acid sequence of a TCR may be altered using methods which are well
 known in the art. For example, the amino acid sequence of the TCR may be altered
 mutagenesis of a nucleic acid sequence encoding the TCR.

35 Increasing the cell surface expression of a TCR means that a TCR comprising at
 least one amino acid residue according to the present invention has a higher level of

cell surface expression relative to an equivalent TCR comprising the amino acid sequence encoded by the germline sequence. An equivalent TCR comprising the amino acid sequence encoded by the germline sequence refers to a TCR which has not been altered to comprise an amino acid residue according to the present invention – i.e. the unaltered TCR has the wild-type amino acid residue at the specific position.

Cell surface expression of a TCR may be determined using any of the methods described herein.

The present invention further relates a method for selecting a high expression TCR which comprises the steps of:

- (i) providing a group of TCR amino acid sequences; and
- (ii) selecting a TCR amino acid sequence comprising at least one amino

acid residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

The at least one amino acid residue may be selected from:

L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
T20 of the α chain;
M50 of the α chain;
T5 of the α chain;

Q8 of the α chain;
S86 of the α chain;
F39 of the α chain;
D55 of the α chain; or
R43 of the β chain.

The method may comprise the step of selecting a TCR which comprises any amino acid residue or any plurality of amino acid residues as defined above.

In a further aspect the present invention provides a method for determining the strength of a TCR which comprises the steps of:

- (i) providing a TCR α chain and/or β chain sequence; and
- (ii) determining if the TCR sequence comprises at least one amino acid residue selected from:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain;
V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain;
Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain;
R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain;
L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain;
M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain;
S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain;
R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain;
V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain;
F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain;
K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein a sequence which comprises at least one of the recited amino acid residues is determined to be a high expression TCR and a sequence which does not comprise at least one of the recited amino acid sequences is determined to be a low expression TCR.

The at least one amino acid residue may be selected from:

L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
T20 of the α chain;

M50 of the α chain;
T5 of the α chain;
Q8 of the α chain;
S86 of the α chain;
5 F39 of the α chain;
D55 of the α chain; or
R43 of the β chain.

10 The α chain and/or β chain sequence may be an amino acid sequence or a nucleic acid sequence which encodes an α chain and/or a β chain.

The method may comprise determining the amino acid residue present at any position or plurality of positions as described herein.

15 Such a method is useful for predicting the level of surface expression of a TCR, for example a therapeutic TCR, when it is expressed as an exogenous TCR in a cell, for example a T cell.

COMPUTER PROGRAMME PRODUCT

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Figure 12 schematically illustrates a general purpose computing device 100 of the type that may be used to implement the above described techniques. In the context of the present invention this could for example be an embedded processor forming part of a larger system. The general purpose computing device 100 includes a central processing unit 102, a random access memory 104 and a read only memory 106, connected together via bus 122. It also further comprises a network interface card 108, a hard disk drive 110, a display driver 112 and monitor 114 and a user input/output circuit 116 with a keyboard 118 and mouse 120 all connected via the common bus 122. In operation, such as when forming part of a larger system the central processing unit 102 will execute computer program instructions that may for example be stored in the random access memory 104 and/or the read only memory 106. Program instructions could be additionally retrieved from the hard disk drive 110 or dynamically downloaded via the network interface card 108. The results of the processing performed may be displayed to a user or an engineer via a connected display driver 112 and monitor 114. User inputs for controlling the operation of the general purpose computing device 100 may be received via a connected user input output circuit 116 from the keyboard 118 or the mouse 120. It will be appreciated that

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the computer program could be written in a variety of different computer languages. The computer program may be stored locally on a recording medium or dynamically downloaded to the general purpose computing device 100. When operating under control of an appropriate computer program, the general purpose computing device 100 can perform the above described techniques and can be considered to form an apparatus for performing any of the above described techniques. The architecture of the general purpose computing device 100 could vary considerably and Figure 13 is only one example. The general purpose computing device 100 can also have a configuration which allows it to provide an instruction execution environment (i.e. a virtual machine).

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1 – Determination of TCR variable segments which correlate with strong or weak TCR expression

Following T cell transduction with TCRs, the introduced TCRs differ greatly in their ability to be expressed on the cell surface relatively to endogenous TCRs. Strongly expressed exogenous TCRs are co-expressed with the endogenous TCR or can even out-compete the endogenous TCR for cell surface expression. Weakly expressed exogenous TCRs are absent from the cell surface or are poorly expressed when co-expressed with a strong TCR (Figure 1A).

Activated primary T cells were transduced with a pMP71 vector encoding WT1 (Wilms tumour 1) TCR, containing a murinised constant domain and an additional cysteine in the constant domain (the whole TCR was codon optimised).

This modified WT1 TCR shows TCR dominance (Figure 2). 72 hours after transduction the T cells were stained with anti-CD3 and CD8 antibodies and anti-human TCR constant domain and anti-murine TCR constant domain antibodies. T cells were gated on the CD3+ and CD8+ T cell population. CD8+ T cells expressing strong endogenous TCRs are co-stained with the anti-murine and anti-human TCR

constant domain antibodies. CD8⁺ T cells expressing weak endogenous TCRs are stained only with the anti-murine TCR constant domain antibodies.

The CD8⁺ T cell populations expressing the strong and weak endogenous TCRs were FACs sorted and clonotyped to determine their variable alpha and beta chain usage. Displayed in the table shown in Figure 3 is (i) the total number of strong and weak alpha and beta variable segments that were sequenced and (ii) the alpha variable segments and beta variable segments that show dominance in either the strong or weak endogenous TCR population, alongside the total number of each of the variable segments within each population. The alpha and beta segments have been classified using the IMGT nomenclature.

Example 2 – Expression of generic strong and weak TCRs in non-competitive and competitive environments

The clonotyping data was used to determine the alpha and beta variable segments most prevalently used in the strong and weak TCR populations. Generic strong and weak TCR were engineered using this information.

The strong TCR is variable alpha 38.2 and variable beta 7.8. The weak TCR is variable alpha 13.2 and variable beta 7.3. As monoclonal antibodies are not commercial available to the above alpha and beta variable segments, in order to determine TCR expression in transduced T cells a V5 Tag is present at the start of the alpha chain and a Myc Tag is present at the start of the beta chain. In addition, in order to be able to normalize transduction efficiency a truncated murine CD19 is present at the end of the constructs (Figure 4).

The two constructs contained the same alpha and beta constant domains and the whole construct was non-codon optimized. Furthermore, unique restrictions sites border the alpha and beta segments in order to allow easy swapping of the alpha and beta segments. The TCRs are cloned into the pMP71 retrovirus for transduction experiments.

Expression of strong and weak TCRs in a non-competitive environment

Jurkat cells that do not contain endogenous TCR alpha or beta chains were transduced with pMP71 vectors encoding the generic strong or weak TCRs. 72 hours

after transduction, the Jurkat cells were stained with anti-CD19, V5 Tag and Myc Tag antibodies (Figure 5). Jurkat cells were gated on high CD19 expression and the V5 Tag and Myc Tag expression on these CD19+ high expressing cells was determined. In addition, the MFI of V5 Tag and Myc Tag were also determined for the CD19+ high
5 expressing cells.

Expression of strong and weak TCRs in a competitive environment

Jurkat cells expressing the modified WT1 TCR were transduced with pMP71 vectors encoding the generic strong or weak TCRs. 72 hours after transduction, Jurkat cells
10 were stained with anti-CD19, V5 Tag and Myc Tag antibodies (Figure 6). Jurkat cells were gated on high CD19 expression and the V5 Tag and Myc Tag expression was determined. The MFI of V5 Tag and Myc Tag were also determined for the CD19+ high expressing cells.

Expression of strong and weak TCRs in Jurkat cells expressing low, medium and high expression of the modified WT1 TCR

Jurkat cells expressing the modified WT1 TCR were transduced with pMP71 vectors encoding the generic strong or weak TCRs. 72 hours after transduction, Jurkat cells
20 were stained with anti-CD19, murine TCR constant domain, V5 Tag and Myc Tag antibodies (Figure 7). Jurkat cells were first gated on high CD19 expression and then on high, medium and low expression of murine TCR constant domain. For all three separate WT1 TCR populations and the WT1 expressing cells as a whole, the V5 Tag and Myc Tag expression was determined. The MFI of V5 Tag and Myc Tag were
25 also determined.

Example 3 – Bioinformatics analysis to identify key features correlating to strength of TCR expression

The sequencing information from the clonotyping was used to compare all the residues at each position for all the strong and weak TCRs that had been sequenced. After TCR alignment, residues that had a high occurrence in particular positions in the strong TCR, compared to the weak TCR are indicated (*) in the figure using TRAV38-1 as an example (Figure 8A).
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The residues which showed prevalence in the strong TCR was further analysed and refined to only include residues in the framework regions that have implications in
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stabilising the structure and pairing of the TCR chains. These residues are hi-lighted (*) using TRAV38-2 and TRBV7-8 as the backbone (Figure 8B). The top line is the residues in the strong TCR. The bottom line is the equivalent residues in the weak TCR.

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Example 4 – Engineering of strong expression and weak expression TCRs

To engineer the strong expression TCR with weak residues, the strong TCR was used as a backbone and all strong residues that were predicated to be important in TCR strength were replaced with the weak TCR residues in the relevant position.

To engineer the weak TCR with strong residues, the weak TCR was used as a backbone and all strong residues that were predicated to be important in TCR strength replaced with weak TCR residues in the relevant position.

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Jurkat cells without TCR (top row), and Jurkat cell expressing the WT1 murinised TCR (bottom row), were transduced with the stated TCR and 72 hours later stained with anti-CD19, V5 Tag and Myc Tag antibodies. Jurkat cells were gated on high CD19 expression and the V5 Tag and Myc Tag expression was determined. The MFI of V5 Tag and Myc Tag were also determined for the CD19+ high expressing cells (Figure 9).

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Example 5 – Effect of mutating individual residues on TCR expression

The individual effect of strong residues on TCR expression

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The generic weak TCR was used as the backbone and the quick change PCR mutagenesis technique was used to make a series of TCRs which had only one of the predicted strong residues replacing the weak TCR residue at the relevant position whilst retaining the rest of the weak TCR backbone.

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The mutated TCRs are labelled so that the letter represents the amino-acid the number is the position in the TCR framework and the α or β indicates whether the residue is in the alpha chain or the beta chain. The last plot, bottom row, right side has 3 residue changes, V19 on the alpha chain and R9, Y10 on the beta chain (Figures 10 and 11).

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Jurkat cells without TCR (Figure 10) and Jurkat cells expressing the modified WT1 TCR (Figure 11), where transduced with the pMP71 retrovirus encoding the indicated TCR. 72 hours after transduction, transduced cells were stained with anti-CD19, V5 Tag and Myc Tag antibodies. Cells were gated on high CD19 expression and the V5 Tag and Myc Tag expression was determined.

Figure 13 shows the results of experiments where Jurkat cells without TCR (A) and Jurkat cells expressing the modified WT1 TCR (B) where transduced with the pMP71 retrovirus encoding the indicated TCRs. 72 hours after transduction, the cells were stained with anti-CD19, V5 Tag, Myc Tag and murine constant beta TCR antibodies. Cells were gated on high CD19 expression and the V5 Tag, Myc Tag and murine constant beta TCR expression was determined. The MFI of V5 Tag, Myc Tag and murine constant beta TCR expression were also determined for the CD19+ high expressing cells. (C) MFI of V5 Tag and Myc Tag for Jurkat cells without TCR. (D) MFI of V5 Tag, Myc Tag and murine constant beta TCR for Jurkat cells expressing the modified WT1 TCR

The results show that F39 of the α chain, D55 of the α chain and R43 of the β chain increases the surface expression of the TCR. Furthermore, the mutation of the above three residues was also shown to decrease the amount of WT1 TCR expressed on the cell surface in Jurkat cell competition experiments.

Example 6 – Effect of mutating variable domain residues to L96 α , R9 β and Y10 β in antigen-specific TCRs

Cell surface expression in Jurkat cells

Jurkat cells (which do not contain endogenous TCR alpha or beta chains), were transduced with pMP71 vectors encoding either unmodified wild-type CMV or WT1 TCRs, or the same TCRs that were mutated to contain L96 α , R9 β and Y10 β . 72h after transduction, Jurkat cells were stained with anti-CD19 Abs and either CMV tetramer or V β 2.1 (to determine WT1 variable beta expression) Abs.

Cells were first gated on high CD19 expression and then CMV tetramer and or V β 2.1 expression was determined (Figure 14).

Cell surface expression in primary T cells

Activated, primary T cells were transduced with pMP71 vectors encoding either unmodified wild-type CMV or WT1 TCRs, or the same TCRs that were mutated to

contain L96 α , R9 β and Y10 β . 72 h after transduction, the T cells were stained with anti-CD3, CD8 and CD19 Abs and either CMV tetramer or V β 2.1 Abs. Cells were gated on CD3+ and CD19 high T cells, and then TCR expression was determined using tetramers or anti-v β 2.1 antibodies to detect the CMV- and WT1-TCR, respectively (Figure 15).

Activated, primary T cells were transduced with pMP71 vectors encoding either unmodified wild-type CMV or the same TCR mutated to contain L96 α , R9 β and Y10 β . 5 days after transduction, T cells were co-cultured with APCs expression the pCMV peptide or the irrelevant peptide pWT235. After 18h co-culture, the supernatant was collected and IL-2 and IFN- γ production was determined by ELISA (Figure 16).

Cells transduced with well expressed and poorly expressed TCRs contain similar amounts of TCR mRNA

Jurkat cells, without endogenous TCRs, were transduced with pMP71 vectors encoding either the generic strong TCR, or the generic weak TCR, or the generic strong TCR containing the predicted weak residues, or the generic weak TCR containing the predicted strong residues. The affymetrix primeflow RNA assay was used to determine the percentage of CD19+ high cells expressing the constant alpha mRNA and the constant beta mRNA. The MFI of the constant alpha and beta mRNA was determined for the four populations. In addition, the percentage of CD19+ high Jurkat cells expressing V5 Tag was also determined (Figure 17).

Example 7 – Contribution of TCR α vs TCR β to TCR cell surface expression

Jurkat cells, without endogenous TCRs, were transduced with pMP71 vectors encoding either the strong TCR, or the weak TCR, or a TCR engineered to contain the strong alpha variable chain and the weak beta variable chain, or a TCR engineered to contain the weak alpha variable chain and the strong beta variable chain. 72h after transduction, Jurkat cells were stained with anti-CD19, V5 Tag and Myc Tag antibodies. Jurkat cells were gated on high CD19 and the V5 Tag and Myc Tag expression was determined. The MFI of the V5 Tag and Myc Tag were also determined in the CD19+ high cells (Figure 18).

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the

invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology, cellular immunology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. An engineered T cell receptor (TCR) comprising at least one of the following amino acid residues, numbered according to the International ImMunoGeneTics information system (IMGT):

L96 of the α chain; R9 of the β chain; T24 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; P9 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein the at least one amino acid residue is not present in the corresponding germline TCR amino acid sequence, preferably wherein the at least one amino acid residue is selected from:

L96 of the α chain;
R9 of the β chain;
T24 of the α chain;
T20 of the α chain;
M50 of the α chain;
T5 of the α chain;
Q8 of the α chain;
S86 of the α chain;
F39 of the α chain;
D55 of the α chain; or
R43 of the β chain.

2. An engineered cell surface expressed T cell receptor (TCR) comprising at least one of the following amino acid residues, numbered according to the IMGT system:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α

chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein the cell is human, and the at least one amino acid residue is not present in the corresponding germline TCR amino acid sequence, preferably wherein the at least one amino acid residue is selected from:

L96 of the α chain;
R9 of the β chain;
Y10 of the β chain;
T24 of the α chain;
V19 of the α chain;
T20 of the α chain;
M50 of the α chain;
T5 of the α chain;
Q8 of the α chain;
S86 of the α chain;
F39 of the α chain;
D55 of the α chain; or
R43 of the β chain.

3. An engineered TCR according to any preceding claim which comprises:

(i) L96 of the α chain; and/or(ii) R9 of the β chain.

4. An engineered TCR according to claim 1 or claim 2 which comprises T24 of the α chain.

5. An engineered TCR according to any of claims 1 to 4 which comprises a plurality of amino acid residues as defined in claim 1 or claim 2.

6. An engineered TCR according to claim 5:

(i) further comprising V19 of the α chain; V19 of the β chain; S7 of the α chain; or M11 the α chain;

(ii) which comprises L96 of the α chain; R9 of the β chain and Y10 of the β chain; or(iii) which comprises V19 of the α chain; R9 of the β chain and Y10 of the β chain.

7. An engineered TCR according to any preceding claim which comprises:

(i) each of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; and R43 of the β chain;

(ii) each of L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; and S86 of the α chain; and/or(iii) each of:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

8. A nucleic acid sequence encoding a TCR α chain and/or a β chain, wherein the α chain and/or a β chain is altered to comprise one or more amino acid residues as defined in claim 1, preferably

which comprises a nucleic acid sequence encoding an α chain and a nucleic acid sequence encoding a β chain linked by an internal self-cleaving sequence or an internal ribosome entry site.

9. A vector comprising a nucleic acid sequence according to claim 8, preferably wherein the vector is a retrovirus vector, lentivirus vector or a transposon.

10. A cell comprising a nucleic acid sequence according to claim 8 or a vector according to claim 9, preferably wherein the cell is a T-cell, a natural killer cell or a stem cell, more preferably wherein the cell is derived from a T-cell, a natural killer cell or a stem cell isolated from a subject.

11. A pharmaceutical composition comprising a vector according to claim 9 or a cell which expresses a TCR as defined in claim 1.

12. A pharmaceutical composition according to claim 11 for use in treating and/or preventing a disease.
13. A vector according to claim 9 or a cell according to claim 10 for use in the manufacture of a medicament for treating and/or preventing a disease.
14. A method for producing a cell according to claim 10 which comprises the step of transducing a cell *in vitro* or *ex vivo* with a vector according to claim 9.
15. A method for determining the strength of a TCR which comprises the steps of:
- (i) providing a TCR α chain and/or β chain sequence; and
 - (ii) determining if the TCR sequence comprises at least one amino acid residue selected from:
L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;
- wherein the amino acid residues are numbered according to the IMGT system, further wherein a sequence which comprises at least one of the recited amino acid residues is determined to be a high expression TCR and a sequence which does not comprise at least one of the recited amino acid sequences is determined to be a low expression TCR.
16. Use of an engineered TCR comprising at least one of the following amino acid residues, numbered according to the IMGT system:
L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of

the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain;

wherein the at least one amino acid residue is not present in the corresponding germline TCR amino acid sequence or an engineered TCR according to any of claims 1 to 7; a nucleic acid sequence encoding the α chain and/or a β chain of said engineered TCR or a vector comprising said nucleic acid sequence encoding the α chain and/or a β chain of said engineered TCR to increase the cell surface expression of the TCR.

17. A cell, isolated from a human subject and transduced with a nucleic acid sequence or vector encoding an engineered TCR comprising at least one of the following residues, numbered according to the IMGT system:

L96 of the α chain; R9 of the β chain; Y10 of the β chain; T24 of the α chain; V19 of the α chain; T20 of the α chain; M50 of the α chain; T5 of the α chain; Q8 of the α chain; S86 of the α chain; F39 of the α chain; D55 of the α chain; R43 of the β chain; A66 of the α chain; V19 of the β chain; L21 of the β chain; L103 of the β chain; T3 of the α chain; S7 of the α chain; P9 of the α chain; M11 of the α chain; A16 of the α chain; T18 of the α chain; L21 of the α chain; S22 of the α chain; D26 of the α chain; F40 of the α chain; S47 of the α chain; R48 of the α chain; Q49 of the α chain; I51 of the α chain; L52 of the α chain; V53 of the α chain; T67 of the α chain; E68 of the α chain; N74 of the α chain; F76 of the α chain; N79 of the α chain; Q81 of the α chain; A83 of the α chain; K90 of the α chain; S92 of the α chain; D93 of the α chain; and M101 of the α chain.

18. The cell of claim 17 for use in the treatment and/or prevention of a disease.