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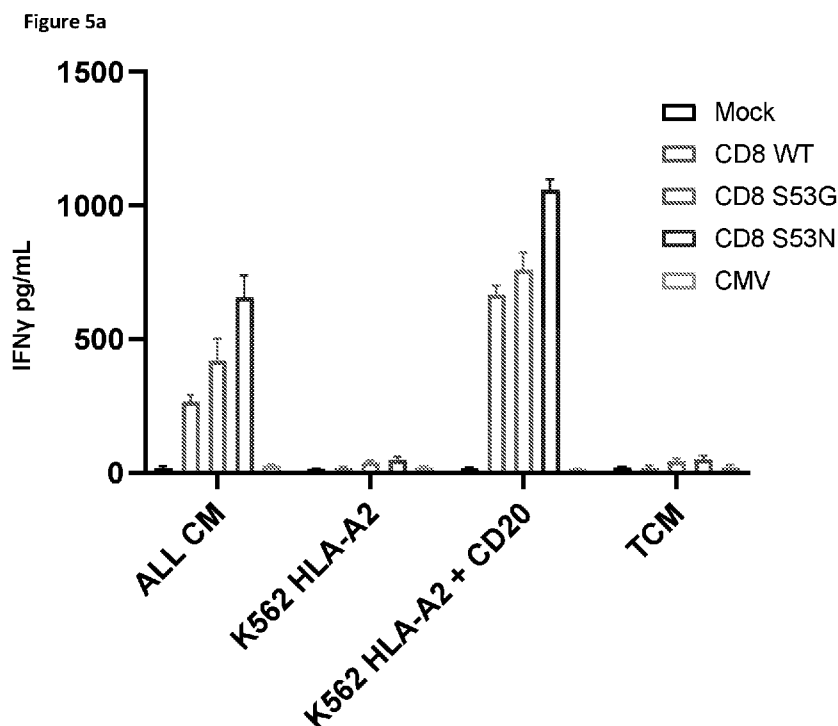
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(54) Title: CD8 VARIANTS WITH INCREASED AFFINITY TO MHCI



(57) **Abstract:** The invention relates to a mutant CD8 polypeptide for use in increasing the affinity of a MHCI-CD8 interaction, and pharmaceutical compositions comprising such polypeptides. The invention also relates to the polypeptides and pharmaceutical compositions for use in a method of treating or preventing cancer and/or infection.



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## CD8 VARIANTS WITH INCREASED AFFINITY TO MHCI

**TECHNICAL FIELD**

The present invention relates to a mutant CD8 alpha chain molecule and uses thereof.

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**BACKGROUND OF THE INVENTION**

CD8+ T-cells can recognize short peptides derived from intracellular antigens which are presented at the surface of cells by Major Histocompatibility Complex Class I (MHCI) molecules. On recognition of tumour derived peptide-MHCI (pMHCI) molecules, CD8+ T-cells can kill tumour cells making them a highly desirable cell  
10 molecules, CD8+ T-cells can kill tumour cells making them a highly desirable cell type for use in adoptive transfer therapy for the treatment of cancer.

The interaction between the MHC Class I-restricted T cell receptor (TCR) and the peptide-MHC complex is stabilized by the glycoprotein CD8 (cluster of differentiation  
15 8), which also recruits the Src-family kinase Lck, and potentiates signalling. CD8 binding to the constant portion of MHC class I results in increased affinity of binding and decreased threshold of response to antigen on target cells (Gao, Nature. 1997 Jun 5; 387(6633): 630-4; Artyomov et al Proc Natl Acad Sci USA. 20 10 Sep 28; 107(39): 1691 6-21). Addition of a CD8 transgene into a TCR lentiviral vector could confer to CD4+ T cells a similar increased response, augmenting their ability to provide helper function to CD8+ T cells as well as additional direct tumour cell killing, possibly resulting in enhanced clinical efficacy.

The use of CD8+ T-cells engineered to express anti-tumour T-cell receptors (TCRs) or chimeric antigen receptors (CARs) is appealing as they can target a wide range of  
25 antigens presented on tumour cells and have shown promise in clinical trials. However, efficacy issues persist largely due to the low affinity of many anti-tumour TCRs.

Mutations in MHCI have been described which can increase the strength of the pMHCI/CD8 interaction (Wooldridge L, Lissina A, Vernazza J, et al. Enhanced immunogenicity of CTL antigens through mutation of the CD8 binding MHC class I invariant region. Eur J Immunol. 2007;37(5):1323-1333).  
30

However, there is a need in the art to provide further means of optimising the affinity of the MHCI-CD8 interaction and antigen sensitivity of the T-cell response, to enhance the magnitude and/or duration of T-cell responses in subjects.

## 5 SUMMARY OF INVENTION

In a first aspect, there is provided a mutant CD8 polypeptide for use in increasing the affinity of a MHCI-CD8 interaction. The increase in affinity of the MHCI-CD8 interaction may increase T-cell antigen sensitivity.

10 CD8 (cluster of differentiation 8) is a transmembrane glycoprotein that serves as a co-receptor for the T-cell receptor (TCR). The CD8 co-receptor is expressed at the surface of CD8<sup>+</sup> T-cells where it interacts with MHCI and acts to enhance T-cell antigen sensitivity. To function, CD8 forms either a hetero- or homodimer composed of either an  $\alpha$  and a  $\beta$  chain or two  $\alpha$  chains, respectively.

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The invention is in part based on the finding that a specific mutation at a single position in the human CD8 alpha chain molecule which is expressed as part of a CD8 co-receptor at the cell surface can unexpectedly increase the affinity of the MHCI-CD8 interaction. In increasing the affinity of the MHCI-CD8 interaction, the antigen  
20 sensitivity of a T-cell is increased, as seen by an increase in the level of activation of the T-cell, in particular at low levels of antigen. Thus, increasing the affinity of the MHCI-CD8 interaction at the cell surface of T-cells that express TCRs with a weak or relatively weak affinity for a target antigen, may enhance the activation of a subject's own T-cells or engineered T-cells in response to the target antigen bound to MHCI,  
25 without compromising the cell's specificity towards that antigen. The invention therefore pushes otherwise relatively low-efficacy T-cells to become therapeutically beneficial, and in addition reduces the research and cost burden of undertaking large scale experiments to identify a high affinity, high-efficacy TCR.

30 In a second aspect, there is provided a polypeptide comprising or consisting of SEQ ID NO: 1 (SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASP TFLLYLGQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYFYFCSALSNSI MYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD FACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVKSGDKPSLSAR  
35 YV) which is the S53G mutant CD8 alpha chain without an N-terminal signal peptide,

or a sequence with at least 90% identity, such as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1. The amino acid at position 53 SEQ ID NO:1 or the position equivalent thereto may be glycine or asparagine. The amino acid at position 53 SEQ ID NO:1 or the position equivalent thereto may be glycine. The polypeptide  
 5 may further comprise a sequence encoding a signal peptide. The signal peptide may comprise or consist of the sequence of SEQ ID NO: 6 (MALPVTALLLPLALLHAARP), which is the canonical signal peptide for human CD8 alpha.

10 In a third aspect, there is provided a mutant human CD8 alpha chain polypeptide which is derived from UNIPROT accession P01732, which is the wild-type human CD8 alpha chain including the N-terminal signal peptide (SEQ ID NO: 2)(MALPVTALLLPLALLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSG  
 15 CSWLFQPRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRE  
 PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRP  
 VVKSGDKPSLSARYV), or a sequence with at least 90% identity, such as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 2, wherein the CD8 alpha chain polypeptide has a mutation of the serine at position 74. The mutation of serine  
 20 at position 74 may be to asparagine. The mutation of serine at position 74 may be to a glycine.

In a fourth aspect, there is provided a polypeptide comprising or consisting of SEQ ID NO: 3 (MALPVTALLLPLALLHAARPSQFRVSPLDRTWNLGETVELKCQVLL  
 25 SNPTSGCSWLFQPRGAAASPTFLLYLGQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRE  
 NEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSL  
 RPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRV  
 CKCPRPVVKSGDKPSLSARYV) which is the S74G mutant CD8 alpha chain including the N-terminal signal peptide, or a sequence with at least 90% identity, such  
 30 as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 3.

In a fifth aspect, there is provided a polypeptide comprising the sequence of any of the polypeptides of the above aspects, and further comprising a sequence encoding the human CD8 beta chain, which may include or exclude a signal peptide. The signal  
 35 peptide may comprise or consist of the sequence of SEQ ID NO: 7 (MRPRLWLLLA

AQLTVLHGNS) which is the canonical signal peptide for human CD8 beta. The human CD8 beta chain may comprise or consist of the sequence of UNIRPOT accession P10966 (SEQ ID NO: 4) (MRPRLWLLAAQLTVLHGNSV LQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDS

5 AKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQL SVVDLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVSLG VAIHLCCRRRRARLRFMKQFYK) which is the wild-type full length CD8 beta chain isoform 1, or SEQ ID NO: 5 (LQQTPAYIKVQTNKMVMLSCEAKISLS NMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNL

10 TSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDLPTTAQPTKKSTLKKRVCRLPR PETQKGPLCSPITLGLLVAGVLVLLVSLGVAIHLCCRRRRARLRFMKQFYK) which is the wild-type CD8 beta chain isoform 1 without an N-terminal signal peptide, or SEQ ID NO: 8 (MRPRLWLLA AQLTVLHGNSVLQQTPAYIKVQTNKM VMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIA

15 VFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDLPTTAQPTKKS TLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVSLGVAIHLCCRRRRARLRF MKQLRLHPLEKCSRMDY) which is the wild-type full length CD8 beta chain isoform 2, or SEQ ID NO: 9 (VLQQTPAYIKVQTNKM VMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIA

20 VFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDLPTTAQPTKKS TLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVSLGVAIHLCCRRRRARLRF MKQLRLHPLEKCSRMDY) which is the wild-type CD8 beta chain isoform 2 without an N-terminal signal peptide, or SEQ ID NO: 10 (MRPRLWLLA AQLTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSD

25 SHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVG SPELTFGKGTQLSVVDLPTTAQPTKKSTLKKRVCRLPRPETQKGRRRRARLRFM KQPQGEGISGTFVPQCLHGYYSNTTTSQKLLNPWILK) which is the wild-type full length CD8 beta chain isoform 3, or SEQ ID NO: 11 (VLQQTPAYIKVQTNKM VMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIA

30 VFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDLPTTAQPTKKS TLKKRVCRLPRPETQKGRRRRARLRFMKQPQGEGISGTFVPQCLHGYYSNTTTSQ KLLNPWILK) ) which is the wild-type CD8 beta chain isoform 3 without an N-terminal signal peptide, or SEQ ID NO: 12 (MRPRLWLLAAQLTVLHGNSVLQQ TPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKG

35 TIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVV

DFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVSLGVAI  
 HLCCRRRRARLRFMKQKFNIVCLKISGFTTCCCFQILQISREYGFGVLLQKDIGQ)  
 which is the wild-type full length CD8 beta chain isoform 4, or SEQ ID NO: 13  
 (VLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALW  
 5 DSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGT  
 QLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLLVAGVLVLLVS  
 LGVAIHLCCRRRRARLRFMKQKFNIVCLKISGFTTCCCFQILQISREYGFGVLLQK  
 DIGQ) ) which is the wild-type CD8 beta chain isoform 4 without an N-terminal  
 signal peptide, or SEQ ID NO: 14 (MRPRLWLLLA  
 10 AQLTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSD  
 SHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVG  
 SPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGPLCSPITLGLL  
 VAGVLVLLVSLGVAIHLCCRRRRARLRFMKQPQGEGISGTFVPQCLHGYYSNTTT  
 SQKLLNPWILKT) which is the wild-type full length CD8 beta chain isoform 5, or  
 15 SEQ ID NO: 15 (VLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIY  
 WLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKP  
 EDSGIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQK  
 GPLCSPITLGLLVAGVLVLLVSLGVAIHLCCRRRRARLRFMKQPQGEGISGTFVPQ  
 CLHGYYSNTTTSQKLLNPWILKT) which is the wild-type CD8 beta chain isoform 5  
 20 without an N-terminal signal peptide, or SEQ ID NO: 16  
 (MRPRLWLLLAQLTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIY  
 WLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKP  
 EDSGIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQK  
 GRRRRARLRFMKQ) which is the wild-type full length CD8 beta chain isoform 6, or  
 25 SEQ ID NO: 17 (VLQQTPAYIKVQTNKMVMLSCEAKISLSNMRI  
 YWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVK  
 PEDSGIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQ  
 KRRRRARLRFMKQ) which is the wild-type CD8 beta chain isoform 6 without an  
 N-terminal signal peptide, or SEQ ID NO: 18 (MRPRLWLLLAQ  
 30 LTVLHGNSVLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSH  
 HEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSP  
 ELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKDFTNKQRIGFWCP  
 ATKRHR SVMSTMWKNERRDTFNPGEFNGC) which is the wild-type full length  
 CD8 beta chain isoform 7, or SEQ ID NO: 19 (VLQQTPAYIKVQTNKM  
 35 VMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSAKGTIHGEEVEQEKIA

VFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKDFTNKQRIGFWCPATKRHR SVMSTMWKNERRDTFNPGE FNGC) which is the wild-type CD8 beta chain isoform 7 without an N-terminal signal peptide, or SEQ ID NO: 20 (MRPRLWLLLAQLTVLHGNSVL

5 QQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPSSDSHHEFLALWDSA KGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMIVGSPELTFGKGTQLS VVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGLKGKQVYQEPLSPNACMDTTAILQ PHRSCLTHGS) which is the wild-type full length CD8 beta chain isoform 8, or SEQ ID NO: 21 (VLQQTPAYIKVQTNKMVMLSCEAKISLSNMRIYWLRQRQAPS

10 SDSHHEFLALWDSAKGTIHGEEVEQEKIAVFRDASRFILNLTSVKPEDSGIYFCMI VGSPELTFGKGTQLSVVDFLPTTAQPTKKSTLKKRVCRLPRPETQKGLKGKQVYQE PLSPNACMDTTAILQPHRSCLTHGS) which is the wild-type CD8 beta chain isoform 8 without an N-terminal signal peptide. The polypeptide may comprise a linker sequence between the sequence of a polypeptide of any of the above aspects and the

15 human CD8 beta chain.

In a sixth aspect, there is provided a polypeptide comprising the sequence of any of the second to fourth aspect, and further comprising the sequence of any one of SEQ ID NOs: 1-3, or a sequence with at least 90% identity, such as 90%, 95%, 96%, 97%,

20 98%, 99% or 100% identity to any of SEQ ID NOs: 1-3. The polypeptide may comprise a linker sequence between the sequence of any of the second to fourth aspect and the sequence of any one of SEQ ID NOs: 1-3, or sequences with at least 90% identity, such as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NOs: 1-3.

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In another aspect, there is provided a nucleic acid encoding any of the polypeptides disclosed herein.

The nucleic acid may encode a polypeptide comprising or consisting of SEQ ID NO: 1

30 or a sequence with at least 90% identity, such as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 1.

The nucleic acid may encode a polypeptide comprising or consisting of SEQ ID NO: 3

35 or a sequence with at least 90% identity, such as 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 3.

The nucleic acid may be a DNA or RNA molecule. The DNA may be a cDNA. The RNA may be mRNA. The nucleic acid may be non-naturally occurring and/or purified and/or engineered. The nucleic acid may be codon optimised.

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In another aspect, there is provided a vector comprising a nucleic acid disclosed herein. The vector may be an expression vector. The vector may be a plasmid. The vector may be a viral vector, such as a retroviral vector, lentiviral vector or adenoviral vector.

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In another aspect, there is provided a viral particle comprising a nucleic acid or vector disclosed herein.

A polypeptide, nucleic acid and/or vector disclosed herein may be recombinant and/or isolated.

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In another aspect, there is provided a cell or population of cells comprising one or more, such as one, two, three, four or more polypeptide of any of the above aspects.

In another aspect, there is provided a cell or population of cells comprising one or more, such as one, two, three, four or more nucleic acid of any of the above aspects.

20

The cell may be a T-cell. The T-cell may be a CD4<sup>+</sup> T-cell. The T-cell may be a CD8<sup>+</sup> T-cell. The T-cell may be a gamma-delta T-cell. The T-cell may be a T-reg cell. The population of cells may be made up of CD4<sup>+</sup> T-cells, or CD8<sup>+</sup> T-cells, or gamma delta T-cells, or T-reg cells, or a mixture of CD4<sup>+</sup> T-cells and CD8<sup>+</sup> T-cells, or a mixture of CD4<sup>+</sup> T-cells and CD8<sup>+</sup> T-cells and gamma-delta T-cells.

25

The cell or population of cells may comprise or consist of a non-naturally occurring and/or purified and/or engineered cell, such as a CD4<sup>+</sup> T-cell and/or CD8<sup>+</sup> T-cell harbouring a nucleic acid disclosed herein and/or expressing a polypeptide disclosed herein. The cell or population of cells may also harbour or express an MHC-1 restricted TCR, which may be homologous or heterologous to that cell or population of cells. An MHC-1 restricted TCR may refer to a TCR which specifically recognises

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an MHC-I molecule which is bound to peptide antigen. The MHC-I molecule may be HLA-A, HLA-B, HLA-C, or HLA-E.

5 Preferably, the cell is a human cell, or the population of cells is made up from human cells.

There are a number of methods suitable for the transfection of cells with nucleic acid (such as DNA, cDNA or RNA) encoding the polypeptides disclosed herein (see for example Robbins et al., (2008) J Immunol. 180: 6 116-61 3 1).

10

In another aspect, there is provided a pharmaceutical composition comprising one or more polypeptide, nucleic acid, vector, viral particle, cell or population of cells disclosed herein.

15 In another aspect, there is provided a method of producing a T-cell or T-cell progenitor cell, or population of T-cells or T-cell progenitor cells, in which the affinity of a MHCI-CD8 interaction is increased, comprising the steps of:

- a. providing a T-cell or T-cell progenitor cell, or population of T-cells or T-cell progenitor cells, obtained from a subject; and
- 20 b. transducing or transfecting the cell or cells with a nucleic acid, vector, viral particle or polypeptide disclosed herein, such that the cell or cells harbour a nucleic acid, vector, viral particle or polypeptide disclosed herein and express a polypeptide disclosed herein.

25 The cell or cells may optionally be expanded. The cells may be expanded before or after transducing or transfecting the cell or cells with a nucleic acid, vector, viral particle, or polypeptide disclosed herein.

Where a T-cell progenitor or population of T-cell progenitor cells is obtained from a subject, said cell or cells may optionally be expanded and/or differentiated into a T-cell, such as a CD4<sup>+</sup> T-cell or CD8<sup>+</sup> T-cell, or a mixture of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. The cell or cells may be expanded and/or differentiated before or after transducing or transfecting the cell or cells with a nucleic acid, vector, viral particle or polypeptide disclosed herein.

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In another aspect, there is provided a method of increasing the affinity of an MHCI-CD8 interaction in a T-cell or population of T-cells comprising transducing or transfecting the cell or cells with a nucleic acid, vector, viral particle or polypeptide disclosed herein such that the cell or cells harbour a nucleic acid, vector, viral particle, or polypeptide disclosed herein and/or express a polypeptide disclosed herein. Preferably, the cell is a CD8+ T-cell or the population of cells is made up of CD8+ T-cells.

In another aspect, there is provided a polypeptide, nucleic acid, vector, viral particle, cell, population of cells or pharmaceutical composition disclosed herein, or a cell or population of cells produced by any method disclosed herein, for use in medicine.

In another aspect, there is provided a polypeptide, nucleic acid, vector, viral particle, cell, population of cells or pharmaceutical composition disclosed herein, or a cell or population of cells produced by any method disclosed herein, for use in treating or preventing cancer and/or infection. The cancer may manifest as a solid tumour, such as melanoma, lung carcinoma, renal cell carcinoma. The cancer may include AML, CML, lymphomas, such as Hodgkin lymphoma, non-Hodgkin lymphoma and multiple myeloma, and solid cancers such as sarcomas, skin cancer, melanoma, bladder cancer, brain cancer, breast cancer, uterus cancer, ovary cancer, prostate cancer, lung cancer, colorectal cancer, cervical cancer, liver cancer, head and neck cancer, oesophageal cancer, pancreas cancer, renal cancer, adrenal cancer, stomach cancer, testicular cancer, cancer of the gall bladder and biliary tracts, thyroid cancer, thymus cancer, cancer of bone, and cerebral cancer, as well as cancer of unknown primary (CUP).

The infection may be from Epstein Barr virus (EBV), Hepatitis B (HBV), Hepatitis C (HCV), Human immunodeficiency virus (HIV), such as HIV-1 or HIV-2, or a coronavirus such as SARS-CoV2.

In another aspect, there is provided a method of treating or preventing cancer and/or infection, comprising administering to a subject in need thereof: a therapeutically effective amount of one or more polypeptide, nucleic acid, vector, viral particle, cell, population of cells or pharmaceutical composition disclosed herein, or a cell or population of cells produced by any method disclosed herein.

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In another aspect, there is provided the use of one or more polypeptide, nucleic acid, vector, viral particle, cell, population of cells or pharmaceutical composition disclosed herein, or a cell or population of cells produced by any method disclosed herein, for the manufacture of a medicament for treating or preventing cancer and/or infection in  
5 a subject.

The T-cell or population of T-cells disclosed herein may be autologous i.e. originally obtained from the same individual to whom they are subsequently administered (i.e. the donor and recipient individual are the same).

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The T-cell or population of T-cells disclosed herein may be allogeneic i.e. originally obtained from a different individual to the individual to whom they are subsequently administered (i.e. the donor and recipient individual are different). Allogeneic as used herein refers to a graft derived from a different animal of the same species. The donor  
15 and recipient individuals may be HLA matched to avoid GVHD and other undesirable immune effects.

A polypeptide, nucleic acid, vector, viral particle, or pharmaceutical composition disclosed herein, may be provided to a therapeutic T-cell, such that the cell harbours a  
20 nucleic acid, vector, viral particle, or polypeptide disclosed herein and expresses a polypeptide disclosed herein. This may enhance any therapeutic activity of the therapeutic T-cell.

The T-cell may be an engineered T-cell. Alternatively, the T-cell may be a natural T-  
25 cell with an endogenous TCR, such as a T-cell isolated from a subject.

A therapeutic T-cell may be a CD8<sup>+</sup> T-cell, CD4<sup>+</sup> T-cell, T-reg cell or  $\gamma\delta$  T-cell.

Any T-cell or population of T-cells disclosed herein may be used for adoptive T-cell  
30 therapy, for example to prevent or treat cancer and/or infection.

Any T-cell or population of T-cells disclosed herein may express a heterologous TCR, for example a TCR which has been introduced into the cell via transduction of nucleic acid encoding the TCR. The TCR may recognise a peptide bound to an MHC-I  
35 molecule, i.e. be an MHC-I restricted TCR.

In any aspect where cancer and/or infection is to be treated or prevented, the cell or population of cells may be derived from the subject or from an allogeneic donor.

## 5 DETAILED DESCRIPTION

The skilled person will appreciate that the level of activation of the T-cell may be measured in a number of ways, which may vary depending on the specific type of T-cell. Examples of markers of T-cell activation include the production of cytokines such as one or more of IFN $\gamma$ , TNF $\alpha$ , IL-2, IL-4, IL-5, IL-10, GM-CSF, IL-13, IL-17, CCL5, MIP-1 $\alpha$ , MIP-1 $\beta$ . Other markers of T-cell activation may include upregulation of proteins such as CD69, CD27, CD45RO, CD44 and/or CD137, amongst others. The affinity of the MHCI-CD8 interaction may be measured biophysically, for example by measuring the  $K_D$  of the interaction using Surface Plasmon Resonance (SPR).

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The phrase "MHCI-CD8" interaction as used herein refers to the interaction of an MHCI molecule, including an MHCI molecule which has a peptide antigen bound, with CD8.

20 Any reference to an increase in the affinity of the MHCI-CD8 interaction may be defined as at least a 5% more, 10% or more, 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more decrease in the  $K_D$  value. Preferably, the  $K_D$  value of the MHCI-CD8 interaction is no lower than about 30 $\mu$ M. An affinity higher than this (corresponding to a  $K_D$  value lower than this) may induce a loss of specificity of the TCR.

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As a result of an increase in the affinity of the MHCI-CD8 interaction, an increase in T-cell activation may be defined by at least a 2 fold or more, 5 fold or more, 10 fold or more, 50 fold or more, 100 fold or more, 500 fold or more, 1000 fold or more increase in the detectable level of interferon gamma compared to the level observed in the absence of the mutated CD8.

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Within the scope of the invention are phenotypically silent variants of any polypeptide disclosed herein. As used herein the term "phenotypically silent variants" is understood to refer to a polypeptide which incorporates one or more further amino

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acid changes, in which a polypeptide has a similar phenotype to the corresponding polypeptide without said change(s). For the purposes of this application, the CD8 phenotype comprises the MHCI-CD8 affinity ( $K_D$  and/or binding half-life). A phenotypically silent variant may have a  $K_D$  and/or binding half-life for the SEQ ID NO: 1: or SEQ ID NO: 3 within about 10% of the measured  $K_D$  and/or binding half-life of the corresponding CD8 without said change(s), when measured under identical conditions (for example at 25°C and on the same SPR chip).

Mutations can be carried out using any appropriate method including, but not limited to, those based on polymerase chain reaction (PCR), restriction enzyme-based cloning, or ligation independent cloning (LIC) procedures. These methods are detailed in many of the standard molecular biology texts. For further details regarding polymerase chain reaction (PCR) and restriction enzyme-based cloning, see Sambrook & Russell, (2001) *Molecular Cloning – A Laboratory Manual* (3rd Ed.) CSHL Press. Further information on ligation independent cloning (LIC) procedures can be found in Rashtchian, (1995) *Curr Opin Biotechnol* 6(1): 30-6.

"Identity" as known in the art is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. While there exist a number of methods to measure identity between two polypeptide or two polynucleotide sequences, methods commonly employed to determine identity are codified in computer programs. Preferred computer programs to determine identity between two sequences include, but are not limited to, GCG program package (Devereux, et al., *Nucleic Acids Research*, 12 , 387 (1984), BLASTP, BLASTN, and FASTA (Atschul et al., *J. Molec. Biol.* 215, 403 (1990)).

A program such as the CLUSTAL program can be used to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a

comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are suitable.

5 The percent identity of two amino acid sequences or of two nucleic acid sequences is determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent identity. The percent identity is determined by the number of identical amino acid  
10 residues or nucleotides in the sequences being compared (i.e., % identity = number of identical positions/total number of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a  
15 mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873- 5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with  
20 the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules for use in the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilised as described in  
25 Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilising BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>. Another example of a mathematical algorithm  
30 utilised for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the CGC sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis and Robotti (1994) Comput. Appl. Biosci., 10 :3-5; and FASTA

described in Pearson and Lipman (1988) Proc. Natl. Acad. Sci. 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

Any pharmaceutical composition referred to herein may further comprise one or more pharmaceutically acceptable carriers or excipients.

For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include, but are not limited to, stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents and buffers. The compositions can be in any suitable form, for example suspensions, emulsions, solutions, aerosols, sterile injectable solutions, and sterile packaged powders. Such compositions may be prepared by any known method, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Suitable pharmaceutical compositions for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate for oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and /or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

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A pharmaceutical composition may be for systemic administration.

The physical and chemical characteristics of the pharmaceutical compositions disclosed herein may be modified or optimized according to the skill in the art, depending on the mode of administration and the particular disease or disorder to be treated. The compositions may be provided in unit dosage form, a sealed container, or as part of a kit, which may include instructions for use and/or a plurality of unit dosage forms.

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A variety of administration routes for the pharmaceutical compositions of the invention are available. The particular mode selected will depend upon the particular composition selected, whether the administration is for prevention, or treatment of disease, the severity of the medical disorder being treated and dosage required for therapeutic efficacy. The methods of this invention may be practiced using any mode of administration that is medically acceptable, and produces effective levels of the active compounds without causing clinically unacceptable adverse effects. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

As used herein, the term “therapeutically effective amount” refers to the total amount of each active component of the pharmaceutical composition or method that is sufficient to provide patient benefit, i.e., prevention or amelioration of the condition to be treated, a reduction in symptoms, an increase in rate of healing, or a detectable change in the levels of a substance in the treated or surrounding tissue. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in concurrently, sequentially or separately.

The precise dose to be employed in the pharmaceutical composition of the present invention may depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient’s circumstances and can be determined by standard clinical techniques. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. The particular dosage regimen, i.e., dose, timing and repetition, will thus depend on the particular individual and that individual's medical history, as well as the route of administration.

The pharmaceutical compositions may be delivered at intervals ranging from about 24 hours to about 2 days, to about 1 week, to about 2 weeks, to about 3 weeks to about 1 month to about 2 months, to about 3 months, to about 4 months, to about 5 months, to

about 6 months, to about 12 months, or more. The scheduling of such dosage regimens can be optimized by the practitioner.

5 The pharmaceutical compositions may be administered using a treatment regimen comprising one or more doses, wherein the treatment regimen is administered over 2 days, 3 days, 4 days, 5 days, 6 days or 7 days, 14 days, 30 days, 1 month, 2 months, 3 months, 6 months, 12 months or more.

10 The term “patient” or “subject,” as used interchangeably herein, refers to any mammal, preferably a human.

The “subject in need thereof” may be a subject who has been diagnosed with cancer and or/infection.

15 The method of any aspect of the invention may be *in vivo*, *ex vivo* or *in vitro*.

The skilled person will appreciate that preferred features of any one embodiment and/or aspect of the invention may be applied to all other embodiments and/or aspects of the invention.

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## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** – illustrates the results of molecular modelling and prediction of point mutations in the human CD8 $\alpha$  chain. A) shows the  $\Delta\Delta G$  values indicating the predicted change in Gibbs free energy when each amino acid is substituted with alanine B) demonstrates a visual representation of the between amino acids 223-229 of  $\alpha 3$  chain of HLA-A2, reported to make the most prominent contact with the CD8 $\alpha\alpha$  co-receptor. C) Shows the chemical structures of the mutations from Serine (S) at position 53, to amino Threonine (T), Glutamine (Q) and Asparagine (N). Substitution with Glycine (G) was added as an expected negative control with the prediction that the small side chain of Glycine would diminish the interaction with the HLA $\alpha 3$  chain.

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**Figure 2** – demonstrates tetramer staining of Jurkat cell lines transduced with RLA TCR only or with RLA TCR and different CD8 variants. HLA-A2 tetramers were refolded around RLARLALVL peptide, recognised by RLA TCR. Variants S53N and S53G show the highest percentage of tetramer positive cells.

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**Figure 3** – demonstrates a comparison of functional sensitivities (pEC50, obtained from peptide titration curves) of RLA TCR<sup>+</sup> Jurkats expressing no CD8, CD8 WT or one of the CD8 variants. There is a significant improvement in functional sensitivity of RLA TCR<sup>+</sup> Jurkats expressing variants S53G and S53N, compared to RLA TCR<sup>+</sup> Jurkats expressing CD8 WT (p=0.045 and p=0.011, respectively, One-Way ANOVA, Dunnet's post hoc test).

**Figure 4** – shows surface plasmon resonance (SPR) measurements of soluble CD8aa binding affinity for immobilised HLA-A2 monomers demonstrate enhanced affinity of CD8alpha chain variants S53G and S53N (measured as dissociation constant K<sub>D</sub>) compared to wild-type CD8alpha molecules.

**Figure 5** – demonstrates activation of primary CD4 T-cells transduced with 1E9 TCR and either CD8 WT, CD8 S53G or CD8 S53N, in response to various tumour lines. IFN-  $\gamma$  production in T-cell media (TCM) was included as a control. CD4 T-cells were transduced sequentially with either CMV TCR or 1E9 TCR, and subsequently with CD8 variant, flow sorted and co-cultured overnight with indicated tumour lines. a) IFN-  $\gamma$  production after overnight culture, measured by ELISA. b) IL-2 production after overnight culture, measured by ELISA. c) T-cells count after 5-day co-culture of T-cells with target tumour lines, measured by flow cytometry. Data shown is representative of data from 3 different donors. For each sample the bars in the graph are in the following order (from left to right) mock, CD8 WT, CD8 S53G, CD\* S53N and CMV.

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## MATERIALS AND METHODS

### Molecular modelling

The crystal structures of human CD8 $\alpha\alpha$  complexed with HLA-A\*0201 (1AKJ) or HLA-A\*2402 (3QZW) and crystal structure of murine CD8 $\alpha\beta$  complexed with H-2D<sub>a</sub> (3DMM) were uploaded to BUDE software (Ibarra, A. A. et al. Predicting and Experimentally Validating Hot-Spot Residues at Protein-Protein Interfaces. ACS Chem. Biol. 14, 2252–2263 (2019)). The software performs an alanine scan by substituting every interacting amino acid of the ligand with alanine, and returns a list of  $\Delta\Delta G$  (change in Gibbs free energy) values, which indicate the strength of the

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interaction between the two side chains. Alanine is used in mutagenesis studies as its radical group is small and uncharged, thereby eliminating any effect that the side chain has on the interaction with the receptor. Positions with values around 0 do not have a very strong interaction with the receptor so there is room for improvement, with less chance of rendering the protein dysfunctional. Since there is no crystal structure of the human CD8 $\alpha\beta$  complexed with HLA, the crystal structure uploaded into BUDE was that of human CD8 $\alpha\alpha$ . The crystal structures were then uploaded into Chimera software and examined visually to identify amino acids which would interact when one of the CD8 $\alpha$  chains was replaced by CD8 $\beta$  chain.

### **Production of soluble CD8 proteins**

For the expression of soluble inclusion bodies, human CD8 $\alpha$  chain was codon optimised and synthesised into a pGMT vector, driven by a T7 promoter. Rosetta E.coli competent cells were transformed with pGMT vectors containing human wild type CD8 $\alpha$  chain or human CD8 $\alpha$  chain with S53N or S53G mutation. Expression of insoluble inclusion bodies was induced with isopropyl-1-thio- $\beta$ -D-galactopyranoside (IPTG) for 3 hours, and the bacteria subsequently lysed with BugBuster Protein Extraction Reagent (Novagen) and washed with 0.5% Triton X-100 buffer. The final protein was resuspended in 6M Guanidine buffer.

CD8 inclusion bodies were denatured in 6M Guanidine buffer containing 10mM DTT, for 30min at 37 °C. Denatured inclusion bodies were added in three steps to 2-mercaptoethylamine buffer (100mM TRIS buffer, pH 8.1, 1mM EDTA, 600mM L-Arginine, 6mM cytamine and 4mM cyteamine) and stirred vigorously at 4 degrees for two hours. After filtration and concentration to 200mL, the refold mixture was placed in dialysis tubing (8kDa) and dialysed overnight in water, then two times for twelve hours in 10mM MES solution. The refold was then purified using fast performance liquid chromatography and a cation-exchange column (HiTrap SP-HP).

### **Biophysical measurements -BIAcore**

After buffer exchange into HBS-EP, the soluble CD8 $\alpha\alpha$  proteins were concentrated to less than 10ug/uL, using Vivaflow 6 (10,000 MCWO). CM5 sensor chip surface was activated using 50uL of NHS/EDC. 0.2mg/mL of streptavidin (Sigma) in 10mM acetate solution was coupled to the activated chip. Four biotinylated HLA-A\*0201 monomers were diluted in HBS-EP buffer and immobilised at ~1000 response (RU)

units in each cell. The CD8 $\alpha$  was serially diluted seven times and flowed over the chip in 10 $\mu$ L injections starting at the lowest protein concentration. The data was analysed using BIAeval software, MS Excel and GraphPad Prism.

## 5 Vectors and production of viral particles

For the lentiviral system, human CD8 $\beta$  and  $\alpha$  chains were codon optimised and synthesised into a third-generation lentiviral vector driven by the elongation factor one- $\alpha$  promoter (pSF.EF1, Oxford genetics). The chains were separated by internal ribosomal entry site sequence (IRES) and cloned in the order of CD8 $\beta$ -IRES-CD8 $\alpha$ .  
10 Human TCR $\alpha$  and  $\beta$  chains recognising the HLA-A\*0201 restricted 5T417-25 (RLA) antigen were codon optimised, partly murinised and cysteine modified. The chains were separated by P2A cleavage sequence and cloned into the pSF.EF1 vector using NotI and NheI restriction sites. Point mutations were introduced into vectors using Q5 Mutagenesis kit (New England Biolabs). Lentiviral particles were produced through  
15 transfection of HEK 293T cell line using Turbofect transfection reagent (Thermo Fisher) and the particles thereafter concentrated using Lenti-X concentrator (Takara Bio).

For the retroviral system, human TCR $\alpha$  and  $\beta$  chains recognising HLA-A\*0201  
20 restricted CD20 antigen (SLFLGILSV) were codon optimised and the human constant domains substituted for murine constant domains to facilitate correct chain pairing. The TCR was termed 1E9 and described in Jahn et al. 20162. The chains were separated by 2A cleavage sequence and cloned into MP71 retroviral vector. Human CD8 $\beta$  and CD8 $\alpha$  chains were separated by a 2A sequence and CD8 $\alpha$  mutations  
25 introduced using Q5 mutagenesis kit (New England Biolabs). The construct was cloned into a MP71 vector containing an IRES site downstream of the cloning site, followed by truncated NGFR sequence, which was used as marker of expression of CD8 in CD8 $^{+}$  T-cells. HLA-A\*0201 restricted TCR targeting CMV epitope NLVPMVATM was used as a negative control. Viral particles were produced through  
30 transfection of Phoenix Amphi cells using Fugene (Promega) transfection reagent.

### Transduction of Jurkat cell lines

200,000 Jurkat cells were transduced with lentivirus containing TCR genes, expanded and MACS sorted for TCR $^{+}$  cells. The sorted cells were then transduced with lentivirus carrying either CD8 WT, or one of the variants thereof (S53T, S53Q, S53N

or S53G). Double positive cells were then flow sorted on similar levels of TCR/CD8 expression and expanded.

#### **Transduction of primary human CD4 and CD8 T-cells**

5 Primary human CD4 and CD8 T-cells were isolated from cryopreserved peripheral blood mononuclear cells (PBMC) using anti-CD4 and anti-CD8 microbeads. The cells were restimulated with feeder mix consisting of irradiated PBMC of the same donor in the presence of 1 $\mu$ g/mL PHA. 48 hours after activation, 250,000 CD4 and CD8 T-cells were transduced with 500 $\mu$ L of retrovirus containing the construct of interest. The  
10 cells were cultured for 7 days after isolation before being MACS enriched for transduced populations.

#### **Activation of Jurkats using C1R presenting cells**

C1R cells expressing HLA-A2 or T2 cells expressing HLA-A2 were incubated with  
15 indicated peptide (RLARLALVL or ILTGIGLTV) for one hour in RPMI media. The cells were washed and 150,000 C1R or T2 cells co-cultured with 50,000 transduced and sorted Jurkat cells for 16h. Following the incubation, the cells were surface stained and analysed by flow cytometry.

#### **Co-cultures of CD4 or CD8 T-cells with tumour lines**

20 1,000 transduced and sorted CD4 T-cells (CMV TCR, 1E9 TCR only, 1E9 TCR +CD8 WT, 1E9 TCR+CD8 S53N or 1E9 TCR+CD8 S53G) were co-cultured with 5,000 tumour cells (either ALL CM, HLA-A\*0201 transduced K562 cells or HLA-A\*0201 K562 cells transduced with CD20) in IMDM media supplemented with 5% FCS and  
25 5% human serum. After 16 hours, the supernatants of the co-cultures were collected and IFN- $\gamma$  and IL2 secretion measured by enzyme-linked immunosorbent assay (R&D Systems). The co-cultures were supplemented with fresh IMDM media containing 5% FCS, 5% human serum and 200iU/mL of IL2, for 5 days and the cell proliferation analysed by flow cytometry.

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#### **Flow cytometry**

Monoclonal antibodies used in flow cytometry were as follows: anti human TCR V $\beta$ 12-PE (VER2.32.1) from Beckman Coulter, anti-human CD8 $\alpha$  PECy7 (RPA-T8), anti-human CD8 $\beta$  eFluor660 (SIDI8BEE) from Life Technologies, anti-human CD8 $\alpha\beta$   
35 BB700 (2ST8.5H7) from BD Biosciences, anti-human HLA-A2 FITC (BB7.2), anti-

mouse TCR $\beta$  chain PE (H57-597) and anti-human CD69 BV421 from Biolegend. In addition, Live/Dead Aqua 405 from Invitrogen was used to assess the viability of the cells on flow cytometer.

- 5 All cells were stained with Live/Dead Aqua 405 at 1:100 dilution for 15min at room temperature. Cells were stained with monoclonal antibodies for 15min at room temperature PBS, washed twice and fixed in 2% paraformaldehyde before running on Acea Novocyte 3000. The data was analysed in FlowJo software.

Statistical analysis was performed in GraphPad Prism.

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#### **The sequence of human CD8 $\alpha$ used to predict high affinity mutations**

- The design of mutations in the CD8 $\alpha$  chain was based on the available crystal structures of human CD8 $\alpha\alpha$  interacting with HLA-A\*0201 (1AKJ) and HLA-A\*2402 (3QZW) complexes. The full-length amino acid sequence of CD8 $\alpha$  contains a signal peptide (1-21), extracellular portion (22-182), transmembrane domain (183-203) and the cytoplasmic tail (204-235) (Sequence 1). In secreted proteins or proteins expressed on the surface of cells, as is the case with the CD8 co-receptor, the N-terminus signal sequence will be used to guide the ribosome to the endoplasmic reticulum. In most cases, signal peptides are cleaved from the mature protein. When describing mutations in the human CD8 $\alpha$  chain, we refer to position Serine22 of the full sequence as position Serine (S)1, as the signal peptide will not be present in the mature protein. In addition, the sequences of the CD8 $\alpha$  proteins shown in crystal structures will also lack the cytoplasmic and transmembrane regions of the protein (Sequence 2).

- 25 Sequence 1: Full length sequence of human CD8 $\alpha$  (Seq ID No: 2)

MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCS  
WLFQPRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDFRRENE  
GYYFCSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPA  
AGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPV  
30 VKSGDKPSLSARYV

Sequence 2: Sequence of human CD8 $\alpha$  used in molecular modelling studies (1AKJ, 3QZW) (Seq ID No: 22):

SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQN  
 KPAAEGLDTQRFSKGRLGDTFVLTLSDFRRENEGYYFCSALSNSIMYFSHFVPV  
 FLPAKPTTTP

## 5 EXAMPLES

### Example 1- Molecular modelling and prediction of point mutations in the human CD8 $\alpha$ chain

Mutations in the CD8 molecule were designed based on available crystal structures of human CD8 $\alpha\alpha$  complexed with HLA-A\*0201 or HLA-A\*2402, and mouse CD8 $\alpha\beta$  in complex with H-2Dd (Gao, G. F. et al. Crystal structure of the complex between human CD8 $\alpha$ (alpha) and HLA-A2. Nature 387, 630–634 (1997); Shi, Y., Qi, J., Iwamoto, A. & Gao, G. F. Plasticity of human  $\beta\alpha$  binding to peptide–HLA-A\*2402. Mol. Immunol. 48, 2198–2202 (2011); Wang, R., Natarajan, K. & Margulies, D. H. Structural Basis of the CD8 $\alpha\beta$ /MHC Class I Interaction: Focused Recognition Orients CD8 $\beta$  to a T Cell Proximal Position. J. Immunol. 183, 2554 LP – 2564 (2009)), with the aim of designing CD8 molecules within the therapeutic window of affinities (Dockree, T. et al. CD8 + T-cell specificity is compromised at a defined MHCI/CD8 affinity threshold. Immunol. Cell Biol. 95, 68–76 (2017)). Initially, the crystal structure of human CD8 $\alpha\alpha$  complexed with HLA-A\*0201 or HLA-A2402 were uploaded to BUDE software, where CD8 chains were selected as ligands, and HLA chains as receptors. The output was a list of  $\Delta\Delta G$  values indicating the predicted change in Gibbs free energy when each amino acid is substituted with alanine. (Figure 1a). These mutations were further evaluated by visually inspecting the interface between the MHCI chains ( $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  and B2M) and the CD8 molecule. The focus was on amino acids yielding values close to 1. Each amino acid interaction, listed in the software output, was visually examined in order to predict possible substituting amino acids. Position Q2 and K21 were only listed on CD8 $\alpha 1$  chain, and upon inspection it was confirmed that these positions on CD8 $\alpha 2$  chain had no contact points with HLA molecule. Since the current evidence suggests that CD8 $\alpha 1$  chain is replaced by CD8 $\beta$  in the interaction with HLA (Wang, R., Natarajan, K. & Margulies, D. H. Structural Basis of the CD8 $\alpha\beta$ /MHC Class I Interaction: Focused Recognition Orients CD8 $\beta$  to a T Cell Proximal Position. J. Immunol. 183, 2554 LP – 2564 (2009)), those two mutations were not considered in further experiments. Visual inspection of the interface was based on the previous findings that the amino acids 223-229 of  $\alpha 3$  chain

of HLA-A2 make the most prominent contact with the CD8 $\alpha\alpha$  co-receptor (Gao, G. F. et al. Crystal structure of the complex between human CD8 $\alpha$ (alpha) and HLA-A2. Nature 387, 630–634 (1997)). Amino acid substitutions were designed to shorten the distance between the contacting amino acid, while conserving the nature of the interaction. At position Serine (S) 53, amino acid substitutions selected for study were Threonine (T), Glutamine (Q) and Asparagine (N). Substitution with Glycine (G) was added as a negative control with the prediction that the small side chain of Glycine would diminish the interaction with the HLA $\alpha$ 3 chain (Figure 1B and 1C).

#### 10 **Example 2 – Expression of CD8 variants in Jurkat cells alongside low affinity cancer targeting TCRs**

The four CD8 variants (which included the homologous signal peptide) were introduced into Jurkat cell line together with a tumour targeting RLA TCR. RLA TCR is a HLA-A\*0201 restricted TCR, specific for the 5T4 derived peptide RLARLALVL and exhibits low affinity for its cognate peptide ( $K_D=45\mu\text{M}$ ). Jurkat cells were first lentivirally transduced with RLA TCR and enriched for RLA TCR+ cells using magnetic activated cell sorting (MACS). The enriched cells were then transduced with either wild type CD8 (CD8 WT) or one of the CD8 variants (S53N, S53T, S53Q and S53G, where position 53 is the position after cleavage of the signal peptide). Transduced cells were then flow sorted for CD8+/TCR+ cells. The first indication of an improved antigen recognition of the TCR was seen upon performing tetramer staining with the double positive Jurkats (Figure 2). Variants S53G and S53N exhibited better staining with HLA-A2 tetramers refolded around RLARLALVL antigen indicating enhanced affinity of the variants for the pMHCI. This was surprising, given that the S53G variant was selected as a negative control, which would diminish CD8/pMHCI affinity.

#### **Example 3- Activation of Jurkat cells expressing RLA TCR and CD8 variants**

In order to determine whether CD8 variants can also enhance activation of cells to cognate antigen, RLA TCR+ Jurkats expressing CD8 WT or one of the variants (S53T, S53Q, S53G and S53N) were incubated with HLA\*0201+ C1R cells pulsed with RLA peptide at concentrations ranging from  $10^{-9}$  to  $10^{-5}\text{M}$ . The response of Jurkat cells was recorded as CD69 upregulation by flow cytometry and the data was plotted using simultaneous least squares curve fitting. The curves were used to calculate the negative decimal logarithm of half maximal concentration, pEC50, which increases in

value as the functional sensitivity of T-cells increases. These values were used to compare the sensitivities of RLA TCR+ Jurkats expressing different CD8 variants to RLARLALVL peptide. In addition, the same assay was repeated with HLA-A\*0201 expressing T2 cells, which unlike C1R cells, are TAP deficient and cannot present most of endogenously processed antigens. In experiments involving either presenting cell line, Jurkats expressing RLA TCR and CD8 variants S53G and S53N demonstrated significantly increased activation to RLARLALVL peptide, compared to Jurkat cells expressing RLA TCR in combination with CD8 WT ( $p=0.045$  and  $p=0.011$ , respectively, one-way ANOVA, Dunnet's post hoc test). Variants S53T and S53Q did not have a significant effect on activation of the cells, compared to CD8 WT (Figure 3).

#### Example 4 - Biophysical measurements of the two best performing CD8 variants

The cells expressing RLA TCR and CD8 variants S53G or S53N activated significantly better to cognate peptide, compared to the cells expressing RLA TCR and CD8 WT. For this reason, surface plasmon resonance measurements were obtained to evaluate their affinity for pMHCI. Soluble CD8 $\alpha\alpha$  WT and the mutants S53N and S53G were flowed over immobilised HLA-A\*0201 molecules folded around three different peptides derived from tumour associated antigens (TAAs). SLLQHLIGL (SLL) and VLDGLDVLL (VLD) are PRAME-derived peptides, while RMFPNAPYL (RMF) is derived from Wilms tumour one (WT1) protein sequence (Kessler, J. H. et al. Efficient identification of novel HLA-A\*0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J. Exp. Med.* 193, 73–88 (2001)). Variants S53G and S53N exhibit higher affinity for all three peptide-HLA complexes, compared to CD8 WT (Figure 4). CD8 WT exhibits affinity of around 100 $\mu$ M for both HLA-A\*0201-SLL and HLA-A\*0201 VLD, which is slightly below the average value reported in the literature (reviewed by Cole et al. The molecular determinants of CD8 co-receptor function. *Immunology* 137, 139–148 (2012)). S53N variant has previously been reported in combination with another mutation, C33A, and the affinity of this molecule was reported to be  $K_D=40.7\mu$ M (Cole, D. K. et al. Computational design and crystal structure of an enhanced affinity mutant human CD8  $\alpha\alpha$  coreceptor. *Proteins Struct. Funct. Bioinforma.* 67, 65–74 (2007)). The average value of  $K_D=48\mu$ M obtained in our measurements is in line with those findings. S53G variant, on the other hand, has a much lower affinity than S53N variant (average  $K_D=109\mu$ M), but higher than wild-

type CD8 (average  $K_D=131\mu\text{M}$ ), making it a potential candidate for improving activation of T-cells with low affinity TCRs. This average affinity of CD8 S53G variant falls within the therapeutic window previously defined by Dockree et al. (Dockree, T. et al. CD8 + T-cell specificity is compromised at a defined MHCI/CD8 affinity threshold. Immunol. Cell Biol. 95, 68–76 (2017)). The affinity of the S53G variant lies on the higher end of the affinity window, but is still significantly improved compared to the affinity of the wild-type CD8 molecule. The affinity on the higher end of the therapeutic window is beneficial as it can provide significant increases in antigen sensitivity of T-cells without incurring losses to specificity (as was previously demonstrated in the Q115E pMHCI variant with comparable affinity; Wooldridge et al 2007). For HLA-A\*0201 folded around RMF peptide, the  $K_D$  values for all three CD8 molecules are substantially higher, but the relationship between the variants remains the same.

#### **Example 5 - Transduction and activation of primary human CD4 T-cells with 1E9 and CD8 WT or variants S53G and S53N**

In order to evaluate the effect of CD8 variants S53G and S53N in primary human cells, primary human CD4 T-cells were transduced with 1E9 TCR, targeting the HLA-A\*0201 restricted peptide SLFLGILSV derived from human CD20 molecule and subsequently MACS enriched the 1E9 TCR+ population. After restimulation using feeder cells, the 1E9 TCR+ cells were transduced with either mock (NGFR only) vector, CD8 WT, CD8 S53G or CD8 S53N. The double positive cells were flow sorted and co-cultured in a 1:5 ratio with 4 tumour lines. ALL BV and ALL CM naturally express CD20 and are HLA-A\*0201+, while CD20+ HLA-A\*0201 K562 cells were transduced with HLA-A\*0201 and CD20 molecules. HLA-A\*0201 K562 cells do not express CD20 antigen and therefore served as a negative control. After an overnight culture, the supernatant was analysed for IFN- $\gamma$  and IL-2 (Figure 5a and b). Both variants S53G and S53N could enhance IFN- $\gamma$  and IL-2 production of 1E9+ CD4 T-cells, compared to CD8 WT. Neither of the two variants enhanced the IFN- $\gamma$  production to HLA-A\*0201 K562 line, suggesting that the sensitivity enhancement was antigen specific. When these experiments were repeated with different PBMC donors, there was clear enhancement in IFN- $\gamma$  production in cells expressing CD8 variants S53G and S53N in response to antigen positive tumour lines, for all experimental repeats. In addition, when the cells were co-cultured with the tumour lines for five days, the cells expressing 1E9 TCR and either CD8 variant S53G or

S53N proliferated better in response to antigen positive lines, compared to cells expressing 1E9 and no CD8 or 1E9 and CD8 WT (Figure 5c). Together with biophysical data, this indicates that S53G (and S53N) variants sits within the therapeutic window of pMHCI/CD8 affinity, and that they could potentially be used as a safe alternative for avidity enhancement of TCR-engineered T-cells. The non-polymorphic nature of CD8 also makes this an attractive strategy as a single CD8 variant could be used to enhance different TCR-engineered T-cells targeting various antigens. In CD4 T-cells, the delivery of pMHCI restricted TCR together with a CD8 variant could confer better helper functions on CD4 T-cells. There is evidence that simultaneous delivery of TCR modified CD4 and CD8 T-cells results in better tumour control in mice (Fujiwara, H. et al. Antileukemia multifunctionality of CD4+ T cells genetically engineered by HLA class I-restricted and WT1-specific T-cell receptor gene transfer. *Leukemia* 29, 2393–2401 (2015)). If the cells were used in combination with TCR-transduced CD8 T-cells, the joint effect of the two cell types could lead to a more sustained anti-tumour response.

#### **Summary of primary CD4 and CD8 T-cell data**

Taken together, the variants represent a valuable tool for transduction of bulk CD3+ cells (which include both CD8 and CD4 T-cells) alongside 1E9 TCR, which would ensure optimal performance of both CD4 and CD8 fraction of cells in response to tumour lines.

#### **Summary**

TCR-engineered T-cells and CAR-T technologies have emerged as two main directions in T-cell engineering. CAR T-cells have shown clinical success and received FDA approval for certain haematological malignancies, but TCR-engineered T-cells still fall behind on efficacy and safety profiles. However, this strategy remains an attractive approach due to versatility of potential antigens that can be targeted by the TCR. In a physiological setting, the TCR determines the specificity of the CD8 T-cell for its antigen, but the CD8 co-receptor fine-tunes the cell's sensitivity. When the TCR binds the peptide:MHCI complex on the antigen presenting cell, the CD8 co-receptor stabilises the interaction and propagates TCR signalling. Increasing the affinity of the peptide:MHCI-CD8 interaction leads to increased antigen sensitivity of the T-cell for its cognate antigen without incurring losses to its specificity. A

threshold for this affinity exists, above which the T-cell loses antigen specificity and becomes promiscuous. The invention exploits this observation by providing a mutant CD8 molecules with enhanced affinity which can be combined with low affinity cancer specific TCRs. It is demonstrated that a moderate increase in CD8 affinity can  
5 lead to enhanced T-cell activation. These findings offer an alternative strategy for enhancing the efficacy of TCR-engineered T-cells without compromising the cell's specificity.

## CLAIMS

1. A mutant CD8 polypeptide for use in increasing the affinity of a MHCI-CD8 interaction.  
5
2. A polypeptide comprising or consisting of SEQ ID NO: 1 or a sequence with at least 90% identity, such as 90% identity to SEQ ID NO: 1, wherein the amino acid at position 53 SEQ ID NO:1, or the position equivalent thereto, is glycine.  
10
3. The polypeptide of claim 2, further comprising a sequence encoding a signal peptide, optionally wherein the signal peptide comprises or consists of the sequence of SEQ ID NO: 6.
- 15 4. A mutant CD8 alpha chain polypeptide which is derived SEQ ID NO: 2 or a sequence with at least 90% identity to SEQ ID NO: 2, wherein the CD8 alpha chain polypeptide has a mutation of the serine at position 74, or the position equivalent thereto, to a glycine.
- 20 5. A polypeptide comprising or consisting of SEQ ID NO: 3 or a sequence with at least 90% identity to SEQ ID NO: 3.
6. A polypeptide of any of claims 2-5, further comprising a sequence encoding the human CD8 beta chain, optionally wherein the CD8 beta chain comprises  
25 or consists of the sequence of SEQ ID NO: 4 or SEQ ID NO: 5.
7. A polypeptide of any of claims 2-5, further comprising the sequence of any one of SEQ ID NOs: 1-3, or a sequence with at least 90% identity to any one of SEQ ID NOs: 1-3.  
30
8. A nucleic acid encoding a polypeptide of any of claims 1-7.
9. The nucleic acid of claim 8, wherein the nucleic acid is a DNA or RNA molecule.  
35
10. The nucleic acid of claim 9, wherein the RNA molecule is an mRNA molecule.

11. A vector comprising a nucleic acid of any of claims 8-10.
12. The vector of claim 11, wherein the vector is an expression vector, plasmid or  
5 viral vector.
13. The vector of claim 12, wherein the viral vector is a retroviral vector, lentiviral  
vector or adenoviral vector.
- 10 14. A viral particle comprising a nucleic acid of any of claims 8-10 or the vector  
of any of claims 11-13.
- 15 15. A cell or population of cells comprising the polypeptide of any of claims 1-7,  
the nucleic acid of any of claims 8-10, the vector of any of claims 11-13 or the  
viral particle of claim 14.
16. The cell or population of cells of claim 15, wherein the cell is a CD4+ T-cell,  
CD8+ T-cell, gamma delta T-cell, or Treg cell, or  
the population of cells is made up of CD4+ T-cells, CD8+ T-cells,  
20 gamma delta T-cells, Treg cells or a mixture thereof.
17. The cell or population of claim 15 or claim 16, wherein the cell or population  
of cells is harbouring nucleic acid encoding an MHC-I restricted TCR, or  
wherein the cell or population of cells comprises or expresses an MHC-I  
25 restricted TCR.
18. A pharmaceutical composition comprising the polypeptide of any of claims 1-  
7, the nucleic acid of any of claims 8-10, the vector of any of claims 11-13,  
the viral particle of claim 14, or the cell or population of cells of any of claims  
30 15-17.
19. A method of producing a T-cell or T-cell progenitor cell, or population of T-  
cells or T-cell progenitor cells, in which the affinity of a MHCI-CD8  
interaction is increased, comprising the steps of:

(a) providing a T-cell or T-cell progenitor cell, or population of T-cells or T-cell progenitor cells, obtained from a subject; and

(b) transducing or transfecting the cell or cells comprising the polypeptide of any of claims 1-7, the nucleic acid of any of claims 8-10, the vector of any of claims 11-13 or the viral particle of claim 14.

20. A method of increasing the affinity of MHCI-CD8 interaction in a T-cell or population of T-cells comprising transducing or transfecting the cell or cells with the polypeptide of any of claims 1-7, the nucleic acid of any of claims 8-10, the vector of any of claims 11-13 or the viral particle of claim 14.

21. The polypeptide of any of claims 1-7, the nucleic acid of any of claims 8-10, the vector of any of claims 11-13, the viral particle of claim 14, the cell or population of cells of any of claims 15-17, or the pharmaceutical composition of claim 18, for use in medicine.

22. The polypeptide of any of claims 1-7, the nucleic acid of any of claims 8-10, the vector of any of claims 11-13, the viral particle of claim 14, the cell or population of cells of any of claims 15-17, or the pharmaceutical composition of claim 18, for use in treating or preventing cancer and/or infection.

23. The polypeptide, nucleic acid, vector, viral particle, cell or population of cells, or pharmaceutical composition for use of claim 22, wherein the cancer is a melanoma, lung carcinoma, or renal cell carcinoma.

24. The polypeptide, nucleic acid, vector, viral particle, cell or population of cells, or pharmaceutical composition for use of claim 22, wherein the infection is from Epstein Barr virus (EBV), Hepatitis B (HBV), Hepatitis C (HCV) or Human immunodeficiency virus (HIV), such as HIV-1 or HIV-2.

25. A method of treating or preventing cancer and/or infection, comprising administering to a subject in need thereof: a therapeutically effective amount of one or more polypeptide of any of claims 1-7, nucleic acid of any of claims 8-10, vector of any of claims 11-13, viral particle of claim 14, cell or

population of cells of any of claims 15-17, or pharmaceutical composition of claim 18.

Figure 1a

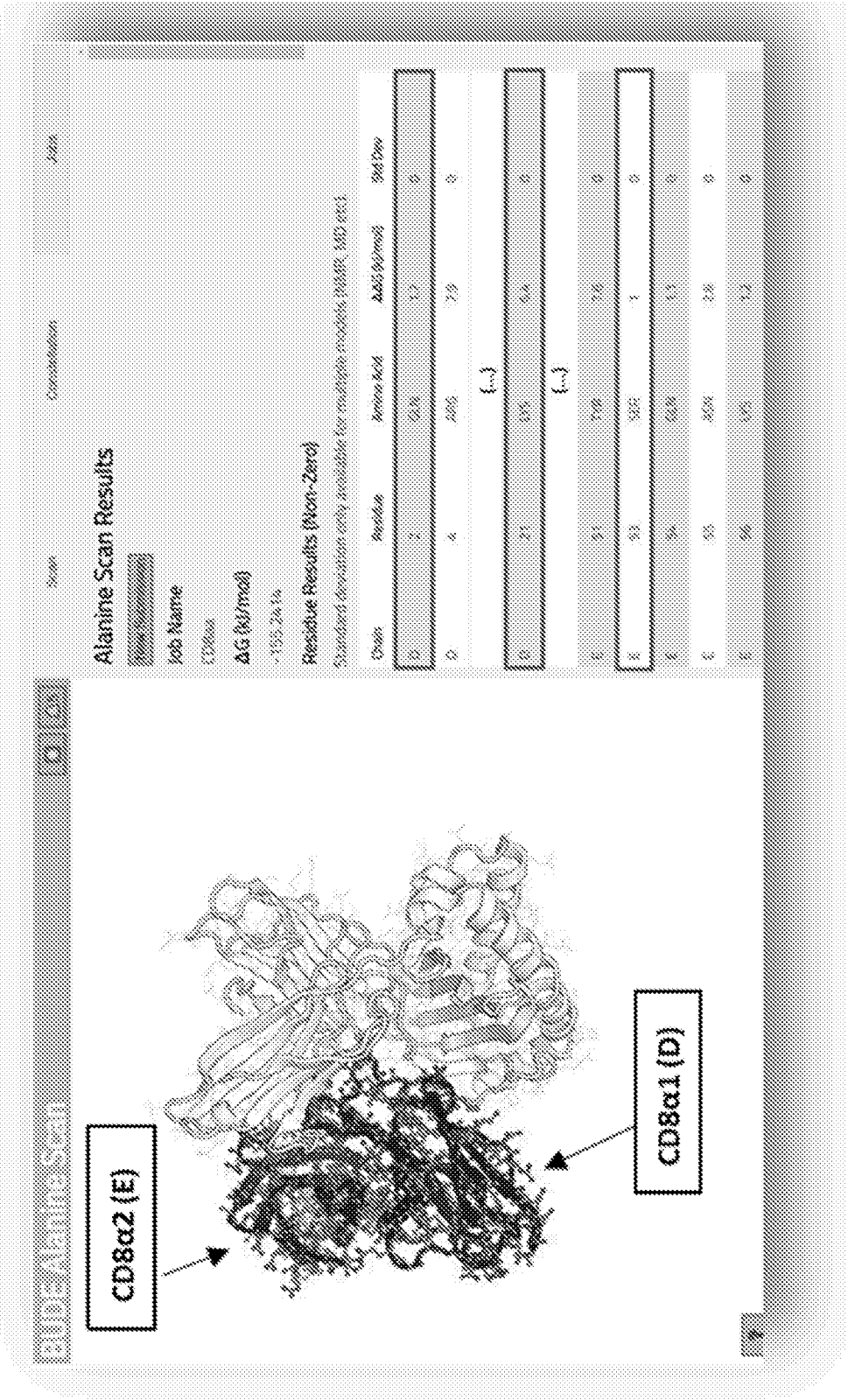


Figure 1 continued

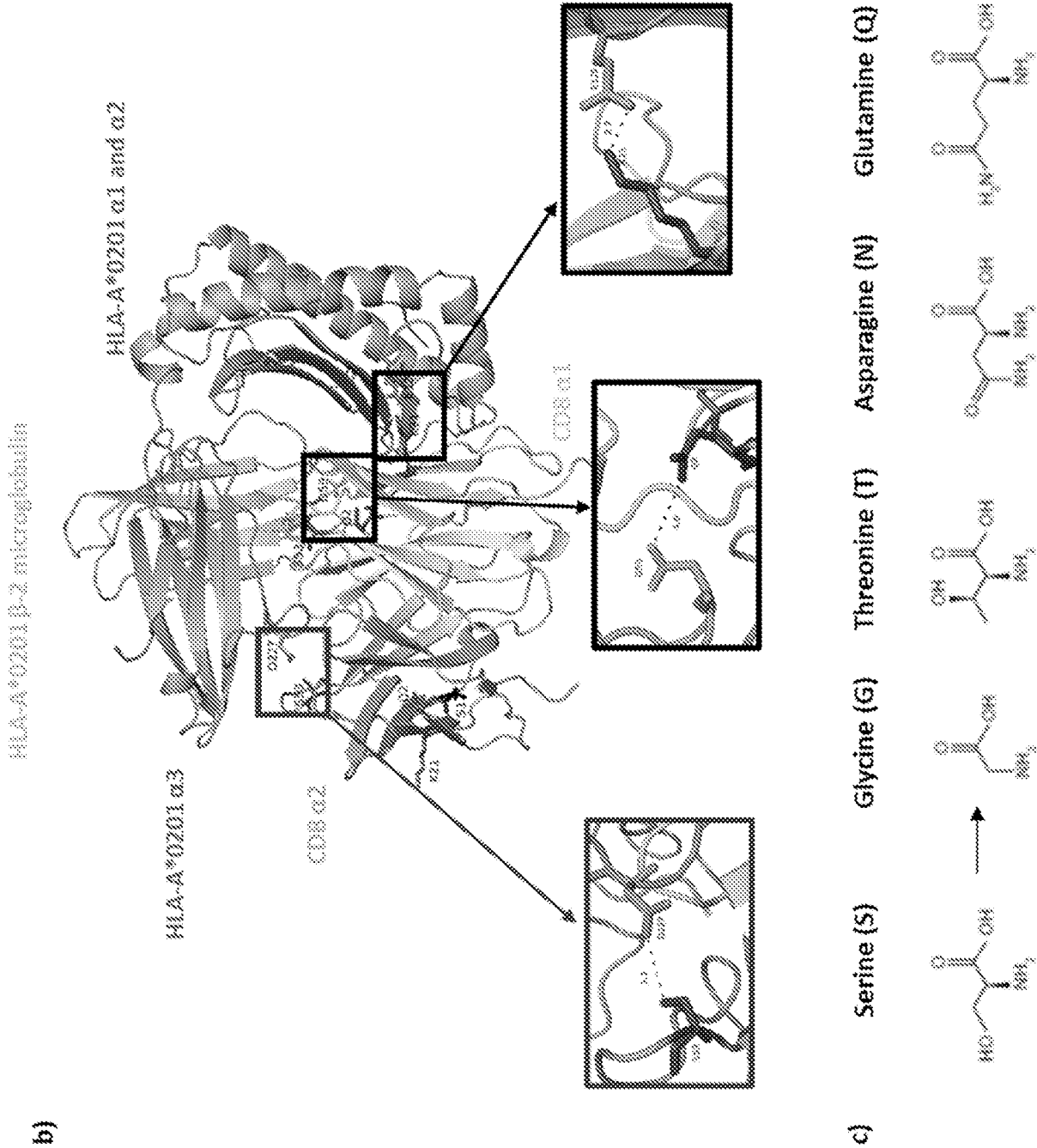


Figure 2

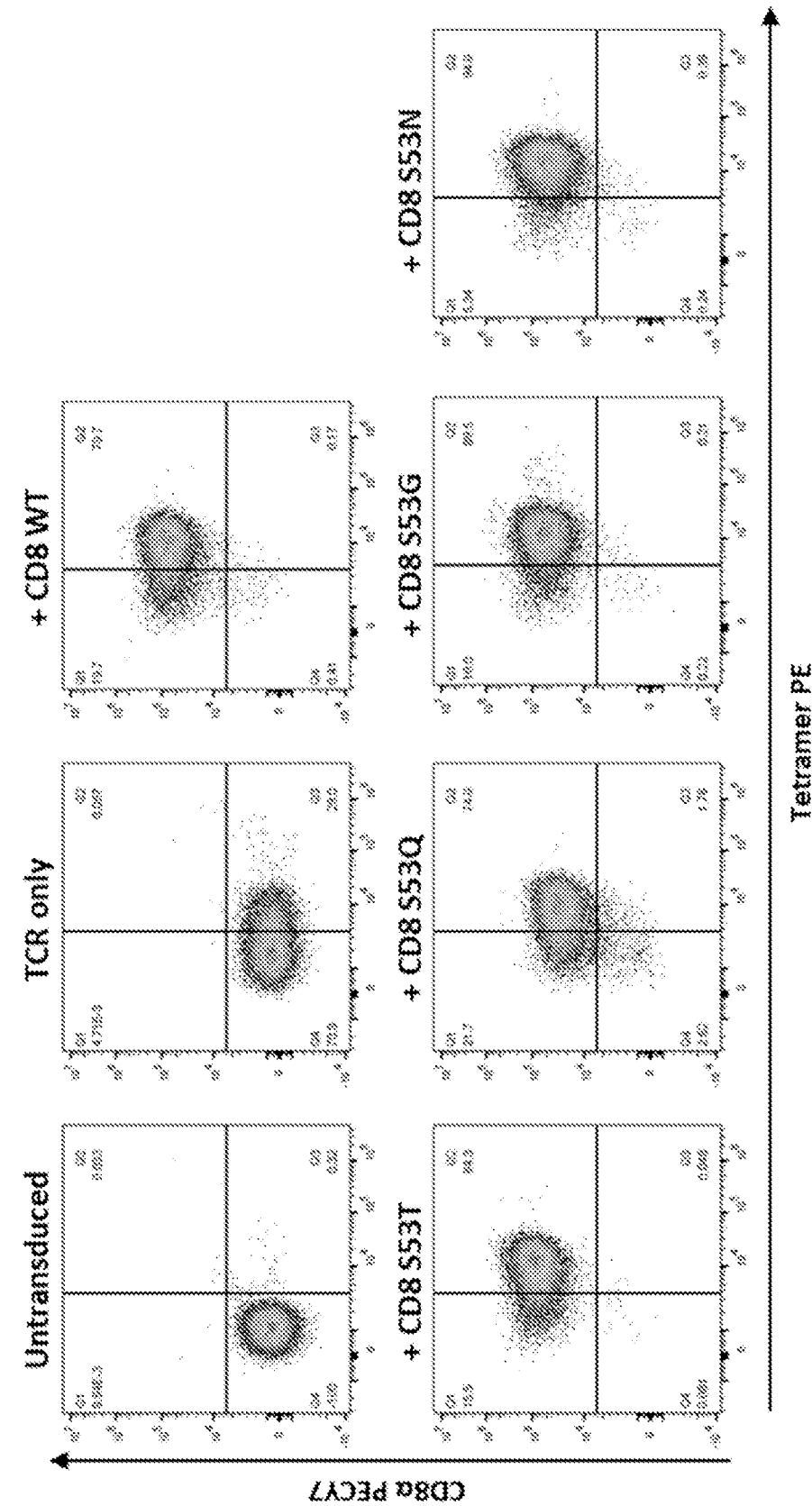


Figure 3

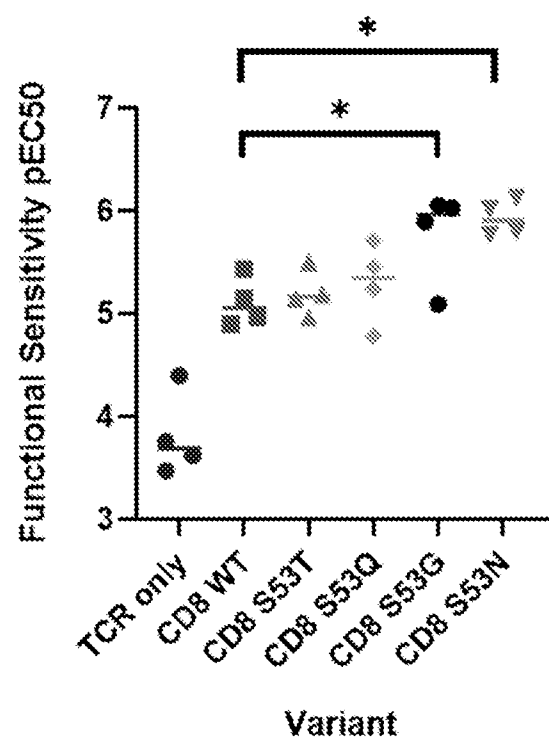


Figure 4

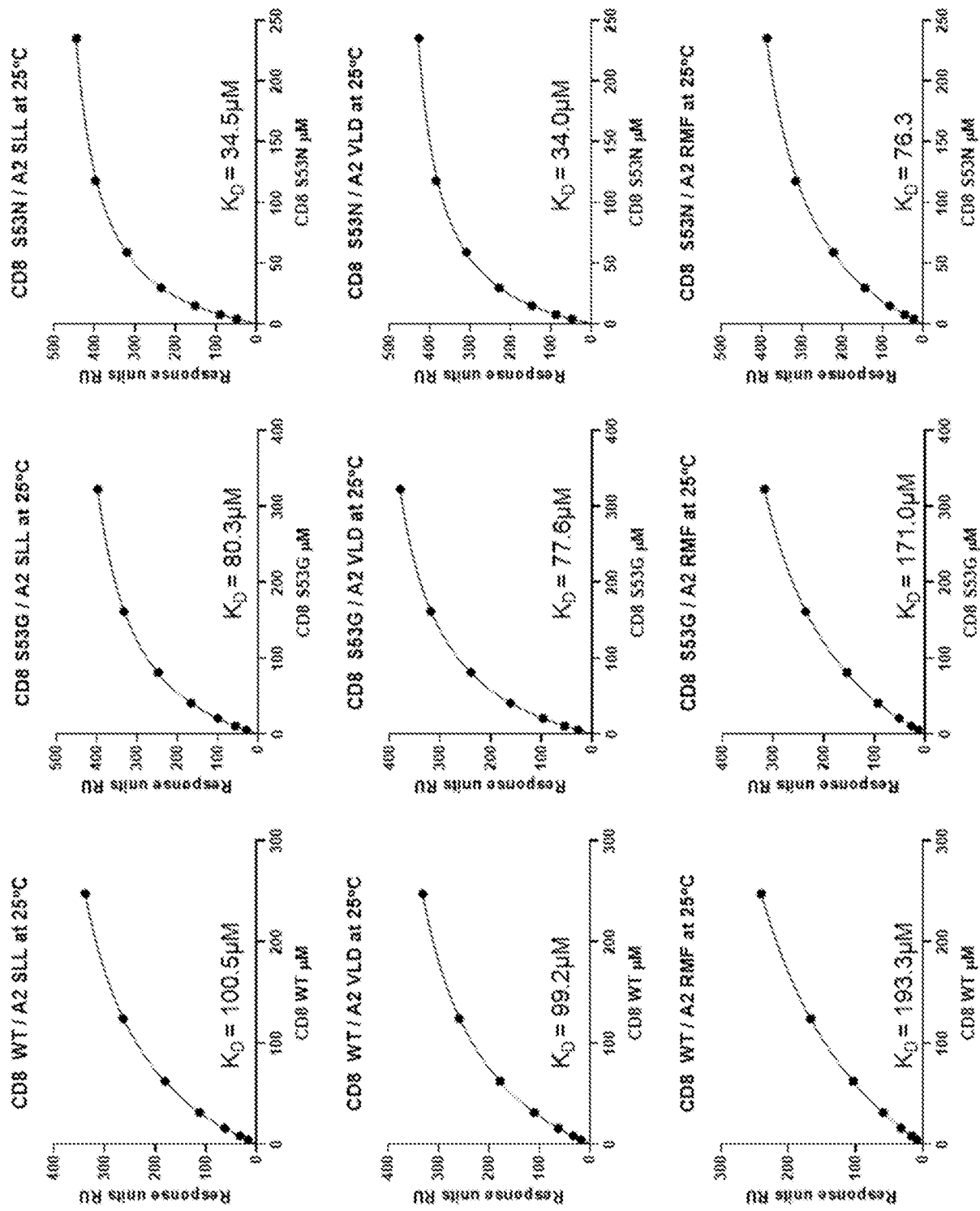


Figure 5a

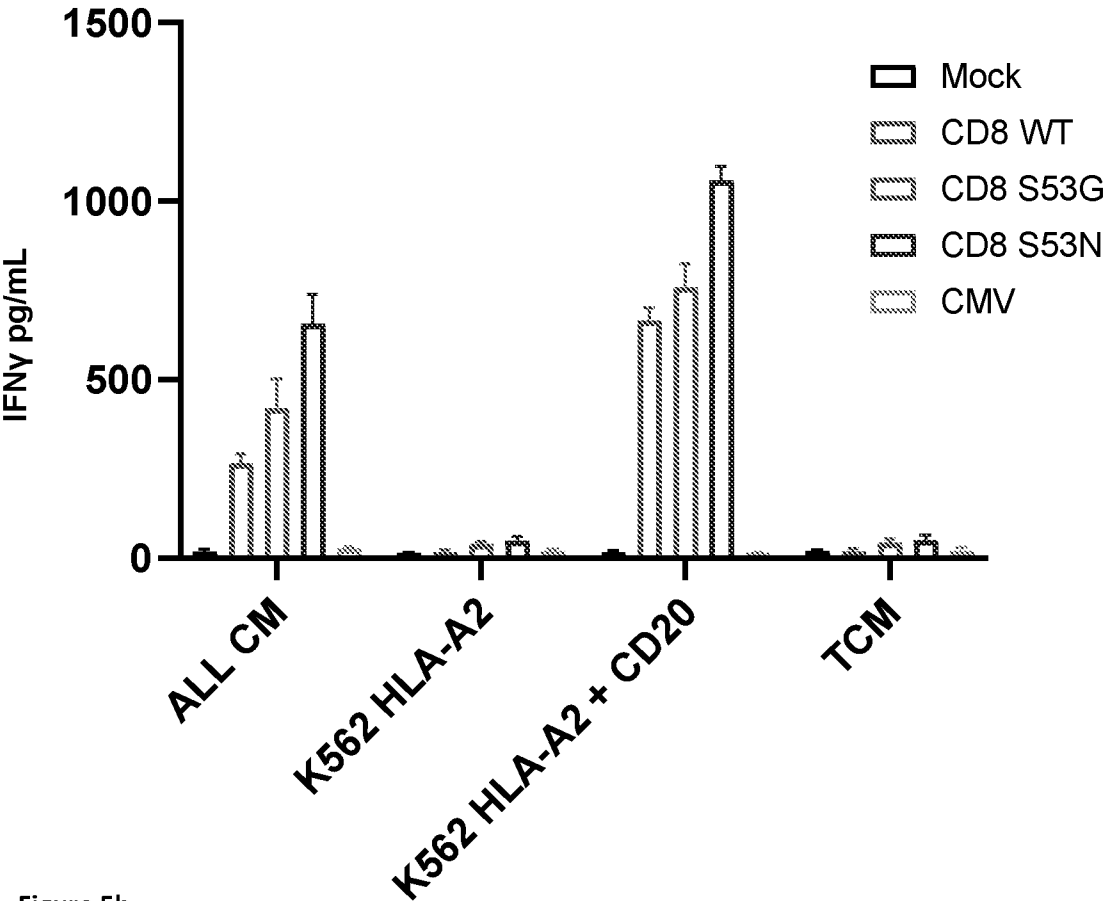


Figure 5b

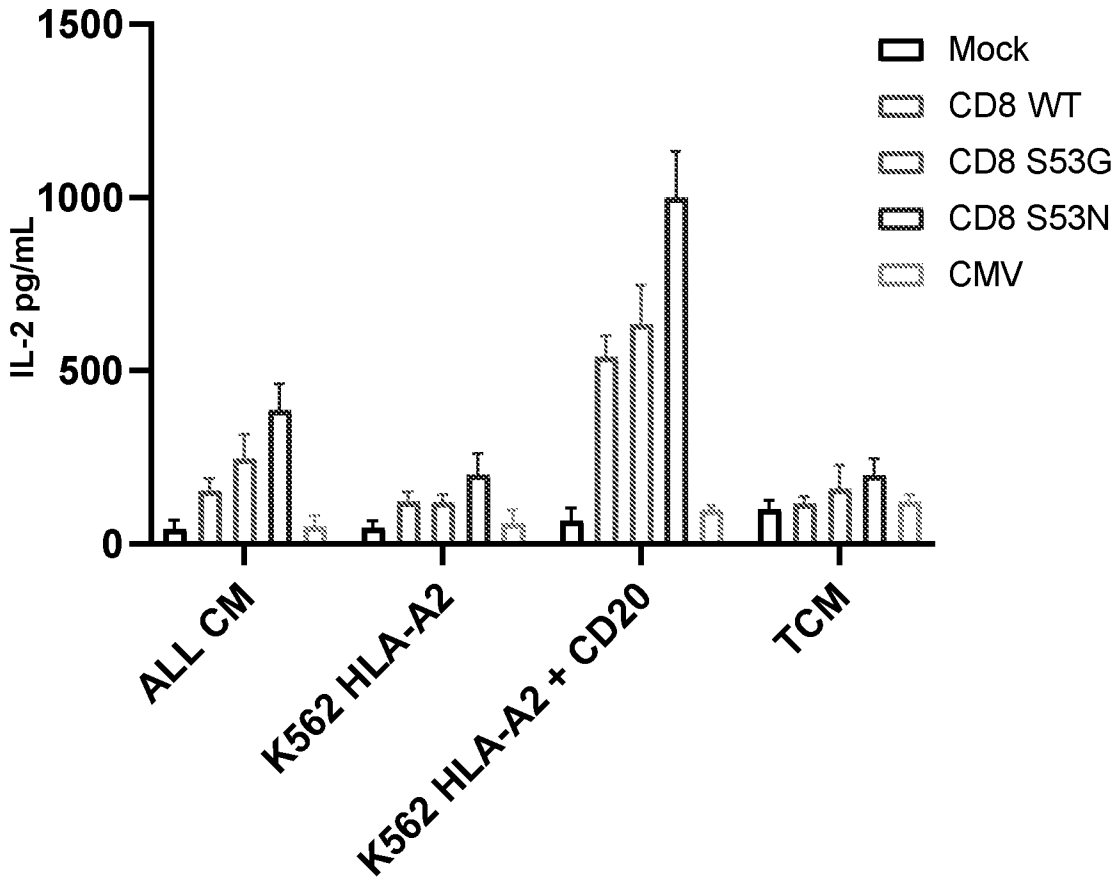
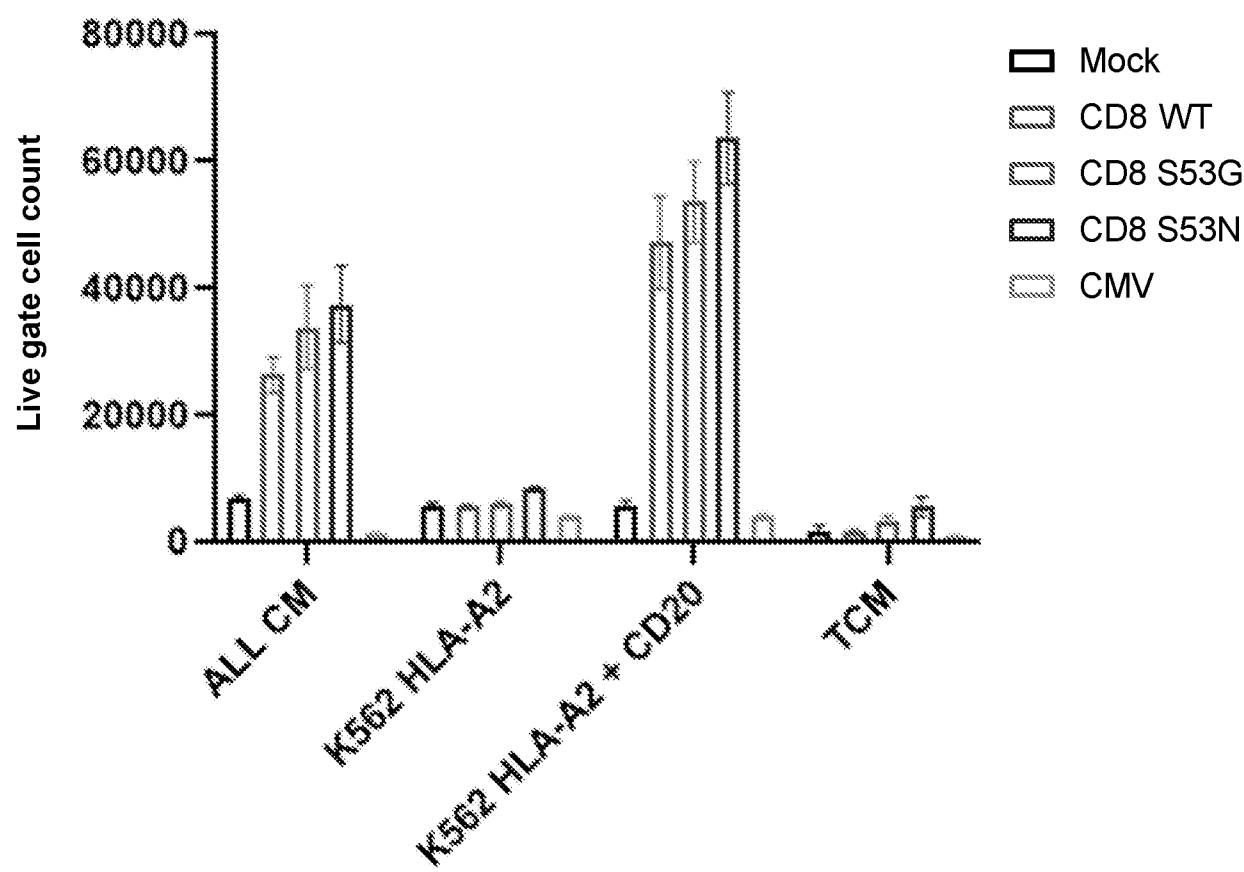


Figure 5c



# INTERNATIONAL SEARCH REPORT

International application No  
**PCT/GB2021/053037**

## A. CLASSIFICATION OF SUBJECT MATTER

**INV. C07K14/705**

**ADD.**

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

**C07K**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE**

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                          | Relevant to claim No. |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| <b>X</b>  | <b>WO 02/102852 A2 (AVIDEX LTD [GB]; JAKOBSEN BENT KARSTEN [GB]; GLICK MEIR [US])</b><br><b>27 December 2002 (2002-12-27)</b><br><b>claims 1, 3; example 1</b><br><b>abstract</b>                                                                                                                                                                                           | <b>1-25</b>           |
| <b>X</b>  | <b>COLE DAVID K. ET AL: "Computational design and crystal structure of an enhanced affinity mutant human CD8 [alpha][alpha] coreceptor", PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS, vol. 67, no. 1, 1 April 2007 (2007-04-01), pages 65-74, XP055897816, US</b><br><b>ISSN: 0887-3585, DOI: 10.1002/prot.21176</b><br><b>abstract</b><br><b>figure 1; table III</b> | <b>1-25</b>           |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

**4 March 2022**

Date of mailing of the international search report

**15/03/2022**

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Authorized officer

**Petri, Bernhard**

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2021/053037

### Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☒ forming part of the international application as filed:
    - ☒ in the form of an Annex C/ST.25 text file.
    - ☐ on paper or in the form of an image file.
  - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
    - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
    - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

## INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2021/053037

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                             |                       |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                          | Relevant to claim No. |
| X                                                    | <p>LESLEY DEVINE ET AL: "Mapping the Binding Site on CD8&amp;#946; for MHC Class I Reveals Mutants with Enhanced Binding", THE JOURNAL OF IMMUNOLOGY, vol. 177, no. 6, 15 September 2006 (2006-09-15), pages 3930-3938, XP055741952, US<br/>ISSN: 0022-1767, DOI: 10.4049/jimmunol.177.6.3930<br/>figure 1</p> <p>-----</p>                                                                                                                 | 1                     |
| A                                                    | <p>SANDERS S K ET AL: "Mutations in CD8 that affect interactions with HLA class I and monoclonal anti-CD8 antibodies.", JOURNAL OF EXPERIMENTAL MEDICINE, vol. 174, no. 2, 1 August 1991 (1991-08-01), pages 371-379, XP055897570, US<br/>ISSN: 0022-1007, DOI: 10.1084/jem.174.2.371<br/>Retrieved from the Internet:<br/>URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2118910/pdf/je1742371.pdf&gt;<br/>figures 3, 4</p> <p>-----</p> | 1-25                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

**PCT/GB2021/053037**

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| WO 02102852      A2                       | 27-12-2002          | AU 2002310633 A1           | 02-01-2003          |
|                                           |                     | EP 1404715 A2              | 07-04-2004          |
|                                           |                     | US 2004157288 A1           | 12-08-2004          |
|                                           |                     | WO 02102852 A2             | 27-12-2002          |
| -----                                     |                     |                            |                     |