

CHIMERIC CYTOKINE RECEPTOR

FIELD OF THE INVENTION

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The present invention relates to a chimeric cytokine receptor (CCR), and a cell which expresses such a chimeric cytokine receptor and optionally a chimeric antigen receptor at the cell surface.

10 BACKGROUND TO THE INVENTION

Chimeric antigen receptors (CARs)

15 A number of immunotherapeutic agents have been described for use in cancer treatment, including therapeutic monoclonal antibodies (mAbs), bi-specific T-cell engagers and chimeric antigen receptors (CARs).

Chimeric antigen receptors are proteins which graft the specificity of a monoclonal antibody (mAb) to the effector function of a T-cell. Their usual form is that of a type I
20 transmembrane domain protein with an antigen recognizing amino terminus, a spacer, a transmembrane domain all connected to a compound endodomain which transmits T-cell survival and activation signals.

The most common form of these molecules are fusions of single-chain variable
25 fragments (scFv) derived from monoclonal antibodies which recognize a target antigen, fused via a spacer and a trans-membrane domain to a signaling endodomain. Such molecules result in activation of the T-cell in response to recognition by the scFv of its target. When T cells express such a CAR, they recognize and kill target cells that express the target antigen. Several CARs have
30 been developed against tumour associated antigens, and adoptive transfer approaches using such CAR-expressing T cells are currently in clinical trial for the treatment of various cancers.

CAR-based approaches to treat Prostate Cancer

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Prostate cancer is the second most common cancer in men worldwide, and the sixth leading cause of cancer-related death. Globally, there are approximately 1,100,000

new cases and 300,000 mortalities every year, comprising 4 percent of all cancer deaths. It is estimated that 1 in every 6 men will be diagnosed with the disease during his lifetime.

5 Initial treatment for prostate cancer may consist of surgery, radiation, or hormone therapy, or any combination of each. Hormone therapy consists of lowering the levels of testosterone, the male hormone that fuels out-of-control cell growth. Chemotherapy is typically reserved for advanced-stage cancers.

10 When prostate cancers grow despite the lowering of testosterone levels by hormone therapy, treatment options are limited. Typically, the cancer vaccine sipuleucel-T (Provenge®) a dendritic cell-based therapeutic cancer vaccine designed to induce an immune response targeted against the prostatic acid phosphatase ((PAP) antigen), a radiopharmaceutical agent (such as radium-223 chloride), secondary hormone
15 therapies (such as abiraterone or enzalutamide), and/or chemotherapies (docetaxel and cabazitaxel) are added to the hormonal therapy in sequence. While each of these treatments can delay growth of the cancer for several months and palliate symptoms produced by the disease, the disease ultimately becomes resistant to them.

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Preclinically, two antigens associated with prostate cancer have been targeted with CAR T-cell based therapies: prostate-specific membrane antigen (PSMA) and prostate stem cell antigen (PSCA).

25 Mice treated with PSCA CAR-engineered T cells showed delayed tumour growth (Hillerdal et al (2014) BMC Cancer 14:30; and Abate-Daga et al (2014) 25:1003-1012). Although the cells showed high in vitro cytotoxicity, in vivo, tumour growth was delayed but tumour-bearing mice were not cured.

30 This may be because, *in vivo*, CAR T-cells struggle to overcome the hostile microenvironment of a carcinoma. In particular CAR T-cells may fail to engraft and expand within a prostate cancer tumour bed.

CAR T-cell persistence and activity can be enhanced by administration of cytokines,
35 or by the CAR T-cells producing cytokines constitutively. However, these approaches have limitations: systemic administration of cytokines can be toxic; constitutive production of cytokines may lead to uncontrolled proliferation and transformation

(Nagarkatti et al (1994) PNAS 91:7638-7642; Hassuneh et al (1997) Blood 89:610-620).

There is therefore a need for alternative CAR T-cell approaches, which facilitate engraftment and expansion of T cells to counteract the effects of the hostile tumour microenvironment.

On-target off-tumour toxicity

It is relatively rare for the presence of a single antigen effectively to describe a cancer, which can lead to a lack of specificity.

Most cancers cannot be differentiated from normal tissues on the basis of a single antigen. Hence, considerable “on-target off-tumour” toxicity occurs whereby normal tissues are damaged by the therapy. For instance, whilst targeting CD20 to treat B-cell lymphomas with Rituximab, the entire normal B-cell compartment is depleted, whilst targeting CD52 to treat chronic lymphocytic leukaemia, the entire lymphoid compartment is depleted, whilst targeting CD33 to treat acute myeloid leukaemia, the entire myeloid compartment is damaged etc.

The predicted problem of “on-target off-tumour” toxicity has been borne out by clinical trials. For example, an approach targeting ERBB2 caused death to a patient with colon cancer metastatic to the lungs and liver. ERBB2 is over-expressed in colon cancer in some patients, but it is also expressed on several normal tissues, including heart and normal vasculature.

There is therefore a need for improved approaches to cancer therapy in which such “on-target off-tumour” toxicity is reduced or eliminated.

DESCRIPTION OF THE FIGURES

Figure 1: Schematic diagram summarising the structure of various cytokine receptors, the cell types which produce the cytokines and the cell types which express the cytokine receptors.

Figure 2: Schematic diagram showing proposed chimeric cytokine receptor

(a) Cytokine IL2 and IL7 cytokine receptors signal through a common gamma chain and a cytokine specific alpha/beta chain.

(b) One implementation of a chimeric cytokine receptor is to replace the ectodomain of the cytokine alpha/beta and gamma chain with different scFvs (or any other suitable binder) which recognize different epitopes of PSA.

(c) An alternative approach is to replace the ectodomains of alpha/beta and gamma with the VH / VL of a PSA specific antibody, where both VH and VL are involved in binding so that binding brings them together.

Figure 3: Aggregation-based cytokine signalling enhancer

Schematic diagram showing a chimeric cytokine receptor and CAR combination system. The cell comprises two chimeric cytokine receptors which bind different epitopes on the same soluble ligand. In the absence of soluble ligand (e.g. PSA) but the presence of the cell-membrane antigen (e.g. PSMA) signalling occurs through the CAR. In the presence of the soluble ligand, aggregation of the two chimeric cytokine receptors occurs, leading to cytokine-based signal enhancement.

Figure 4: Theoretical construct map for the chimeric cytokine receptor/CAR combination system illustrated in Figure 3.

Figure 5: Schematic diagram illustrating an example of a structure for the chimeric transmembrane protein of the present invention. The chimeric transmembrane protein comprises a dimerization domain and a cytokine receptor endodomain. The embodiment shown has a “Fab” type architecture, as the dimerization domain comprises antibody-type heavy and light chain constant regions. Constant dimerization between these domains brings together the IL2 receptor common γ chain with either the IL-2 receptor β chain or the IL-7 receptor α chain, leading to constitutive cytokine signalling.

Figure 6: IL-2 signalling by the chimeric transmembrane protein.

Two chimeric transmembrane proteins having the general structure shown in Figure 5 were tested for their ability to induce IL-2 signalling. One chimeric transmembrane protein comprised an IL2 receptor endodomain and the other comprised an IL-7 receptor endodomain. IL-2 signalling was tested using the murine cell line CTLL2 which is dependent on IL-2 signalling for growth. As a positive control, CTLL2 cells were cultured with 100 u/mL murine IL2. Cells expressing the chimeric transmembrane protein comprising the IL2 receptor endodomain (Fab_IL2endo)

supported CTLL2 cell survival and growth, whereas cells expressing the chimeric transmembrane protein comprising the IL-7 receptor (Fab_IL7endo) did not.

Figure 7: Schematic diagram illustrating panel of PSA chimeric cytokine receptors

A panel of chimeric cytokine receptors (CCRs) targeting PSA was developed using scFvs derived from two antibodies which bind to different PSA epitopes: 5D5A5 and 5D3D11.

Top-left panel: A CCR with an IL-2R endodomain having A5 on the chain with IL2R β chain and D11 on the chain with common γ chain;

Top-right panel: A CCR with an IL7R endodomain having A5 on the chain with IL7R α chain and D11 on the chain with common γ chain;

Bottom-left panel: A CCR with an IL-2R endodomain having D11 on the chain with IL2R β chain and A5 on the chain with common γ chain; and

Bottom-right hand panel: A CCR with an IL-7R endodomain having D11 on the chain with IL7R α chain and A5 on the chain with common γ chain.

A negative control was also created for each CCR, in which the IL2R γ chain was replaced by a rigid linker.

Figure 8: IL2 signalling from cells expressing a PSA chimeric cytokine receptor in the presence of PSA – CTLL2 proliferation

CTLL2 cells were transduced with constructs expressing some of the PSA chimeric cytokine receptors illustrated in Figure 7. Cells were cultured in the presence of absence of IL2 (positive control) and the presence of absence of 5ng/mL or 5 μ g/mL PSA. CTLL2 proliferation was assessed after 3 and 7 days.

The anti-PSA chimeric cytokine receptor with an IL2R endodomain supported CTLL2 cell proliferation in the absence of IL2 and the presence of PSA, but not the receptor having an IL7R endodomain or any of the CCRs comprising a rigid linker in the place of the common γ chain.

Figure 9: IL2 signalling from cells expressing a PSA chimeric cytokine receptor in the presence of PSA – CTLL2 STAT5 phosphorylation

CTLL2 cells were either left untransduced (WT); or transduced with a vector expressing a CCR against PSA (D11-CD8STK-IL2Rg_A5-Hinge-IL2Rb) or an equivalent construct having a rigid linker in the place of the common γ chain (D11-CD8STK-RL_A5-Hinge-IL2Rb). Cells were incubated with either 500 μ M Pervanadate or 500ng/mL PSA for 1 or 4 hours. Phosphorylation of Y694 of STAT5 was then investigated using phosphoflow.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have developed "chimeric cytokine receptors" (CCR) which graft the binding specificity of a non-cytokine binding molecule on to the endodomain of a cytokine receptor. The co-expression of such a CCR with a chimeric antigen receptor (CAR) helps a CAR T-cell to engraft and expand in the hostile tumour microenvironment. The requirement for the ligand for the CCR as well as the ligand for the CAR to be present add another layer of selectivity and helps prevent on-target off-tumour toxicity.

For example, they have developed a cell which co-expresses a CAR with a chimeric cytokine receptor which detects PSA and transmits an IL2/15 or an IL7 signal to the CAR T-cell. In this way, the CAR T-cell is stimulated to proliferate selectively only in a prostate cancer microenvironment, and in the absence of PSA (i.e. after the patient is in remission), the cytokine stimulation is lost.

In a first aspect, the present invention provides a chimeric cytokine receptor (CCR) comprising:

- an exodomain which binds to a ligand selected from a tumour secreted factor, a chemokine and a cell-surface antigen; and
- a cytokine receptor endodomain.

In a first embodiment of the first aspect of the invention, the chimeric cytokine receptor comprises two polypeptides:

(i) a first polypeptide which comprises:

(a) a first antigen-binding domain which binds a first epitope of the ligand

(b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

(a) a second antigen-binding domain which binds a second epitope of the ligand

(b) a second chain of the cytokine-receptor endodomain. Figure 2b illustrates such an arrangement.

Each of the first and second antigen-binding domains may be, for example, single-chain variable fragments (scFvs) or single domain binders.

In a second embodiment of the first aspect of the invention, the chimeric cytokine receptor which comprises two polypeptides:

(i) a first polypeptide which comprises:

(a) a heavy chain variable domain (VH)

(b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

(a) a light chain variable domain (VL)

(b) a second chain of the cytokine-receptor endodomain.

Figure 2c illustrates such an arrangement.

The first and second chains for the cytokine receptor endodomains may be different and may be selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

Alternatively the first and second chains for the cytokine receptor endodomains may be the same and may be selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

For example, the cytokine receptor endodomain may comprise:

(i) IL-2 receptor β -chain endodomain

(ii) IL-7 receptor α -chain endodomain;

(iii) IL-15 receptor α -chain endodomain; or

(iv) common γ -chain receptor endodomain.

The cytokine receptor endodomain may comprise (i), (ii) or (iii); and (iv).

The ligand may be a tumour secreted factor, for example a tumour secreted factor selected from: prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), vascular endothelial growth factor (VEGF) and CA125.

The ligand may be a chemokine, for example a chemokine selected from chemokine selected from: CXCL12, CCL2, CCL4, CCL5 and CCL22.

The ligand may be a cell-surface molecule, such as a transmembrane protein. The ligand may be, for example, CD22.

In a second aspect, the present invention provides a cell which comprises a chimeric cytokine receptor according to the first aspect of the invention.

The cell may comprise a first chimeric cytokine receptor and a second chimeric cytokine receptor which bind different epitopes on the same ligand.

The cell may comprise a first chimeric cytokine receptor which comprises a type I cytokine receptor endodomain α - or β -chain, and a second chimeric cytokine receptor which comprises a type I cytokine receptor endodomain γ -chain, such that when the first chimeric cytokine receptor and the second cytokine receptor bind the ligand, combined signalling through the α -/ β -chain and γ -chain occurs.

The cell may also comprise a chimeric antigen receptor, for example a chimeric antigen receptor which binds a tumour-associated cell surface antigen.

The chimeric antigen receptor may bind a cell surface antigen associated with prostate cancer, such as prostate stem-cell antigen (PSCA) or prostate-specific membrane antigen (PSMA).

Where the CCR recognises a cell-surface antigen, the CCR and CAR may recognise cell-surface antigens which are co-expressed on the same target (e.g. tumour) cell. For example, for B-cell malignancies, the CAR may recognize a cell-surface antigen such as CD19 and the CCR may recognize a molecule which is co-expressed on the target cell surface, such CD22, thereby enhancing engraftment.

In a third aspect, the present invention provides a nucleic acid sequence encoding a chimeric cytokine receptor (CCR) according to the first aspect of the invention.

In a fourth aspect the present invention provides a nucleic acid construct which comprises a first nucleic acid sequence encoding a first CCR and a second nucleic acid sequence encoding a second CCR, the nucleic acid construct having the structure:

AgB1-spacer1-TM1-endo1-coexpr-AbB2-spacer2-TM2-endo2

in which

AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CCR;

spacer 1 is a nucleic acid sequence encoding the spacer of the first CCR;

TM1 is a a nucleic acid sequence encoding the transmembrane domain of the first CCR;

endo 1 is a nucleic acid sequence encoding the endodomain of the first CCR;

coexpr is a nucleic acid sequence enabling co-expression of both CCRs

AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR;

spacer 2 is a nucleic acid sequence encoding the spacer of the second CCR;

TM2 is a a nucleic acid sequence encoding the transmembrane domain of the second CCR;

endo 2 is a nucleic acid sequence encoding the endodomain of the second CCR.

The nucleic acid construct may also encode a chimeric antigen receptor (CAR). In this embodiment, the nucleic acid construct may have the structure:

(i) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2-coexpr2-CARAgB-CARspacer-CARTM-CARendo;

(ii) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CARAgB-CARspacer-CARTM-CARendo-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2; or

(iii) CARAgB-CARspacer-CARTM-CARendo-coexpr1-CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2;

in which

CCRAgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CCR;

CCRspacer1 is a nucleic acid sequence encoding the spacer of the first CCR;

CCRTM1 is a nucleic acid sequence encoding the transmembrane domain of the first CCR;

CCRendo1 is a nucleic acid sequence encoding the endodomain of the first CCR;

CCRAgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR;

5 CCRspacer2 is a nucleic acid sequence encoding the spacer of the second CCR;

CCRTM2 is a nucleic acid sequence encoding the transmembrane domain of the second CCR;

10 CCRendo2 is a nucleic acid sequence encoding the endodomain of the second CCR;

Coexpr1 and coexpr2 are nucleic acid sequences enabling co-expression of the two flanking sequences;

CARAgB is a nucleic acid sequence encoding the antigen-binding domain of the CAR;

15 CARspacer is a nucleic acid sequence encoding the spacer of the CAR;

CARTM is a nucleic acid sequence encoding the transmembrane domain of the CAR; and

CARendo is a nucleic acid sequence encoding the endodomain of the CAR.

20 Any or all of the sequences coexpr, coexpr1, coexpr2 may encode a sequence comprising a self-cleaving peptide.

Alternative codons may be used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

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In a fifth aspect, the present invention provides a vector comprising a nucleic acid construct according to the fourth aspect of the invention.

30 The vector may be, for example, a retroviral vector or a lentiviral vector or a transposon.

In a sixth aspect, the present invention provides a kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a first CCR according to the first aspect of the invention; and

35 ii) a vector comprising a nucleic acid sequence encoding a second CCR according to the second aspect of the invention.

The kit may also comprise a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

The kit may comprise:

- i) a vector comprising a nucleic acid sequence encoding a CCR according to the first aspect of the invention; and
- ii) a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

In a seventh aspect, the present invention provides a method for making a cell according to the second aspect of the invention, which comprises the step of introducing: a nucleic acid sequence according to the third aspect of the invention; a nucleic acid construct according to the fourth aspect of the invention; a vector according to the fifth aspect of the invention; or a kit of vectors according to the sixth aspect of the invention, into a cell.

The cell may be from a sample isolated from a subject.

In an eighth aspect, there is provided a pharmaceutical composition comprising a plurality of cells according to the second aspect of the invention.

In a ninth aspect, there is provided a method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to the eighth aspect of the invention to a subject.

The method may comprise the following steps:

- (i) isolation of a cell-containing sample from a subject;
- (ii) transduction or transfection of the cells with: a nucleic acid sequence according to the third aspect of the invention; a nucleic acid construct according to the fourth aspect of the invention; a vector according to the fifth aspect of the invention; or a kit of vectors according to the sixth aspect of the invention; and
- (iii) administering the cells from (ii) to a the subject.

The sample may be a T-cell containing sample.

The disease may be a cancer.

There is also provided a pharmaceutical composition according to the eighth aspect of the invention for use in treating and/or preventing a disease.

There is also provided the use of a cell according to the second aspect of the invention in the manufacture of a medicament for treating and/or preventing a disease.

Further aspects of the invention are summarised in the following numbered paragraphs:

1. A chimeric transmembrane protein comprising:
a dimerization domain; and
a cytokine receptor endodomain.

2. A chimeric transmembrane protein according to paragraph 1, wherein the dimerization domain comprises the dimerization portion of a heavy chain constant domain (C_H) and a light chain constant domain (C_L).

3. A chimeric transmembrane protein according to any preceding paragraph, which comprises two polypeptides:

(i) a first polypeptide which comprises:

- (a) a first dimerisation domain; and
- (b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

- (a) a second dimerization domain, which dimerises with the first dimerization domain; and
- (b) a second chain of the cytokine-receptor endodomain.

4. A chimeric transmembrane protein according to paragraph 3, wherein the first and second dimerization domains either dimerise spontaneously, or in the presence of a chemical inducer of dimerization (CID).

5. A chimeric transmembrane protein according to paragraph 2, 3 or 4 which comprises two polypeptides:

(i) a first polypeptide which comprises:

- (a) a heavy chain constant domain (C_H)
- (b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

(a) a light chain constant domain (CL)

(b) a second chain of the cytokine-receptor endodomain.

5 6. A chimeric transmembrane protein according to paragraph 5 wherein the first and second chains for the cytokine receptor endodomains are different and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

10 7. A chimeric transmembrane protein according to paragraph 5 wherein the first and second chains for the cytokine receptor endodomains are the same and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

15 8. A chimeric transmembrane protein according to any preceding paragraph, wherein the cytokine receptor endodomain comprises:
(i) IL-2 receptor β -chain endodomain
(ii) IL-7 receptor α -chain endodomain; or
(iii) IL-15 receptor α -chain endodomain; and/or
(iv) common γ -chain receptor endodomain.

20 9. A chimeric transmembrane protein according to paragraph 5, wherein the first polypeptide comprises a heavy chain variable domain (VH) and a heavy chain constant domain (CH); and the second polypeptide comprises a light chain variable domain (VL) and a light chain constant domain (CL).

25 10. A chimeric transmembrane protein according to paragraph 9 which comprises a Fab exodomain.

30 11. A cell which comprises a chimeric transmembrane protein according to any preceding paragraph.

12. A cell according to paragraph 11, which also comprises a chimeric antigen receptor.

35 13. A cell according to paragraph 12, wherein the chimeric antigen receptor binds a tumour-associated cell surface antigen.

14. A nucleic acid sequence encoding a chimeric transmembrane protein according to any of paragraphs 1 to 10.

15. A nucleic acid construct which comprises a first nucleic acid sequence encoding a first polypeptide as defined in paragraph 3 and a second nucleic acid sequence encoding a second polypeptide as defined in paragraph 3, the nucleic acid construct having the structure:

Dim1 -TM1-endo1-coexpr-Dim2 -TM2-endo2

in which

Dim1 is a nucleic acid sequence encoding the first dimerisation domain;

TM1 is a nucleic acid sequence encoding the transmembrane domain of the first polypeptide;

endo 1 is a nucleic acid sequence encoding the endodomain of the first polypeptide;

coexpr is a nucleic acid sequence enabling co-expression of both CCRs

Dim2 is a nucleic acid sequence encoding the second dimerization domain;

TM2 is a nucleic acid sequence encoding the transmembrane domain of the second polypeptide;

endo 2 is a nucleic acid sequence encoding the endodomain of the second polypeptide.

16. A nucleic acid construct according to paragraph 15 which also encodes a chimeric antigen receptor (CAR).

17. A nucleic acid construct according to paragraph 15 or 16, wherein coexpr encodes a sequence comprising a self-cleaving peptide.

18. A nucleic acid construct according to any of paragraphs 15 to 17, wherein alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

19. A vector comprising a nucleic acid construct according to any of paragraphs 15 to 18.

20. A retroviral vector or a lentiviral vector or a transposon according to paragraph 19.

21. A kit which comprises:

5 i) a vector comprising a nucleic acid sequence encoding a first polypeptide as defined in paragraph 3; and

ii) a vector comprising a nucleic acid sequence encoding a second polypeptide as defined in paragraph 3.

10 22. A kit according to paragraph 21 which also comprises a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

23. A kit which comprises:

15 i) a vector comprising a nucleic acid sequence encoding a chimeric transmembrane protein as defined in any of paragraphs 1 to 10; and

ii) a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

20 24. A method for making a cell according to any of paragraphs 11 to 13, which comprises the step of introducing: a nucleic acid sequence according to paragraph 14; a nucleic acid construct according to any of paragraphs 15 to 18; a vector according to paragraph 19 or 20; or a kit of vectors according to any paragraphs 21 to 23, into a cell.

25 25. A method according to paragraph 24, wherein the cell is from a sample isolated from a subject.

26. A pharmaceutical composition comprising a plurality of cells according to any of paragraphs 11 to 13.

30 27. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to paragraph 26 to a subject.

35 28. A method according to claim 27, which comprises the following steps:
(i) isolation of a cell-containing sample from a subject;

(ii) transduction or transfection of the cells with: a nucleic acid sequence according to paragraph 14; a nucleic acid construct according to any of paragraphs 15 to 18; a vector according to paragraph 19 or 20; or a kit of vectors according to any paragraphs 21 to 23; and

5 (iii) administering the cells from (ii) to a the subject.

29. A method according to paragraph 28, wherein the sample is a T-cell containing sample.

10 30. A method according to paragraph 28 or 29, wherein the disease is a cancer.

31. A pharmaceutical composition according to paragraph 26 for use in treating and/or preventing a disease.

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32. The use of a cell according to any of paragraphs 11 to 13 in the manufacture of a medicament for treating and/or preventing a disease.

DETAILED DESCRIPTION

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CHIMERIC CYTOKINE RECEPTOR (CCR)

A chimeric cytokine receptor (CCR) is a molecule which comprises a cytokine receptor endodomain and a heterologous ligand-binding exodomain. The heterologous exodomain binds a ligand other than the cytokine for which the cytokine receptor from which the endodomain was derived is selective. In this way, it is possible to alter the ligand specificity of a cytokine receptor by grafting on a heterologous binding specificity.

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30 A chimeric cytokine receptor comprises:

- (i) a ligand binding exodomain;
- (ii) an optional spacer;
- (iii) a transmembrane domain; and
- (iv) a cytokine-receptor endodomain.

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CYTOKINE RECEPTORS AND SIGNALLING

Many cell functions are regulated by members of the cytokine receptor superfamily. Signalling by these receptors depends upon their association with Janus kinases (JAKs), which couple ligand binding to tyrosine phosphorylation of signalling proteins recruited to the receptor complex. Among these are the signal transducers and activators of transcription (STATs), a family of transcription factors that contribute to the diversity of cytokine responses.

When the chimeric cytokine receptor of the invention binds its ligand, one or more of the following intracellular signaling pathways may be initiated:

- (i) the JAK-STAT pathway
- (ii) the MAP kinase pathway; and
- (iii) the Phosphoinositide 3-kinase (PI3K) pathway.

The JAK-STAT system consists of three main components: (1) a receptor (2) Janus kinase (JAK) and (3) Signal Transducer and Activator of Transcription (STAT).

JAKs, which have tyrosine kinase activity, bind to cell surface cytokine receptors. The binding of the ligand to the receptor triggers activation of JAKs. With increased kinase activity, they phosphorylate tyrosine residues on the receptor and create sites for interaction with proteins that contain phosphotyrosine-binding SH2 domains. STATs possessing SH2 domains capable of binding these phosphotyrosine residues are recruited to the receptors, and are themselves tyrosine-phosphorylated by JAKs. These phosphotyrosines then act as binding sites for SH2 domains of other STATs, mediating their dimerization. Different STATs form hetero- or homodimers. Activated STAT dimers accumulate in the cell nucleus and activate transcription of their target genes.

CYTOKINE RECEPTOR ENDODOMAIN

The chimeric cytokine receptor of the present invention comprises an endodomain which causes "cytokine-type" cell signalling (either alone or when in the presence of another chimeric cytokine receptor) when the exodomain binds its ligand.

The endodomain may be a cytokine receptor endodomain.

The endodomain may be derived from a type I cytokine receptor. Type I cytokine receptors share a common amino acid motif (WSXWS) in the extracellular portion adjacent to the cell membrane.

- 5 The endodomain may be derived from a type II cytokine receptor. Type II cytokine receptors include those that bind type I and type II interferons, and those that bind members of the interleukin-10 family (interleukin-10, interleukin-20 and interleukin-22).
- 10 Type I cytokine receptors include:
 - (i) Interleukin receptors, such as the receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23 and IL-27;
 - (ii) Colony stimulating factor receptors, such as the receptors for erythropoietin, GM-CSF, and G-CSF; and
 - 15 (iii) Hormone receptor/neuropeptide receptor, such as hormone receptor and prolactin receptor

Members of the type I cytokine receptor family comprise different chains, some of which are involved in ligand/cytokine interaction and others that are involved in signal transduction. For example the IL-2 receptor comprises an α -chain, a β -chain and a γ -chain.

The IL-2 receptor common gamma chain (also known as CD132) is shared between the IL-2 receptor, IL-4 receptor, IL-7 receptor, IL-9 receptor, IL-13 receptor and IL-15 receptor.

IL-2

IL-2 binds to the IL-2 receptor, which has three forms, generated by different combinations of three different proteins, often referred to as "chains": α , β and γ ; these subunits are also parts of receptors for other cytokines. The β and γ chains of the IL-2R are members of the type I cytokine receptor family.

The three receptor chains are expressed separately and differently on various cell types and can assemble in different combinations and orders to generate low, intermediate, and high affinity IL-2 receptors.

The α chain binds IL-2 with low affinity, the combination of β and γ together form a complex that binds IL-2 with intermediate affinity, primarily on memory T cells and NK cells; and all three receptor chains form a complex that binds IL-2 with high affinity ($K_d \sim 10^{-11}$ M) on activated T cells and regulatory T cells.

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The three IL-2 receptor chains span the cell membrane and extend into the cell, thereby delivering biochemical signals to the cell interior. The alpha chain does not participate in signalling, but the beta chain is complexed with the tyrosine phosphatase JAK1. Similarly the gamma chain complexes with another tyrosine kinase called JAK3. These enzymes are activated by IL-2 binding to the external domains of the IL-2R.

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IL-2 signalling promotes the differentiation of T cells into effector T cells and into memory T cells when the initial T cells are also stimulated by an antigen. Through their role in the development of T cell immunologic memory, which depends upon the expansion of the number and function of antigen-selected T cell clones, they also have a key role in long-term cell-mediated immunity.

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The chimeric cytokine receptor of the present invention may comprise the IL-2 receptor β -chain and/or the IL-2 receptor (i.e. common) γ -chain

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The amino acid sequences for the endodomains of the IL-2 β -chain and common γ -chain are shown as SEQ ID No. 1 and 2

SEQ ID No. 1: Endodomain derived from human common gamma chain:

ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPCKGG
ALGEGPGASPCNQHSPYWAPPCYTLKPET

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SEQ ID No. 2: Endodomain derived from human IL-2R β :

NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAP
EISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEAC
QVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSL
LGGPSPSTAPGGSGAGEERMPPSLQERVPRDWDQPPLGPPTPGVPDLVDFQPP
PELVLREAGEEVPDAGPREGVSFPSRPPGQGEFRALNARLPLNTDAYLSLQELQ
GQDPHTLV

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35

The term "derived from" means that the endodomain of the chimeric cytokine receptor of the invention has the same sequence as the wild-type sequence of the endogenous molecule, or a variant thereof which retains the ability to form a complex with JAK-1 or JAK-3 and activate one of the signalling pathways mentioned above.

5

A "variant" sequence having at least 80, 85, 90, 95, 98 or 99% sequence identity to the wild-type sequence (e.g. SEQ ID Nos. 1 or 2), providing that the variant sequence retains the function of the wild-type sequence i.e. the ability to form a complex with JAK-1 or JAK-3 and activate, for example, the JAK-STAT signalling pathway.

10

The percentage identity between two polypeptide sequences may be readily determined by programs such as BLAST which is freely available at <http://blast.ncbi.nlm.nih.gov>.

15 IL-7

The interleukin-7 receptor is made up of two chains: the interleukin-7 receptor- α chain (CD127) and common- γ chain receptor (CD132). The common- γ chain receptors is shared with various cytokines, including interleukin-2, -4, -9, and -15. Interleukin-7 receptor is expressed on various cell types, including naive and memory T cells.

20

The interleukin-7 receptor plays a critical role in the development of lymphocytes, especially in V(D)J recombination. IL-7R also controls the accessibility of a region of the genome that contains the T-cell receptor gamma gene, by STAT5 and histone acetylation. Knockout studies in mice suggest that blocking apoptosis is an essential function of this protein during differentiation and activation of T lymphocytes.

25

The chimeric cytokine receptor of the present invention may comprise the IL-7 receptor α -chain and/or the IL-7 receptor (i.e. common) γ -chain, or a variant thereof.

30

The amino acid sequence for the endodomain of the IL-7 α -chain is shown as SEQ ID No. 3.

SEQ ID No. 3 - Endodomain derived from human IL-7R α :

35

KKRIKPIVWPSLPDHKKTLLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEG
FLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACD

APILSSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQ
 PILTSLGSNQEEAYVTMSSFYQNNQ

IL-15

5

Interleukin 15 (IL-15) is a cytokine with structural similarity to IL-2. Like IL-2, IL-15 binds to and signals through a complex composed of IL-2/IL-15 receptor beta chain (CD122) and the common gamma chain (gamma-C, CD132). IL-15 is secreted by mononuclear phagocytes (and some other cells) following viral infection. IL-15 induces cell proliferation of natural killer cells.

10

Interleukin-15 receptor consists of an interleukin 15 receptor alpha subunit and shares common beta and gamma subunits with the IL-2 receptor.

15 SPACER

The chimeric cytokine receptor of the present invention may comprise a spacer to connect the antigen-binding domain with the transmembrane domain and spatially separate the antigen-binding domain from the endodomain. A flexible spacer allows to the antigen-binding domain to orient in different directions to enable antigen binding.

20

Where the cell of the present invention comprises two or more chimeric cytokine receptors, the spacers may be the same or different. Where the cell of the present invention comprises a chimeric cytokine receptor (CCR) and a chimeric antigen receptor (CAR), the spacer of the CCR and the CAR may be different, for example, having a different length. The spacer of the CAR may be longer than the spacer of the or each CCR.

25

The spacer sequence may, for example, comprise an IgG1 Fc region, an IgG1 hinge or a CD8 stalk. The linker may alternatively comprise an alternative linker sequence which has similar length and/or domain spacing properties as an IgG1 Fc region, an IgG1 hinge or a CD8 stalk.

30

A human IgG1 spacer may be altered to remove Fc binding motifs.

35

Examples of amino acid sequences for these spacers are given below:

SEQ ID No. 4 (hinge-CH₂CH₃ of human IgG1)

AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
5 LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSGSSFFLYSKLTVDKSRWQQGNVDFSCSVMHEALHNHYTQK
SLSLSPGKKD

SEQ ID No. 5 (human CD8 stalk):

10 TTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

SEQ ID No. 6 (human IgG1 hinge):

AEPKSPDKTHTCPPCPKDPK

15 TRANSMEMBRANE DOMAIN

The transmembrane domain is the sequence of a CCR that spans the membrane. It may comprise a hydrophobic alpha helix. The transmembrane domain may be derived from CD28, which gives good receptor stability.

20

Alternatively the transmembrane domain may be derived from a cytokine receptor, for example the same cytokine from which the endodomain is derived.

The transmembrane domain may, for example be derived from IL-2R, IL-7R or IL-
25 15R.

SEQ ID No. 7 - Transmembrane derived from human common gamma chain:

VVISVGSMGLIISLLCVYFWL

30 SEQ ID No. 8 - Transmembrane derived from human IL-2R β :

IPWLGHLLVGLSGAFGFILVYLLI

SEQ ID No. 9 - Transmembrane derived from human IL-7R α :

PILLTISILSFFSVALLVILACVLW

35

SEQ ID No. 10 - Transmembrane derived from human IL-15R α :

AISTSTVLLCGLSAVSLLACYL

LIGAND-BINDING EXODOMAIN

The ligand binding domain comprises an antigen binding domain. The antigen binding domain binds the target ligand for the CCR, i.e. the tumour secreted factor or chemokine or cell surface antigen.

Numerous antigen-binding domains are known in the art, including those based on the antigen binding site of an antibody, antibody mimetics, and T-cell receptors. For example, the antigen-binding domain may comprise: a single-chain variable fragment (scFv) derived from a monoclonal antibody; the binding domain from a natural receptor for the target antigen; a peptide with sufficient affinity for the target ligand; a single domain binder such as a camelid; an artificial binder single as a Darpin; or a single-chain derived from a T-cell receptor.

The term "ligand" is used synonymously with "antigen" to mean an entity which is specifically recognised and bound by the antigen-binding domain of the CCR.

Where the ligand is a tumour secreted factor, the antigen binding domain may comprise an immunoglobulin-based antigen binding site, such as an scFv or a single domain binder.

Where the ligand is a chemokine, the antigen binding domain may comprise the chemokine-binding portion of a natural receptor for the chemokine.

LIGAND

The CCR of the present invention binds ligand.

The ligand may be a soluble ligand such as a tumour secreted factor or a chemokine.

Alternatively, the ligand may be a membrane bound ligand, such as a cell surface antigen.

The term "soluble ligand" is used to indicate a ligand or antigen which is not part of or attached to a cell but which moves freely in the extracellular space, for example in a

bodily fluid of the tissue of interest. The soluble ligand may exist in a cell-free state in the serum, plasma or other bodily fluid of an individual.

The soluble ligand may be associated with the presence or pathology of a particular disease, such as cancer.

The soluble ligand may be part of the cancer secretome, i.e. the collection of factors secreted by a tumour, be it from cancer stem cells, non-stem cells or the surrounding stroma. The soluble ligand may be secreted or shed by tumour cells (see next section).

The soluble ligand may be characteristic of a disease or of diseased tissue. It may be found exclusively, or at a higher level in a subject having the disease vs a healthy subject; or in diseased tissue vs healthy tissue. The soluble ligand may be expressed at at least a 2-fold, 5-fold, 10-fold, 100-fold, 1000-fold, 10,000-fold or 100,000 fold higher level a subject having the disease vs a healthy subject; or in diseased tissue vs healthy tissue.

The terms “cell-surface antigen” and “cell-surface ligand” is used synonymously with “membrane-bound antigen” and “membrane-bound ligand” to mean a ligand which is attached to or expressed on the surface of the cell. The cell-surface ligand may, for example, be a transmembrane protein.

The cell on which the cell-surface ligand is found may be a target cell, such as a cancer cell.

The cell-surface ligand may be associated with the presence or pathology of a particular disease, such as cancer. Alternatively the cell-surface ligand may be characteristic of the cell type of the target cell (e.g. B-cell) without being necessarily associated with the diseased state.

Where the cell-surface ligand is characteristic of a disease or of diseased tissue it may be found exclusively, or at a higher level on the relevant cells a subject having the disease vs a healthy subject; or in diseased tissue vs healthy tissue. The cell-surface ligand may be expressed at at least a 2-fold, 5-fold, 10-fold, 100-fold, 1000-fold, 10,000-fold or 100,000 fold higher level on a cell of a subject having the disease vs a healthy subject; or in diseased tissue vs healthy tissue.

TUMOUR SECRETED FACTOR

The ligand recognised by the CCR may be a soluble ligand secreted by or shedded
5 from a tumour.

This “tumour secreted factor” may, for example, be prostate-specific antigen (PSA),
carcinoembryonic antigen (CEA), vascular endothelial growth factor (VEGF) or
Cancer Antigen -125 (CA-125).

10 The tumour secreted factor may be a soluble ligand which is not a cytokine. The
CCR of the present invention therefore grafts the binding specificity for a non-cytokine
ligand on to the endodomain of a cytokine receptor.

15 PROSTATE-SPECIFIC ANTIGEN (PSA)

The soluble ligand may be prostate-specific antigen (PSA).

Prostate-specific antigen (PSA), also known as gamma-seminoprotein or kallikrein-3
20 (KLK3), is a glycoprotein enzyme encoded in humans by the KLK3 gene. PSA is a
member of the kallikrein-related peptidase family and is secreted by the epithelial
cells of the prostate gland.

PSA is present in small quantities in the serum of men with healthy prostates, but is
25 elevated in individuals with prostate cancer and other prostate disorders.

PSA is a 237-residue glycoprotein and is activated by KLK2. Its physiological role is
the liquefaction of the coagulum components of the semen leading to liberation of
spermatozoa. In cancer, PSA may participate in the processes of neoplastic growth
30 and metastasis.

PSA is a chymotrypsin-like serine protease with a typical His-Asp-Ser triad and a
catalytic domain similar to those of other kallikrein-related peptidases. The crystal
structure of PSA has been obtained i) in complex with the monoclonal antibody (mAb)
35 8G8F5 and ii) in a sandwich complex with two mAbs 5D5A5 and 5D3D11 (Stura et al
(J. Mol. Biol. (2011) 414:530-544).

Various monoclonal antibodies are known, including clones 2G2-B2, 2D8-E8, IgG1/K described in Bavat et al Avicenna J. Med. Biotechnol. 2015, 7:2-7; and Leinonen (2004) 289:157-67.

- 5 The CCR of the present invention may, for example, comprise the 6 CDRs or the VH and/or VL domain(s) from a PSA-binding mAb such as 8G8F5, 5D5A5 or 5D3D11.

Where the CCR comprises two antigen binding specificities, binding different epitopes on PSA, one may be based on, for example 5D3D11 and one may be based on, for
10 example, 5D5A5.

The amino acid sequences for 5D3D11 and 5D5A5 VH and VL are given below. The complementarity determining regions (CDRs) are highlighted in bold.

- 15 5D3D11 VH (SEQ ID No. 11)

QVQLQQSGPELVKPGASVKISCKVSGYAIS**SSWMN**WVKQRPGQGLEWIG**RIYPGD**
GDTKYNGKFKDKATLTVDKSSSTAYMQLSSLTSVDSAVYFCAR**DGYRYYFDY**WGQ
GTSVTVSS

- 20 5D3D11 VL (SEQ ID No. 12)

DIVMTQTAPSVFVTPGESVSIS**CRSSKSL****HSNGNTYLY**WFLQRPGQSPQLLIY**RMS**
NLASGVDPDRFSGSGSGTDFTLRISRVEAEDVGVYYC**MQHLEYPVT**FGAGTKVEIK

- 25 5D5A5 VH (SEQ ID No. 13)

QVQLQQSGAELAKPGASVKMSCKTSGYSFSS**SYWMH**WVKQRPGQGLEWIG**YINPS**
TGYTENNQKFKDKVTLTADKSSNTAYMQLNSLTSEDSAVYYCAR**SGRLYFDV**WGA
GTTVTVSS

- 30 5D5A5 VL (SEQ ID No. 14)

DIVLTQSPPSLAVSLGQRATIS**CRASESIDLYGFTFMH**WYQQKPGQPPKILY**RASNL**
ESGIPARFSGSGSRTDFTLTINPVEADDVATYYC**QQTHEDPYT**FGGGTKLEIK

ScFv based on 5D5A5 (SEQ ID No. 15)

- 35 QVQLQQSGAELAKPGASVKMSCKTSGYSFSSYWMHWWVKQRPGQGLEWIGYINPS
TGYTENNQKFKDKVTLTADKSSNTAYMQLNSLTSEDSAVYYCARSGRLYFDVWGA
GTTVTVSSGGGGSGGGGSGGGGSGGGGSDIVLTQSPPSLAVSLGQRATISCRASE

SIDLYGFTFMHWYQQKPGQPPKILYRASNL ESGIPARFSGSGSRTDFTLTINPVEAD
DVATYYCQQTHEDPYTFGGG TKLEIK

ScFv based on 5D3D11 (SEQ ID No. 16)

5 QVQLQQSGPELVKPGASVKISCKVSGYAISSSWMNWVKQRPGQGLEWIGRIYPGD
GDTKYNGKFKDKATLTVDKSSSTAYMQLSSLTSVDSAVYFCARDGYRYYFDYWGQ
GTSVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQTAPSVFVTPGESVSISCRSS
KSLHLSNGNTYLYWFLQRPQGSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLRISR
VEAEDVGVYYCMQHLEYPVTFGAGTKVEIK

10

Where a cell comprises two CCRs, the antigen-binding domain of the first CCR may comprise the 6 CDRs from 5D5A5 and the antigen-binding domain of the second CCR may comprise the 6 CDRs from 5D3D11.

15 The antigen-binding domain of the first CCR may comprise the VH and/or VL domain(s) from 5D5A5 or a variant thereof; and the antigen-binding domain of the second CCR may comprise the VH and/or VL domain(s) from 5D3D11 or a variant thereof. Variant VH and VL domains may have at least 80, 90, 95 or 99% identity to the sequences given above, provided that they retain PSA-binding activity.

20

A cell expressing a CCR which binds PSA may be useful in the treatment of prostate cancer.

CARCINOEMBRYONIC ANTIGEN (CEA)

25

The soluble ligand may be CEA.

Carcinoembryonic antigen (CEA) describes a set of highly related glycoproteins involved in cell adhesion. CEA is normally produced in gastrointestinal tissue during fetal development, but the production stops before birth. Therefore CEA is usually present only at very low levels in the blood of healthy adults. However, the serum levels are raised in some types of cancer, which means that it can be used as a tumor marker in clinical tests.

35 CEA are glycosyl phosphatidyl inositol (GPI) cell surface anchored glycoproteins whose specialized sialofucosylated glycoforms serve as functional colon carcinoma L-selectin and E-selectin ligands, which may be critical to the metastatic dissemination

of colon carcinoma cells. Immunologically they are characterized as members of the CD66 cluster of differentiation.

CEA and related genes make up the CEA family belonging to the immunoglobulin superfamily. In humans, the carcinoembryonic antigen family consists of 29 genes, 18 of which are normally expressed. The following is a list of human genes which encode carcinoembryonic antigen-related cell adhesion proteins: CEACAM1, CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7, CEACAM8, CEACAM16, CEACAM18, CEACAM19, CEACAM20, CEACAM21

Various antibodies which target CEA are described in WO 2011/034660.

A cell expressing a CCR against CEA may be useful in the treatment of, for example, colorectal cancer.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

The soluble ligand may be VEGF.

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels.

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize.

VEGF is a sub-family of the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

The VEGF family comprises in mammals five members: VEGF-A, placenta growth factor (PGF), VEGF-B, VEGF-C and VEGF-D.

Various antibodies to VEGF are known, such as bevacizumab (Avastin) and Ranibizumab (Lucentis).

5 CANCER ANTIGEN 125 (CA-125)

CA-125 is associated with ovarian cancer and is the most frequently used biomarker for ovarian cancer detection. While CA-125 is best known as a marker for ovarian cancer, it may also be elevated in other cancers, including endometrial cancer,
10 fallopian tube cancer, lung cancer, breast cancer and gastrointestinal cancer.

The sequence of human CA-125 (also known as mucin-16) is available from NCBI, Accession No. 078966.

15 A number of CA125-binding monoclonal antibodies are known, including OC125 and M11 (Nustad et al 1996, Tumour Biol. 17:196-329). In this study the specificity of 26 monoclonal antibodies against the CA 125 antigen was investigated. It was found that the CA 125 antigen carries only two major antigenic domains, which classifies the antibodies as OC125-like (group A) or M11-like (group B).

20

The chimeric cytokine receptor of the present invention may comprise an antigen-binding domain from such an antibody. A cell comprising such a CCR may be useful in the treatment of, for example, ovarian cancer.

25 The tumour secreted factor (or, if in a membrane-bound form, the transmembrane protein) may be selected from the following non-exhaustive list:

ALK gene rearrangements and overexpression giving mutated forms of ALK proteins

Alpha-fetoprotein (AFP)

30 Beta-2-microglobulin (B2M)

Beta-human chorionic gonadotropin (Beta-hCG)

BRAF V600 mutations giving mutated B-REF protein

C-kit/CD117

CA15-3/CA27.29

35 CA19-9

Calcitonin

CD20

Chromogranin A (CgA)

Cytokeratin fragment 21-1

EGFR gene mutation analysis

Estrogen receptor (ER)/progesterone receptor (PR)

5 Fibrin/fibrinogen

HE4

HER2/neu gene amplification or protein overexpression

Immunoglobulins

KRAS gene mutation analysis

10 Lactate dehydrogenase

Neuron-specific enolase (NSE)

Nuclear matrix protein 22

Programmed death ligand 1 (PD-L1)

Thyroglobulin

15 Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1)

CHEMOKINE

20 Chemokines are chemotactic cytokines. Cell migration is guided by chemokine gradients embedded and immobilized in extracellular matrix. The positively charged chemokines like CXCL12 bind to negatively charged ECM molecules. These gradients provide tracks for cancer cell and immune cell homing. The action on T cells seems to be inhibitory for the homing of cytotoxic T cells, while regulatory T cells appear to be attracted.

25

Chemokines are approximately 8-10 kilodaltons in mass and have four cysteine residues in conserved locations which are key to forming their 3-dimensional shape.

30 Some chemokines are considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to a site of infection, while others are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development.

35 Chemokines have been classified into four main subfamilies : CXC, CC, CX3C and XC. All of these proteins exert their biological effects by interacting with G protein-linked transmembrane receptors called chemokine receptors that are selectively found on the surfaces of their target cells.

The major role of chemokines is to act as a chemoattractant to guide the migration of cells. Cells that are attracted by chemokines follow a signal of increasing chemokine concentration towards the source of the chemokine. Some chemokines control cells of the immune system during processes of immune surveillance, such as directing lymphocytes to the lymph nodes so they can screen for invasion of pathogens by interacting with antigen-presenting cells residing in these tissues. Other chemokines are inflammatory and are released from a wide variety of cells in response to bacterial infection, viruses and other agents. Their release is often stimulated by pro-inflammatory cytokines such as interleukin 1. Inflammatory chemokines function mainly as chemoattractants for leukocytes, recruiting monocytes, neutrophils and other effector cells from the blood to sites of infection or tissue damage. Certain inflammatory chemokines activate cells to initiate an immune response or promote wound healing. They are released by many different cell types and serve to guide cells of both innate immune system and adaptive immune system.

CC chemokines

The CC chemokine (or β -chemokine) proteins have two adjacent cysteines (amino acids), near their amino terminus. There have been at least 27 distinct members of this subgroup reported for mammals, called CC chemokine ligands (CCL)-1 to -28; CCL10 is the same as CCL9. Chemokines of this subfamily usually contain four cysteines (C4-CC chemokines), but a small number of CC chemokines possess six cysteines (C6-CC chemokines). C6-CC chemokines include CCL1, CCL15, CCL21, CCL23 and CCL28. CC chemokines induce the migration of monocytes and other cell types such as NK cells and dendritic cells.

Examples of CC chemokine include monocyte chemoattractant protein-1 (MCP-1 or CCL2) which induces monocytes to leave the bloodstream and enter the surrounding tissue to become tissue macrophages.

CCL5 (or RANTES) attracts cells such as T cells, eosinophils and basophils that express the receptor CCR5.

CXC chemokines

The two N-terminal cysteines of CXC chemokines (or α -chemokines) are separated by one amino acid, represented in this name with an "X". There have been 17 different CXC chemokines described in mammals, that are subdivided into two

categories, those with a specific amino acid sequence (or motif) of glutamic acid-leucine-arginine (or ELR for short) immediately before the first cysteine of the CXC motif (ELR-positive), and those without an ELR motif (ELR-negative). ELR-positive CXC chemokines specifically induce the migration of neutrophils, and interact with chemokine receptors CXCR1 and CXCR2.

C chemokines

The third group of chemokines is known as the C chemokines (or γ chemokines), and is unlike all other chemokines in that it has only two cysteines; one N-terminal cysteine and one cysteine downstream. Two chemokines have been described for this subgroup and are called XCL1 (lymphotactin- α) and XCL2 (lymphotactin- β).

CX3C chemokine

CX3C chemokines have three amino acids between the two cysteines. The only CX3C chemokine discovered to date is called fractalkine (or CX3CL1). It is both secreted and tethered to the surface of the cell that expresses it, thereby serving as both a chemoattractant and as an adhesion molecule.

Chemokine receptors are G protein-coupled receptors containing 7 transmembrane domains that are found on the surface of leukocytes. Approximately 19 different chemokine receptors have been characterized to date, which are divided into four families depending on the type of chemokine they bind; CXCR that bind CXC chemokines, CCR that bind CC chemokines, CX3CR1 that binds the sole CX3C chemokine (CX3CL1), and XCR1 that binds the two XC chemokines (XCL1 and XCL2). They share many structural features; they are similar in size (with about 350 amino acids), have a short, acidic N-terminal end, seven helical transmembrane domains with three intracellular and three extracellular hydrophilic loops, and an intracellular C-terminus containing serine and threonine residues important for receptor regulation. The first two extracellular loops of chemokine receptors each has a conserved cysteine residue that allow formation of a disulfide bridge between these loops. G proteins are coupled to the C-terminal end of the chemokine receptor to allow intracellular signaling after receptor activation, while the N-terminal domain of the chemokine receptor determines ligand binding specificity.

CXCL12

CXCL12 is strongly chemotactic for lymphocytes. CXCL12 plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow through a CXCR4 dependent mechanism. It is this function of CXCL12 that makes it a very important factor in carcinogenesis and the neovascularisation linked to tumour progression. CXCL12 also has a role in tumour metastasis where cancer cells that express the receptor CXCR4 are attracted to metastasis target tissues that release the ligand, CXCL12.

The receptor for CXCL12 is CXCR4. The CCR of the present invention may comprise the CXCL12-binding domain from CXCR4 linked to an endodomain derived from a cytokine receptor, such as the IL-2 receptor.

CXCR4 coupled expression of IL2 would support engraftment of therapeutic T cell for cancer therapies. In multiple myeloma, a cell expressing such a CCR may mobilize cells and change the bone marrow environment. Such cells also have uses in the treatment of solid cancers by modifying the solid tumour microenvironment.

The amino acid sequence for CXCR4 is shown below as SEQ ID No. 17

SEQ ID No. 17

```

1 msiplplqi ytsdnyteem gsgdydsmke pcfreenanf nkiflptiys iifltgivgn
  61 glvilvmgyq kklrsmtsky rhlsvadll fvitlpfwav davanwyfng flickavhviy
 121 tvnlyssvli lafislrdyl aivhatnsqr prkllaekvv yvgvwipall ltipdfifan
 181 vseaddryic drfypndlwv vvfqfghimv glilpgivil scyciiskl shskghqkrk
 241 alktvilil affacwlpvy igisidsfil leiikgqcef entvhkwisi tealaffhcc
 301 lnpylaflg akfktsaqha ltsvrgssl kilskgkrvg hssvstesesssfhss

```

CXCR7 also binds CXCL12.

CCL2

The chemokine (C-C motif) ligand 2 (CCL2) is also referred to as monocyte chemotactic protein 1 (MCP1) and small inducible cytokine A2. CCL2 recruits monocytes, memory T cells, and dendritic cells to the sites of inflammation produced by either tissue injury or infection.

CCR2 and CCR4 are two cell surface receptors that bind CCL2.

CCR2 has the amino acid sequence shown as SEQ ID No. 18

5 SEQ ID No. 18

1 mlstsrfrfi rntnesgeev ttfdydyga pchkfdvkqi gaqlpplys lvfifgvgn
 61 mlvvlilinc kklkcltdiy llnlaisdll flitlplwah saanewvfgn amcklftgly
 121 higyfggiff iilltidryl aivhavfalk artvtfgvvt svitwlvavf asvpgiiftk
 181 cqkedsvyvc gpyfprgwnn fhtimrnilg lvppllmvi cysgilktil rcrnekkhrh
 10 241 avrviftimi vyflfwtpyn ivillntfqe ffglsncest sqldqatqvt etlgmthcci
 301 npiiyafvge kfrslfhial gcrlaplqkp vcggpgvrpg knkvttqgl ldgrgkgsi
 361 grapeaslqd kega

CCR4 has the amino acid sequence shown as SEQ ID No. 19.

15

SEQ ID No. 19

1 mnptdiadtt ldesiysnyy lyesipkpct kegikafgel flpplyslvf vfgllgnsvv
 61 vlvlfkykrl rsmtdvylln laisdllfvf slpfwgyyaa dqwvfglgc kmiswmylv
 121 fysgiffvml msidrylaiv havfslrart ltygvitsla twsvavfasl pgflfstcyt
 20 181 ernhtycktk yslntstkwv lssleinilg lviplgimlf cysmiirtlq hckneknka
 241 vkmifavvvl flgfwtpyni vlfletlvel evlqdtfer yldyaiqate tlaflvhccln
 301 piiyfflgek frkyilqlfk tcrglfvlcq ycgllqiysa dtpsssyts tmdhdlhdal

25

The CCR of the present invention may comprise the CCL2 binding site of CCR2 or CCR4 in its ligand binding domain.

CELL-SURFACE ANTIGEN

The ligand may be a cell-surface antigen, such as a transmembrane protein.

30

The cell surface antigen may be CD22.

35

CD22, or cluster of differentiation-22, is a molecule belonging to the SIGLEC family of lectins. It is found on the surface of mature B cells and to a lesser extent on some immature B cells. Generally speaking, CD22 is a regulatory molecule that prevents the overactivation of the immune system and the development of autoimmune diseases.

CD22 is a sugar binding transmembrane protein, which specifically binds sialic acid with an immunoglobulin (Ig) domain located at its N-terminus. The presence of Ig domains makes CD22 a member of the immunoglobulin superfamily. CD22 functions as an inhibitory receptor for B cell receptor (BCR) signalling.

Increased expression of CD22 is seen in non-Hodgkin and other lymphomas. Various monoclonal antibodies targeting CD22 are known, including epratuzumab, inotuzumab ozogamicin, m971 and m972.

CHIMERIC ANTIGEN RECEPTORS (CAR)

The cell of the present invention may also comprise one or more chimeric antigen receptor(s). The CAR(s) may be specific for a tumour-associated antigen.

Classical CARs are chimeric type I trans-membrane proteins which connect an extracellular antigen-recognizing domain (binder) to an intracellular signalling domain (endodomain). The binder is typically a single-chain variable fragment (scFv) derived from a monoclonal antibody (mAb), but it can be based on other formats which comprise an antibody-like or ligand-based antigen binding site. A trans-membrane domain anchors the protein in the cell membrane and connects the spacer to the endodomain.

Early CAR designs had endodomains derived from the intracellular parts of either the γ chain of the Fc ϵ R1 or CD3 ζ . Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3 ζ results in second generation receptors which can transmit an activating and co-stimulatory signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most potent co-stimulatory signal - namely immunological signal 2, which triggers T-cell proliferation. Some receptors have also been described which include TNF receptor family endodomains, such as the closely related OX40 and 41BB which transmit survival signals. Even more potent third generation CARs have now been described which have endodomains capable of transmitting activation, proliferation and survival signals.

CAR-encoding nucleic acids may be transferred to T cells using, for example, retroviral vectors. In this way, a large number of antigen-specific T cells can be generated for adoptive cell transfer. When the CAR binds the target-antigen, this results in the transmission of an activating signal to the T-cell it is expressed on. Thus the CAR directs the specificity and cytotoxicity of the T cell towards cells expressing the targeted antigen.

The cell of the present invention may comprise one or more CAR(s).

The CAR(s) may comprise an antigen-binding domain, a spacer domain, a transmembrane domain and an endodomain. The endodomain may comprise or associate with a domain which transmit T-cell activation signals.

CAR ANTIGEN BINDING DOMAIN

The antigen-binding domain is the portion of a CAR which recognizes antigen.

Numerous antigen-binding domains are known in the art, including those based on the antigen binding site of an antibody, antibody mimetics, and T-cell receptors. For example, the antigen-binding domain may comprise: a single-chain variable fragment (scFv) derived from a monoclonal antibody; a natural ligand of the target antigen; a peptide with sufficient affinity for the target; a single domain binder such as a camelid; an artificial binder single as a Darpin; or a single-chain derived from a T-cell receptor.

The term "ligand" is used synonymously with "antigen" to mean an entity which is specifically recognised and bound by the antigen-binding domain of a CAR.

CELL SURFACE ANTIGEN

The CAR may recognise a cell-surface antigen, i.e. an entity, such as a transmembrane protein which is expressed on the surface of a target cell, such as a tumour cell.

The CAR may specifically bind a tumour-associated cell-surface antigen.

Various tumour associated antigens (TAA) are known, some of which are shown in Table 1. The antigen-binding domain used in the present invention may be a domain which is capable of binding a TAA as indicated therein.

5 Table 1

Cancer type	TAA
Diffuse Large B-cell Lymphoma	CD19, CD20, CD22
Breast cancer	ErbB2, MUC1
AML	CD13, CD33
Neuroblastoma	GD2, NCAM, ALK, GD2
B-CLL	CD19, CD52, CD160
Colorectal cancer	Folate binding protein, CA-125
Chronic Lymphocytic Leukaemia	CD5, CD19
Glioma	EGFR, Vimentin
Multiple myeloma	BCMA, CD138
Renal Cell Carcinoma	Carbonic anhydrase IX, G250
Prostate cancer	PSMA
Bowel cancer	A33

Where the CAR recognises a B-cell lymphoma or leukemia antigen (such as CD19, CD20, CD52, CD160 or CD5), the CCR may recognise another B-cell antigen, such
 10 as CD22.

PROSTATE-CANCER ASSOCIATED ANTIGENS

The CAR may specifically bind a cell-surface antigen associated with prostate cancer,
 15 such as prostate stem cell antigen (PSCA) or prostate-specific membrane antigen (PSMA).

PSCA is a glycosylphosphatidylinositol-anchored cell membrane glycoprotein. It is up-regulated in a large proportion of prostate cancers and is also detected in cancers
 20 of the bladder and pancreas.

Various anti-PSCA antibodies are known, such as 7F5 (Morgenroth et al (Prostate (2007) 67:1121-1131); 1G8 (Hillerdal et al (2014) BMC Cancer 14:30); and Ha1-4.117 (Abate-Daga et al (2014) 25:1003-1012).

The CCR-expressing cell of the invention may also express an anti-PSCA CAR which may comprise an antigen binding domain based on one of these antibodies.

PSMA is a zinc metalloenzyme that resides in membranes. PSMA is strongly expressed in the human prostate, being a hundredfold greater than the expression in most other tissues. In cancer, it is upregulated in expression and has been called the second-most-upregulated gene in prostate cancer, with increase of 8- to 12-fold over the noncancerous prostate. In addition to the expression in the human prostate and prostate cancer, PSMA is also found to be highly expressed in tumor neovasculature but not normal vasculature of all types of solid tumors, such as kidney, breast, colon, etc.

Various anti-PSMA antibodies are known, such as 7E11, J591, J415, and Hybritech PEQ226.5 and PM2J004.5 each of which binds a distinct epitope of PSMA (Chang et al (1999) Cancer Res 15:3192-8).

The CCR-expressing cell of the invention may also express an anti-PSMA CAR which may comprise an antigen binding domain based on one of these antibodies.

For example, the CCR may comprise an scFv based on J591, having the sequence shown as SEQ ID No. 20.

SEQ ID No. 20 (J591 scFv)

EVQLQQSGPELKKPGTSVRISCKTSGYTFTEYTIHWVKQSHGKSLEWIGNINPNNG
GTTYNQKFEDKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGQGTTL
TVSSGGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDW
YQQKPGQSPKLLIWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQY
NSYPLTFGAGTMLDLKR

CAR TRANSMEMBRANE DOMAIN

The transmembrane domain is the sequence of a CAR that spans the membrane. It may comprise a hydrophobic alpha helix. The CAR transmembrane domain may be derived from CD28, which gives good receptor stability.

CAR SIGNAL PEPTIDE

The CAR and CCR described herein may comprise a signal peptide so that when it/they is expressed in a cell, such as a T-cell, the nascent protein is directed to the endoplasmic reticulum and subsequently to the cell surface, where it is expressed.

5

The core of the signal peptide may contain a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognized and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

10

15 The signal peptide may be at the amino terminus of the molecule.

The signal peptide may comprise the sequence shown as SEQ ID No. 21, 22 or 23 or a variant thereof having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that the signal peptide still functions to cause cell surface expression of the CAR.

20

SEQ ID No. 21: MGTSLLCWMALCLLGADHADG

The signal peptide of SEQ ID No. 21 is compact and highly efficient and is derived from TCR beta chain. It is predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

25

SEQ ID No. 22: MSLPVTALLLPLALLLHAARP

30 The signal peptide of SEQ ID No. 22 is derived from IgG1.

30

SEQ ID No. 23: MAVPTQVLGLLLLWLTDARC

The signal peptide of SEQ ID No. 23 is derived from CD8a.

35

CAR ENDODOMAIN

The endodomain is the portion of a classical CAR which is located on the intracellular side of the membrane.

5 The endodomain is the signal-transmission portion of a classical CAR. After antigen recognition by the antigen binding domain, individual CAR molecules cluster, native CD45 and CD148 are excluded from the synapse and a signal is transmitted to the cell.

10 The CAR endodomain may be or comprise an intracellular signalling domain. In an alternative embodiment, the endodomain of the present CAR may be capable of interacting with an intracellular signalling molecule which is present in the cytoplasm, leading to signalling.

15 The intracellular signalling domain or separate intracellular signalling molecule may be or comprise a T cell signalling domain.

20 The most commonly used signalling domain component is that of CD3-zeta endodomain, which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3-zeta may not provide a fully competent activation signal and additional co-stimulatory signalling may be needed. For example, chimeric CD28 and OX40 can be used with CD3-Zeta to transmit a proliferative / survival signal, or all three can be used together.

25 The CAR may comprise the CD3-Zeta endodomain alone, the CD3-Zeta endodomain with that of either CD28 or OX40 or the CD28 endodomain and OX40 and CD3-Zeta endodomain.

30 The CAR endodomain may comprise one or more of the following: an ICOS endodomain, a CD27 endodomain, a BTLA endodomain, a CD30 endodomain, a GITR endodomain and an HVEM endodomain.

The endomain may comprise the sequence shown as SEQ ID No. 24 to 32 or a variant thereof having at least 80% sequence identity.

35 SEQ ID No. 24 - CD3 Z endodomain

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

5 SEQ ID No. 25 - CD28 and CD3 Zeta endodomains

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

10 SEQ ID No. 26 - CD28, OX40 and CD3 Zeta endodomains

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRDQRLPPDAHKPPG
GGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPR

15

SEQ ID No. 27 - ICOS endodomain

CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL

SEQ ID No. 28 - CD27 endodomain

20 QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP

SEQ ID No. 29 - BTLA endodomain

RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCF
RMQEGSEVYSNPCLEENKPGIVYASLNHSHVIGPNSRLARNVKEAPTEYASICVRS

25

SEQ ID No. 30 - CD30 endodomain

HRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGL
MSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNKNKIEKIYIM
KADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVML
30 SVEEEGKEDPLPTAASGK

SEQ ID No. 31 - GITR endodomain

QLGLHIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPPEEERGERSAEEKGRLGD
LWW

35

SEQ ID No. 32 - HVEM endodomain

CVKRRRKPRGDVVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRS
PNH

A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence
identity to SEQ ID No. 24 to 32, provided that the sequence provides an effective
intracellular signalling domain.

NUCLEIC ACID

The present invention also provides a nucleic acid encoding a CCR of the invention.

The nucleic acid may have the structure:

AgB-spacer-TM-endo

in which

AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the CCR;

spacer 1 is a nucleic acid sequence encoding the spacer of the CCR;

TM1 is a a nucleic acid sequence encoding the transmembrane domain of the CCR;

endo 1 is a nucleic acid sequence encoding the endodomain of the CCR.

NUCLEIC ACID CONSTRUCT

The present invention further provides a nucleic acid construct which comprises a first
nucleic acid sequence encoding a first CCR as defined in connection with the first
aspect of the invention; and a second nucleic acid sequence encoding a second CCR
as defined in connection with the first aspect of the invention.

The nucleic acid construct may have the following structure:

AgB1-spacer1-TM1-endo1-coexpr-AgB2-spacer2-TM2-endo2

in which

AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first
CCR;

spacer 1 is a nucleic acid sequence encoding the spacer of the first CCR;

TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CCR;

endo 1 is a nucleic acid sequence encoding the endodomain of the first CCR;

coexpr is a nucleic acid sequence enabling co-expression of both CCRs

5 AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR;

spacer 2 is a nucleic acid sequence encoding the spacer of the second CCR;

TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CCR;

10 endo 2 is a nucleic acid sequence encoding the endodomain of the second CCR.

When the nucleic acid construct is expressed in a cell, such as a T-cell, it encodes a polypeptide which is cleaved at the cleavage site such that the first and second CCRs are co-expressed at the cell surface.

15

The first and second CCRs may bind distinct epitopes on the same antigen.

The first and second CCRs may have complementary endodomains e.g. one derived from the α or β chain of a cytokine receptor and one derived from the γ chain of the same cytokine receptor.

20

The present invention also provides a nucleic acid construct encoding a CCR of the invention and a CAR. Such a construct may have the structure:

25 CCRAgB-CCRspacer-CCRTM-CCRendo-coexpr-CARAgB-CARspacer-CARTM-CARendo

or

30 CARAgB-CARspacer-CARTM-CARendo-coexpr-CCRAgB-CCRspacer-CCRTM-CCRendo

in which

CCRAgB is a nucleic acid sequence encoding the antigen-binding domain of the CCR;

35

CCRspacer is a nucleic acid sequence encoding the spacer of the CCR;

CCRTM is a a nucleic acid sequence encoding the transmembrane domain of the CCR;

CCRendo is a nucleic acid sequence encoding the endodomain of the CCR;

coexpr is a nucleic acid sequence enabling co-expression of both the CCR and the
5 CAR

CARAgB is a nucleic acid sequence encoding the antigen-binding domain of the CAR;

CARspacer is a nucleic acid sequence encoding the spacer of the CAR;

CARTM is a nucleic acid sequence encoding the transmembrane domain of the CAR;

10 and

CARendo is a nucleic acid sequence encoding the endodomain of the CAR.

The present invention also provides a nucleic acid construct encoding a first and a second CCR of the invention and a CAR. The first and second CCRs may bind
15 separate epitopes on the same antigen. Such a construct may have the structure:

(i) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CCRAgB2-CCRspacer2-
CCRTM2-CCRendo2-coexpr2-CARAgB-CARspacer-CARTM-CARendo;

20 (ii) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CARAgB-CARspacer-
CARTM-CARendo-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2; or

(iii) CARAgB-CARspacer-CARTM-CARendo-coexpr1-CCRAgB1-CCRspacer1-
CCRTM1-CCRendo1-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2;

25

in which

CCRAgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CCR;

CCRspacer1 is a nucleic acid sequence encoding the spacer of the first CCR;

30 CCRTM1 is a nucleic acid sequence encoding the transmembrane domain of the first CCR;

CCRendo1 is a nucleic acid sequence encoding the endodomain of the first CCR;

CCRAgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR;

35 CCRspacer2 is a nucleic acid sequence encoding the spacer of the second CCR;

CCRTM2 is a nucleic acid sequence encoding the transmembrane domain of the second CCR;

CCRendo2 is a nucleic acid sequence encoding the endodomain of the second CCR;
Coexpr1 and coexpr2 are nucleic acid sequences enabling co-expression of the two
flanking sequences;

CARAgB is a nucleic acid sequence encoding the antigen-binding domain of the
CAR;

CARspacer is a nucleic acid sequence encoding the spacer of the CAR;

CARTM is a nucleic acid sequence encoding the transmembrane domain of the CAR;
and

CARendo is a nucleic acid sequence encoding the endodomain of the CAR.

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 terms “variant”, “homologue” or “derivative” in relation to a nucleotide sequence
include any substitution of, variation of, modification of, replacement of, deletion of or
addition of one (or more) nucleic acid from or to the sequence.

In the structure above, “coexpr” is a nucleic acid sequence enabling co-expression of
both first and second CARs. It may be a sequence encoding a cleavage site, such
that the nucleic acid construct produces comprises two or more CCRs, or a CCR and

a CAR, joined by a cleavage site(s). 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 cleavage site may be any sequence which enables the first and second CCRs, or CCR and CAR, to become separated.

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
 10 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 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
 15 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.

The cleavage site may be a furin cleavage site.

20

Furin is an enzyme which belongs to the subtilisin-like proprotein convertase family. The members of this family are proprotein convertases that process latent precursor proteins into their biologically active products. Furin is a calcium-dependent serine
 25 endoprotease that can efficiently cleave precursor proteins at their paired basic amino acid processing sites. Examples of furin substrates include parathyroid hormone, transforming growth factor beta 1 precursor, proalbumin, pro-beta-secretase, membrane type-1 matrix metalloproteinase, beta subunit of pro-nerve growth factor and von Willebrand factor. Furin cleaves proteins just downstream of a basic amino acid target sequence (canonically, Arg-X-(Arg/Lys)-Arg') and is enriched in the Golgi
 30 apparatus.

The cleavage site may be a Tobacco Etch Virus (TEV) cleavage site.

TEV protease is a highly sequence-specific cysteine protease which is chymotrypsin-
 35 like proteases. It is very specific for its target cleavage site and is therefore frequently used for the controlled cleavage of fusion proteins both in vitro and in vivo. The consensus TEV cleavage site is ENLYFQ\S (where '\ ' denotes the cleaved peptide

bond). Mammalian cells, such as human cells, do not express TEV protease. Thus in embodiments in which the present nucleic acid construct comprises a TEV cleavage site and is expressed in a mammalian cell – exogenous TEV protease must also be expressed in the mammalian cell.

5

The cleavage site may encode a self-cleaving peptide.

A 'self-cleaving peptide' refers to a peptide which functions such that when the polypeptide comprising the proteins and the self-cleaving peptide is produced, it is immediately "cleaved" or separated into distinct and discrete first and second polypeptides without the need for any external cleavage activity.

The self-cleaving peptide may be a 2A self-cleaving peptide from an aphtho- or a cardiovirus. The primary 2A/2B cleavage of the aphtho- and cardioviruses is mediated by 2A "cleaving" at its own C-terminus. In aphthoviruses, such as foot-and-mouth disease viruses (FMDV) and equine rhinitis A virus, the 2A region is a short section of about 18 amino acids, which, together with the N-terminal residue of protein 2B (a conserved proline residue) represents an autonomous element capable of mediating "cleavage" at its own C-terminus (Donnelly et al (2001) as above).

20

"2A-like" sequences have been found in picornaviruses other than aphtho- or cardioviruses, 'picornavirus-like' insect viruses, type C rotaviruses and repeated sequences within Trypanosoma spp and a bacterial sequence (Donnelly et al (2001) as above). The cleavage site may comprise one of these 2A-like sequences, such

25

YHADYYKQRLIHDVEMNPGP (SEQ ID No. 33)

HYAGYFADLLIHDVETNPGP (SEQ ID No. 34)

QCTNYALLKLAGDVESNPGP (SEQ ID No. 35)

30 ATNFSLLKQAGDVEENPGP (SEQ ID No. 36)

AARQMLLLLSGDVETNPGP (SEQ ID No. 37)

RAEGRGSLTCTGDVEENPGP (SEQ ID No. 38)

TRAEIEDELIRAGIESNPGP (SEQ ID No. 39)

TRAEIEDELIRADIESNPGP (SEQ ID No. 40)

35 AKFQIDKILISGDVELNPGP (SEQ ID No. 41)

SSIIRTKMLVSGDVEENPGP (SEQ ID No. 42)

CDAQRRQKLLLSGDIEQNPGP (SEQ ID No. 43)

YPIDFGGFLVKADSEFNPGP (SEQ ID No. 44)

The cleavage site may comprise the 2A-like sequence shown as SEQ ID No. 38 (RAEGRGSLLTCGDVEENPGP).

5

The present invention also provides a kit comprising one or more nucleic acid sequence(s) encoding first and second CCRs according to the first aspect of the present invention, or one or more CCR(s) according to the invention and one or more CAR(s).

10

SEQ ID NOS 45 and 46 give the complete amino acid sequences of a fusion between and anti-PSMA CAR and an anti-PSA CCR. Subheadings are given to label each portion of the sequence but in practice the various elements are connected giving one continuous sequence.

15

The nucleic acid construct of the invention may encode a fusion protein as shown in SEQ ID No. 45 or 46.

SEQ ID NO. 45 - Illustrative construct with IL-2R beta chain

20

Signal sequence derived from human CD8a:

MSLPVTALLPLALLHAA

scFv aPSMA (J591 H/L)

EVQLQQSGPELKKPGTSVRISCKTSGYTFTEYTIHWWKQSHGKSLEWIGNINPNNG

25

GTTYNQKFEDKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGGQTTL

TVSSGGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDW

YQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQY

NSYPLTFGAGTMLDLKR

Linker

30

SDPA

Human IgG1Fc spacer (HCH2CH3pvaa):

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVDVSHEDPEV

KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL

PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG

35

QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS

LSLSPGK

Transmembrane derived from human CD28:

FWWLVVGGVLACYSLLVTVAFIIFWW

Endodomain derived from TCRz:

RRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA

5 LPPR

2A peptide from Thosea asigna virus capsid protein:

RAEGRGSLLTCGDVEENPGP

Signal sequence derived from mouse kappa VIII:

METDTLILWLLLLVPGSTG

10 scFv aPSA (5D5A5 H/L):

QVQLQQSGAELAKPGASVKMSCKTSGYSFSSYWMHWWKQRPQGQLEWIGYINPS
TGYTENNQKFKDKVTLTADKSSNTAYMQLNSLTSEDSAVYYCARSGRLYFDVWGA
GTTVTVSSGGGGSGGGGSGGGGSGGGGSDIVLTQSPPSLAVSLGQRATISCRASE
SIDLYGFTFMHWYQQKPGQPPKILYRASNLESGIPARFSGSGSRTDFTLTINPVEAD

15 DVATYYCQQTHEDPYTFGGGKLEIK

Linker:

SDPA

Human CD8aSTK spacer:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

20 Transmembrane derived from human common gamma chain:

VVISVGSMGLIISLLCVYFWL

Endodomain derived from human common gamma chain:

ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGG
ALGEGPGASPCNQHSPYWAPPCYTLKPET

25 2A peptide from equine rhinitis A virus polyprotein:

QCTNYALLKLAGDVESNPGP

Signal sequence derived from mouse kappa VIII:

METDTLILWLLLLVPGSTG

scFv aPSA (5D3D11 H/L):

30 QVQLQQSGPELVKPGASVKISCKVSGYAISSSWMNWWKQRPQGQLEWIGRIYPGD
GDTKYNGKFKDKATLTVDKSSSTAYMQLSSLTSVDSAVYFCARDGYRYYFDYWGQ
GTSVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQTAPSVFVTPGESVVISCRSS
KSLHNSNGNTYLYWFLQRPQGQSPQLLIYRMSNLASGVPDRFSGSGSGTDFTLRISR
VEAEDVGVYYCMQHLEYPVTFGAGTKVEIK

35 Linker:

SDPA

Human CD28STK spacer:

KIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPFGPSKP

Transmembrane derived from human IL-2R β :

IPWLGHLLVGLSGAFGFILVYLLI

Endodomain derived from human IL-2R β :

5 NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAP
 EISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLSLTSCFTNQGYFFFHLPDALEIEAC
 QVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSL
 LGGPSPSTAPGGSGAGEERMPPSLQERVPRDWDQPPLGPPTPGVPDLVDFQPP
 PELVLRAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQ
 10 GQDPHTLV

SEQ ID No. 46 – Illustrative construct with IL-7R alpha chain

Signal sequence derived from human CD8a:

15 MSLPVTALLPLALLHAA
 scFv aPSMA (J591 H/L)
 EVQLQQSGPELKKPGTSVRISCKTSGYTFTETIHWKQSHGKSLEWIGNINPNNG
 GTTYNQKFEDKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGGQTTL
 TVSSGGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDW
 20 YQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQY
 NSYPLTFGAGTMLDLKR

Linker

SDPA

Human IgG1Fc spacer (HCH2CH3pvaa):

25 EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
 QPENNYKTTTPVLDSGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
 LSLSPGK

30 Transmembrane derived from human CD28:

FWWLVVGGVLACYSLLVTVAFIIFWV

Endodomain derived from TCR ζ :

RRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
 PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
 35 LPPR

2A peptide from Thosea asigna virus capsid protein:

RAEGRGSLLTCGDVEENPGP

Signal sequence derived from mouse kappa VIII:

METDTLILWLLLLVPGSTG

scFv aPSA (5D5A5 H/L):

QVQLQQSGAELAKPGASVKMSCKTSGYSFSSYWMHWWKQRPQGQGLEWIGYINPS

5 TGYTENNQKFKDKVTLTADKSSNTAYMQLNSLTSEDSAVYYCARSGRLYFDVWGA
GTTVTVSSGGGGSGGGGSGGGGSGGGGSDIVLTQSPPSLAVSLGQRATISCRASE
SIDLYGFTFMHWYQQKPGQPPKILYRASNLESGIPARFSGSGSRTDFTLTINPVEAD
DVATYYCQQTHEDPYTFGGGTKEIK

Linker:

10 SDPA

Human CD8aSTK spacer:

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

Transmembrane derived from human common gamma chain:

VVISVGSMGLIISLLCVYFWL

15 Endodomain derived from human common gamma chain:

ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGG
ALGEGPGASPCNQHSPYWAPPCYTLKPET

2A peptide from equine rhinitis A virus polyprotein:

QCTNYALLKLAGDVESNPGP

20 Signal sequence derived from mouse kappa VIII:

METDTLILWLLLLVPGSTG

scFv aPSA (5D3D11 H/L):

QVQLQQSGPELVKPGASVKISCKVSGYAISSSWMNWWKQRPQGQGLEWIGRIYPGD

GDTKYNGKFKDKATLTVDKSSSTAYMQLSSLTSVDSAVYFCARDGYRYFYFDYWQQ

25 GTSVTVSSGGGGSGGGGSGGGGSGGGGSDIVMTQTAPSVFVTPGESVSISCRSS
KSLHNSNGNTYLYWFLQRPQGQSPQLLIYRMSNLASGVDPDRFSGSGSGTDFTLRISR
VEAEDVGVYYCMQHLEYPVTFGAGTKVEIK

Linker:

SDPA

30 Human CD28STK spacer:

KIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKP

Transmembrane derived from human IL-7R α :

PILLTISILSFFSVALLVILACVLW

Endodomain derived from human IL-7R α :

35 KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEG
FLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACD

APILSSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLPVAQGG
 PILTSLGSNQEEAYVTMSSFYQNNQ

VECTOR

5

The present invention also provides a vector, or kit of vectors, which comprises one or more nucleic acid sequence(s) encoding a one or more CCR(s) according to the first aspect of the invention and optionally one or more CAR(s). Such a vector may be used to introduce the nucleic acid sequence(s) into a host cell so that it expresses
 10 a CCR according to the first aspect of the invention.

The vector may, for example, be a plasmid or a viral vector, such as a retroviral vector or a lentiviral vector, or a transposon based vector or synthetic mRNA.

15 The vector may be capable of transfecting or transducing a T cell or a NK cell.

CELL

The present invention provides a cell which comprises one or more CCR(s) of the
 20 invention and optionally one of more CAR(s).

The cell may comprise a nucleic acid or a vector of the present invention.

The cell may be a cytolytic immune cell such as a T cell or an NK cell.

25

T cells or T lymphocytes are a type of lymphocyte that play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. There are various types of T cell, as summarised below.

30

Helper T helper cells (TH cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. TH cells express CD4 on their surface. TH cells become activated when they are presented with peptide antigens
 35 by MHC class II molecules on the surface of antigen presenting cells (APCs). These cells can differentiate into one of several subtypes, including TH1, TH2, TH3, TH17,

Th9, or TFH, which secrete different cytokines to facilitate different types of immune responses.

Cytolytic T cells (TC cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. CTLs express the CD8 at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of all nucleated cells. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis.

Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with "memory" against past infections. Memory T cells comprise three subtypes: central memory T cells (TCM cells) and two types of effector memory T cells (TEM cells and TEMRA cells). Memory cells may be either CD4+ or CD8+. Memory T cells typically express the cell surface protein CD45RO.

Regulatory T cells (Treg cells), formerly known as suppressor T cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress auto-reactive T cells that escaped the process of negative selection in the thymus.

Two major classes of CD4+ Treg cells have been described — naturally occurring Treg cells and adaptive Treg cells.

Naturally occurring Treg cells (also known as CD4+CD25+FoxP3+ Treg cells) arise in the thymus and have been linked to interactions between developing T cells with both myeloid (CD11c+) and plasmacytoid (CD123+) dendritic cells that have been activated with TSLP. Naturally occurring Treg cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP3. Mutations of the FOXP3 gene can prevent regulatory T cell development, causing the fatal autoimmune disease IPEX.

Adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response.

The cell may be a Natural Killer cell (or NK cell). NK cells form part of the innate immune system. NK cells provide rapid responses to innate signals from virally infected cells in an MHC independent manner

5

NK cells (belonging to the group of innate lymphoid cells) are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils and thymus where they then enter into the circulation.

10

The CCR-expressing cells of the invention may be any of the cell types mentioned above.

15

T or NK cells according to the first aspect of the invention may either be created ex vivo either from a patient's own peripheral blood (1st party), or in the setting of a haematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party).

20

Alternatively, T or NK cells according to the first aspect of the invention may be derived from ex vivo differentiation of inducible progenitor cells or embryonic progenitor cells to T or NK cells. Alternatively, an immortalized T-cell line which retains its lytic function and could act as a therapeutic may be used.

25

In all these embodiments, CCR-expressing cells are generated by introducing DNA or RNA coding for the or each CCR(s) by one of many means including transduction with a viral vector, transfection with DNA or RNA.

30

The cell of the invention may be an ex vivo T or NK cell from a subject. The T or NK cell may be from a peripheral blood mononuclear cell (PBMC) sample. T or NK cells may be activated and/or expanded prior to being transduced with nucleic acid encoding the molecules providing the CCR according to the first aspect of the invention, for example by treatment with an anti-CD3 monoclonal antibody.

35

The T or NK cell of the invention may be made by:

(i) isolation of a T or NK cell-containing sample from a subject or other sources listed above; and

(ii) transduction or transfection of the T or NK cells with one or more a nucleic acid sequence(s) encoding a CCR.

The T or NK cells may then be purified, for example, selected on the basis of expression of the antigen-binding domain of the antigen-binding polypeptide.

PHARMACEUTICAL COMPOSITION

The present invention also relates to a pharmaceutical composition containing a plurality of cells according to the 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 compounds. Such a formulation may, for example, be in a form suitable for intravenous infusion.

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 example in a pharmaceutical composition as described above) to a subject.

A method for treating a disease 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 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.

The method for preventing a disease 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 be thought to be at risk of developing, the disease.

The method may involve the steps of:

- (i) isolating a T or NK cell-containing sample;
- (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.

5

The T or NK cell-containing sample may be isolated from a subject or from other sources, for example as described above. The T or NK cells may be isolated from a subject's own peripheral blood (1st party), or in the setting of a haematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an
10 unconnected donor (3rd party).

The present invention provides a CCR-expressing cell of the present invention for use in treating and/or preventing a disease.

- 15 The invention also relates to the use of a CCR-expressing cell of the present invention in the manufacture of a medicament for the treatment and/or prevention of a disease.

- 20 The disease to be treated and/or prevented by the methods of the present invention may be a 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.

Where the ligand recognised by the CCR is PSA, the cancer may be prostate cancer.

25

The cells of the present invention may be capable of killing target cells, such as cancer cells. The target cell may be characterised by the presence of a tumour secreted ligand or chemokine ligand in the vicinity of the target cell. The target cell may be characterised by the presence of a soluble ligand together with the
30 expression of a tumour-associated antigen (TAA) at the target cell surface.

The cells and pharmaceutical compositions of present invention may be for use in the treatment and/or prevention of the diseases described above.

- 35 The cells and pharmaceutical compositions of present invention may be for use in any of the methods described above.

CHIMERIC TRANSMEMBRANE PROTEIN

The present invention also provides a chimeric transmembrane protein comprising a dimerization domain; and a cytokine receptor endodomain.

5

Dimerisation may occur spontaneously, in which case the chimeric transmembrane protein will be constitutively active. Alternatively, dimerization may occur only in the presence of a chemical inducer of dimerization (CID) in which case the transmembrane protein only causes cytokine-type signalling in the presence of the CID.

10

Suitable dimerization domains and CIDs are described in WO2015/150771, the contents of which are hereby incorporated by reference.

15

For example, one dimerization domain may comprise the rapamycin binding domain of FK-binding protein 12 (FKBP12), the other may comprise the FKBP12-Rapamycin Binding (FRB) domain of mTOR; and the CID may be rapamycin or a derivative thereof.

20

One dimerization domain may comprise the FK506 (Tacrolimus) binding domain of FK-binding protein 12 (FKBP12) and the other dimerization domain may comprise the cyclosporin binding domain of cyclophilin A; and the CID may be an FK506/cyclosporin fusion or a derivative thereof.

25

One dimerization domain may comprise an oestrogen-binding domain (EBD) and the other dimerization domain may comprise a streptavidin binding domain; and the CID may be an estrone/biotin fusion protein or a derivative thereof.

30

One dimerization domain may comprise a glucocorticoid-binding domain (GBD) and the other dimerization domain may comprise a dihydrofolate reductase (DHFR) binding domain; and the CID may be a dexamethasone/methotrexate fusion protein or a derivative thereof.

35

One dimerization domain may comprise an O6-alkylguanine-DNA alkyltransferase (AGT) binding domain and the other dimerization domain may comprise a dihydrofolate reductase (DHFR) binding domain; and the CID may be an O6-benzylguanine derivative/methotrexate fusion protein or a derivative thereof.

One dimerization domain may comprise a retinoic acid receptor domain and the other dimerization domain may comprise an ecodysone receptor domain; and the CID may be RSL1 or a derivative thereof.

5

Where the dimerization domain spontaneously heterodimerizes, it may be based on the dimerization domain of an antibody. In particular it may comprise the dimerization portion of a heavy chain constant domain (CH) and a light chain constant domain (CL). The "dimerization portion" of a constant domain is the part of the sequence which forms the inter-chain disulphide bond.

10

The chimeric cytokine receptor may comprise the Fab portion of an antibody as exodomain, for example as illustrated schematically in Figure 5..

15

The chimeric transmembrane protein may comprise two polypeptides:

(i) a first polypeptide which comprises:

(a) a first dimerisation domain; and

(b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

20

(a) a second dimerization domain, which dimerises with the first dimerization domain; and

(b) a second chain of the cytokine-receptor endodomain.

25

The sections above defining the cytokine receptor endodomain of the chimeric cytokine receptor also apply to the chimeric transmembrane protein of the present invention.

30

The sections above relating to nucleic acids, vectors, kits, cells, pharmaceutical compositions and methods also apply to the chimeric transmembrane protein of the present invention.

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.

35

EXAMPLES

Example 1 – In vitro testing

T-cells are transduced with either a PSMA-specific CAR, or transduced with a construct which co-expresses a PSMA-specific CAR with a PSA-specific CCR. T-cells are co-cultured with PSMA expressing target cells which secrete or do not secrete PSA. This co-culture is conducted in the presence or absence of exogenous IL2. This co-culture is conducted at different effector to target ratios. This co-culture is repeated serially with T-cells challenged with repeated target cells. Proliferation of T-cells and killing of target cells is determined. In this way, the contribution to proliferation and survival of T-cells the CCR makes can be measured. Further, the ability contribution to repeated re-challenge the ability of serial

Example 2 - In vivo testing

NSG mice are engrafted with a human prostate cancer cell line which expresses PSMA and secretes PSA and which expresses firefly Luciferase. T-cells are transduced with either a PSMA-specific CAR, or transduced with a construct which co-expresses the PSMA-specific CAR with a PSA-specific CCR. T-cells are administered to the mice. The tumour burden can be serially measured using bioluminescent imaging and the response to CAR T-cells evaluated. Mice within each cohort can be sacrificed at different time-points and tumour burden directly measured by macroscopic measurements and by immunohistochemistry. Further, engraftment / expansion of T-cells at the tumour bed or within lymphoid tissues such as lymph nodes, spleen and bone-marrow measured by flow cytometry of said tissues.

Example 3 – Creation of and testing a constitutively active cytokine-signalling molecule

A constitutively active cytokine-signalling chimeric transmembrane protein was produced by linking cytokine receptor endodomains to a “Fab” type exodomain (Figure 5). This structure uses the natural dimerization components of antibodies, namely the dimerization domain from the heavy and light chain constant regions. The chimeric transmembrane protein has two chains; a first polypeptide which comprises the antibody light κ chain and the IL2 receptor common γ chain as endodomain; and a second polypeptide which comprises the antibody heavy chain CH1 and an endodomain which comprises either: the IL2 receptor β chain (giving a constitutively active IL2-signalling molecule); or the IL7 receptor (giving a constitutively active IL7-signalling molecule). The constitutively active cytokine-signalling chimeric transmembrane proteins tested in this study included the scFv heavy and light chain

variable regions. These domains are not needed for dimerization to occur. The signal is independent of antigen binding and the structure could equally be “headless” (as shown in Figure 5) or comprise another entity such as a protein tag.

- 5 Nucleic acid sequences encoding these two polypeptides were cloned in frame separated by a 2A-peptide encoding sequence.

CTLL-2 (ATCC® TIB-214™) are murine cytotoxic T lymphocyte cells which are dependent upon IL-2 for growth. In the absence of IL-2 the cells undergo apoptosis.

- 10 CTLL-2 cells were transduced with a vector expressing the chimeric protein comprising an IL2-receptor endodomain (Fab_IL2endo) or a vector expressing the chimeric protein comprising an IL7 receptor endodomain (Fab_IL7endo) or left untransduced (WT). As a positive control, cells of all three types were co-cultured with 100 U/ml murine IL2. Cell proliferation was assessed after 3 and 7 days of
15 culture and the results are shown in Figure 6.

Untransduced CTLL2 cells, together with CTLL2 cells transduced with either construct (Fab_IL2endo or Fab_IL7endo) proliferated in the presence of 100U/mL murine IL2 (Figure 6, left-hand panel). However, in the absence of exogenously
20 added IL2, only cells transduced with the construct having an IL2R endodomain (Fab_IL2endo) survived and proliferated. This shows that the chimeric transmembrane receptor provides the CTLL2 cells with the necessary IL2 signal.

Example 4 – Generation and testing of a chimeric cytokine receptor against PSA

25 A panel of chimeric cytokine receptors targeting PSA was developed using scFvs derived from two antibodies which bind to different PSA epitopes: 5D5A5 and 5D3D11. The crystal structure of PSA has been obtained in a sandwich complex with these two (Stura et al (2011) as above).

30 Schematic diagrams illustrating some of the panel of CCRs is illustrated in Figure 7.

The panel included the following constructs:

A5-CD8stk-IL2Rg_D11-Hinge-IL2Rb: A CCR with an IL-2R endodomain having A5 on the chain with common γ chain and D11 on the chain with the IL2R β chain;

D11-CD8stk- IL2Rg_A5-Hinge-IL2Rb: A CCR with an IL-2R endodomain having D11 on the chain with common γ chain and A5 on the chain with IL2R β chain;

D11-CD8stk- RL _A5-Hinge -IL2Rb: A negative control construct which is equivalent D11-CD8stk- IL2Rg_A5-Hinge-IL2Rb, but in which the IL2R γ chain is replaced by a rigid linker;

D11-CD8stk- IL2Rg_A5-Hinge-IL7Ra: A CCR with an IL-7R endodomain having D11 on the chain with common γ chain and A5 on the chain with IL7R α chain; and

D11-CD8stk- RL _A5-Hinge -IL7Ra: A negative control construct which is equivalent D11-CD8stk- IL2Rg_A5-Hinge-IL7Ra, but in which the IL2R γ chain is replaced by a rigid linker;

CTLL2 cells were transduced with vectors expressing these constructs. Cells were cultured in the presence or absence of IL2 (the presence of IL2 acting as a positive control) and the presence or absence of 5ng/mL or 5 μ g/mL PSA. CTLL2 cell proliferation was assessed after 3 and 7 days and the results are shown in Figure 8.

CTLL2 cells expressing a CCR with an IL7 endodomain did not support CTLL2 cell survival and proliferation (Figure 8, last two panels). The presence of murine IL-2 in these cells supported CTLL2 cell growth and proliferation at day 3, but by day 7 the majority of cells had undergone apoptosis.

The anti-PSA chimeric cytokine receptors with an IL2R endodomain supported CTLL2 cell proliferation in the absence of IL2 and the presence of PSA at both 5ng/ml and 5 μ g/ml (Figure 8, first panel), with 5 μ g/ml giving greater survival and proliferation, particularly at day 7.

Both the anti-PSA chimeric cytokine receptors with an IL2R endodomain, i.e. A5-CD8stk-IL2Rg_D11-Hinge-IL2Rb and D11-CD8stk- IL2Rg_A5-Hinge-IL2Rb, indicating that the relative positioning of the two PSA-binding domains: 5D5A5 and 5D3D11, is not important for function.

Substitution of the common γ chain with a rigid linker abolished the capacity of the CCR to support CTLL2 cell survival and proliferation (Figure 8, third panel).

As another read-out for IL2 signalling, the phosphorylation of Y694 of STAT5 was investigated using phosphoflow.

CTLL2 cells were either untransduced (WT); transduced with a PSA CCR constructs having an IL2R endodomain (D11-CD8STK-IL2Rg_A5-Hinge-IL2Rb); or transduced

with an equivalent negative control construct in which the IL2R γ chain is replaced with a rigid linker (D11-CD8STK-RL_A5-Hinge-IL2R β). The cells were incubated overnight in the absence of exogenously added IL-2. The following day, the cells were incubated with either Pervanadate at 500 μ M (a positive control which inhibits phosphatase and will lead to STAT5 phosphorylation) or 500ng/mL PSA for 1 or 4 hours. After incubation the cells were fixed, permeabilised and analysed by flow cytometry.

The results are shown in Figure 9. In the cells expressing the PSA CCR, the presence of PSA lead to increasing STAT5 phosphorylation with time (Figure 9, central panel). No such increase in phosphorylation was seen with untransduced CTLL2 cells, or with CTLL2 cells transduced with an equivalent construct in which the IL2R γ chain is replaced with a rigid linker (Figure 9, right hand panel).

These results are consistent with the CTLL2 survival/proliferation data shown in Figure 8 and demonstrate that a chimeric cytokine receptor against a soluble ligand (here, PSA) can be used to trigger cytokine signalling in a T-cell.

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 or related fields are intended to be within the scope of the following claims.

FURTHER ASPECTS

Further aspects and embodiments of the invention are now set out in the following numbered Paragraphs; it is to be understood that the invention encompasses these aspects:

Paragraph 1. A chimeric cytokine receptor (CCR) comprising: an exodomain which
5 binds to a ligand selected from a tumour secreted factor, a chemokine and a cell-surface antigen; and a cytokine receptor endodomain.

2. A chimeric cytokine receptor according to Paragraph 1, which comprises two polypeptides: (i) a first polypeptide which comprises: (a) a first antigen-binding domain which binds a first epitope of the ligand (b) a first chain of the cytokine receptor endodomain; and
10 (ii) a second polypeptide which comprises: (a) a second antigen-binding domain which binds a second epitope of the ligand (b) a second chain of the cytokine-receptor endodomain.

Paragraph 3. A chimeric cytokine receptor according to Paragraph 2, wherein each of the first and second antigen-binding domains are single-chain variable fragments (scFvs).

Paragraph 4. A chimeric cytokine receptor according to Paragraph 2, wherein each of
15 the first and second antigen-binding domains are single domain binders (dAbs).

Paragraph 5. A chimeric cytokine receptor according to Paragraph 1 which comprises two polypeptides: (i) a first polypeptide which comprises: (a) a heavy chain variable domain (VH) (b) a first chain of the cytokine receptor endodomain; and (ii) a second polypeptide which comprises: (a) a light chain variable domain (VL) (b) a second chain of the cytokine-
20 receptor endodomain.

Paragraph 6. A chimeric cytokine receptor according to Paragraph 5 wherein the first and second chains for the cytokine receptor endodomains are different and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

Paragraph 7. A chimeric cytokine receptor according to Paragraph 5 wherein the first
25 and second chains for the cytokine receptor endodomains are the same and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.

Paragraph 8. A chimeric cytokine receptor according to any preceding Paragraph, wherein the cytokine receptor endodomain comprises: (i) IL-2 receptor β -chain endodomains

(ii) IL-7 receptor α -chain endodomain; or (iii) IL-15 receptor α -chain endodomain; and/or (iv) common γ -chain receptor endodomain.

Paragraph 9. A chimeric cytokine receptor according to any preceding Paragraph, wherein the ligand is a tumour secreted factor selected from: prostate-specific antigen (PSA),
 5 carcinoembryonic antigen (CEA) and vascular endothelial growth factor (VEGF) and CA125.

Paragraph 10. A chimeric cytokine receptor according to any of Paragraphs 1 to 7, wherein the ligand is a chemokine selected from: CXCL12, CCL2, CCL4, CCL5 and CCL22.

Paragraph 11. A cell which comprises a chimeric cytokine receptor according to any preceding Paragraph.

10 Paragraph 12. A cell according to Paragraph 11 which comprises a first chimeric cytokine receptor and a second chimeric cytokine receptor which bind different epitopes on the same ligand.

Paragraph 13. A cell according to Paragraph 12, wherein the first chimeric cytokine receptor comprises a type I cytokine receptor endodomain α - or β -chain, and the second
 15 chimeric cytokine receptor comprises a type I cytokine receptor endodomain γ -chain, such that when the first chimeric cytokine receptor and the second cytokine receptor bind the ligand, combined signalling through the α -/ β -chain and γ -chain occurs.

Paragraph 14. A cell according to any of Paragraphs 11 to 13, which also comprises a chimeric antigen receptor.

20 Paragraph 15. A cell according to Paragraph 14, wherein the chimeric antigen receptor binds a tumour-associated cell surface antigen.

Paragraph 16. A cell according to Paragraph 15, wherein the chimeric antigen receptor binds a cell surface antigen associated with prostate cancer.

Paragraph 17. A cell according to Paragraph 16, wherein the cell surface antigen is
 25 prostate stem-cell antigen (PSCA) or prostate-specific membrane antigen (PSMA).

Paragraph 18. A nucleic acid sequence encoding a chimeric cytokine receptor (CCR) according to any of Paragraphs 1 to 10.

Paragraph 19. A nucleic acid construct which comprises a first nucleic acid sequence encoding a first CCR and a second nucleic acid sequence encoding a second CCR, the nucleic acid construct having the structure: AgB1-spacer1-TM1-endo1-coexpr-AbB2-spacer2-TM2-endo2 in which AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CCR; spacer 1 is a nucleic acid sequence encoding the spacer of the first CCR; TM1 is a nucleic acid sequence encoding the transmembrane domain of the first CCR; endo 1 is a nucleic acid sequence encoding the endodomain of the first CCR; coexpr is a nucleic acid sequence enabling co-expression of both CCRs; AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR; spacer 2 is a nucleic acid sequence encoding the spacer of the second CCR; TM2 is a nucleic acid sequence encoding the transmembrane domain of the second CCR; endo 2 is a nucleic acid sequence encoding the endodomain of the second CCR.

Paragraph 20. A nucleic acid construct according to Paragraph 19 which also encodes a chimeric antigen receptor (CAR), the nucleic acid construct having the structure: (i) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2-coexpr2-CARAgB-CARspacer-CARTM-CAREndo; (ii) CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr1-CARAgB-CARspacer-CARTM-CAREndo-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2; or (iii) CARAgB-CARspacer-CARTM-CAREndo-coexpr1-CCRAgB1-CCRspacer1-CCRTM1-CCRendo1-coexpr2-CCRAgB2-CCRspacer2-CCRTM2-CCRendo2; in which CCRAgB1 is a nucleic acid sequence encoding the antigen-binding domain of the first CCR; CCRspacer1 is a nucleic acid sequence encoding the spacer of the first CCR; CCRTM1 is a nucleic acid sequence encoding the transmembrane domain of the first CCR; CCRendo1 is a nucleic acid sequence encoding the endodomain of the first CCR; CCRAgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second CCR; CCRspacer2 is a nucleic acid sequence encoding the spacer of the second CCR; CCRTM2 is a nucleic acid sequence encoding the transmembrane domain of the second CCR; CCRendo2 is a nucleic acid sequence encoding the endodomain of the second CCR; Coexpr1 and coexpr2 are nucleic acid sequences enabling co-expression of the two flanking sequences; CARAgB is a nucleic acid sequence encoding the antigen-binding domain of the CAR; CARspacer is a nucleic acid sequence encoding the spacer of the CAR; CARTM is a nucleic acid sequence encoding the transmembrane domain of the CAR; and CAREndo is a nucleic acid sequence encoding the endodomain of the CAR.

Paragraph 21. A nucleic acid construct according to Paragraph 19 or 20, wherein coexpr encodes a sequence comprising a self-cleaving peptide.

Paragraph 22. A nucleic acid construct according to any of Paragraphs 19 to 21, wherein alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

Paragraph 23. A vector comprising a nucleic acid construct according to any of Paragraphs 19 to 22.

Paragraph 24. A retroviral vector or a lentiviral vector or a transposon according to Paragraph 23.

Paragraph 25. A kit which comprises: i) a vector comprising a nucleic acid sequence encoding a first CCR as defined in any of Paragraphs 1 to 10; and ii) a vector comprising a nucleic acid sequence encoding a second CCR as defined in any of Paragraphs 1 to 10.

Paragraph 26. A kit according to Paragraph 25 which also comprises a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

Paragraph 27. A kit which comprises: i) a vector comprising a nucleic acid sequence encoding a CCR as defined in any of Paragraphs 1 to 10; and ii) a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.

Paragraph 28. A method for making a cell according to any of Paragraphs 11 to 17, which comprises the step of introducing: a nucleic acid sequence according to Paragraph 18; a nucleic acid construct according to any of Paragraphs 19 to 22; a vector according to Paragraph 23 or 24; or a kit of vectors according to any of Paragraphs 25 to 27, into a cell.

Paragraph 29. A method according to Paragraph 28, wherein the cell is from a sample isolated from a subject.

Paragraph 30. A pharmaceutical composition comprising a plurality of cells according to any of Paragraphs 11 to 17.

Paragraph 31. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to Paragraph 30 to a subject.

Paragraph 32. A method according to Paragraph 31, which comprises the following steps: (i) isolation of a cell-containing sample from a subject; (ii) transduction or transfection of the cells with: a nucleic acid sequence according to Paragraph 18; a nucleic acid construct according to any of Paragraphs 19 to 22; a vector according to Paragraph 23 or 24; or a kit of
5 vectors according to any of Paragraphs 25 to 27; and (iii) administering the cells from (ii) to a the subject.

Paragraph 33. A method according to Paragraph 32, wherein the sample is a T-cell containing sample.

Paragraph 34. A method according to Paragraph 32 or 33, wherein the disease is a
10 cancer.

Paragraph 35. A pharmaceutical composition according to Paragraph 30 for use in treating and/or preventing a disease.

Paragraph 36. The use of a cell according to any of Paragraphs 11 to 17 in the manufacture of a medicament for treating and/or preventing a disease.

CLAIMS

1. A chimeric transmembrane protein comprising:

a dimerization domain; and

a cytokine receptor endodomain.

5 2. A chimeric transmembrane protein according to Claim 1, in which the dimerization domain comprises the dimerization portion of a heavy chain constant domain (C_H) and a light chain constant domain (C_L).

3. A chimeric transmembrane protein according to Claim 1 or 2, which comprises two polypeptides:

10 (i) a first polypeptide which comprises:

(a) a first dimerisation domain; and

(b) a first chain of the cytokine receptor endodomain; and

(ii) a second polypeptide which comprises:

15 (a) a second dimerization domain, which dimerises with the first dimerization domain; and

(b) a second chain of the cytokine-receptor endodomain.

4. A chimeric transmembrane protein according to Claim 3, in which the first and second dimerization domains either dimerise spontaneously, or in the presence of a chemical inducer of dimerization (CID).

20 5. A chimeric transmembrane protein according to Claim 2, 3 or 4 which comprises two polypeptides:

(i) a first polypeptide which comprises:

(a) a heavy chain constant domain (C_H)

(b) a first chain of the cytokine receptor endodomain; and

25 (ii) a second polypeptide which comprises:

(a) a light chain constant domain (CL)

(b) a second chain of the cytokine-receptor endodomain.

6. A chimeric transmembrane protein according to Claim 5, in which the first and second chains for the cytokine receptor endodomains are different and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.
7. A chimeric transmembrane protein according to Claim 5, in which the first and second chains for the cytokine receptor endodomains are the same and are selected from type I cytokine receptor endodomain α -, β -, and γ -chains.
8. A chimeric transmembrane protein according to any preceding claim, in which the cytokine receptor endodomain comprises:
 - (i) IL-2 receptor β -chain endodomain
 - (ii) IL-7 receptor α -chain endodomain; or
 - (iii) IL-15 receptor α -chain endodomain; and/or
 - (iv) common γ -chain receptor endodomain.
9. A chimeric transmembrane protein according to Claim 5, in which the first polypeptide comprises a heavy chain variable domain (VH) and a heavy chain constant domain (CH); and the second polypeptide comprises a light chain variable domain (VL) and a light chain constant domain (CL).
10. A chimeric transmembrane protein according to Claim 9 which comprises a Fab exodomain.
11. A cell which comprises a chimeric transmembrane protein according to any preceding claim.
12. A cell according to Claim 11, which also comprises a chimeric antigen receptor.
13. A cell according to Claim 12, in which the chimeric antigen receptor binds a tumour-associated cell surface antigen.

14. A nucleic acid sequence encoding a chimeric transmembrane protein according to any of Claims 1 to 10.

15. A nucleic acid construct which comprises a first nucleic acid sequence encoding a first polypeptide as defined in Claim 3 and a second nucleic acid sequence encoding a second polypeptide as defined in Claim 3, the nucleic acid construct having the structure:

Dim1 -TM1-endo1-coexpr-Dim2 -TM2-endo2

in which

Dim1 is a nucleic acid sequence encoding the first dimerisation domain;

TM1 is a nucleic acid sequence encoding the transmembrane domain of the first polypeptide;

endo 1 is a nucleic acid sequence encoding the endodomain of the first polypeptide;

coexpr is a nucleic acid sequence enabling co-expression of both CCRs

Dim2 is a nucleic acid sequence encoding the second dimerization domain;

TM2 is a a nucleic acid sequence encoding the transmembrane domain of the second polypeptide;

endo 2 is a nucleic acid sequence encoding the endodomain of the second polypeptide.

16. A nucleic acid construct according to Claim 15 which also encodes a chimeric antigen receptor (CAR).

17. A nucleic acid construct according to Claim 15 or 16, in which coexpr encodes a sequence comprising a self-cleaving peptide.

18. A nucleic acid construct according to any of Claims 15 to 17, in which alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

19. A vector comprising a nucleic acid construct according to any of Claims 15 to 18.

20. A retroviral vector or a lentiviral vector or a transposon according to Claim 19.

21. A kit which comprises:

- (i) a vector comprising a nucleic acid sequence encoding a first polypeptide as defined in Claim 3; and
 - (ii) a vector comprising a nucleic acid sequence encoding a second polypeptide as defined in Claim 3.
- 5 22. A kit according to Claim 21 which also comprises a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.
23. A kit which comprises:
- (i) a vector comprising a nucleic acid sequence encoding a chimeric transmembrane protein as defined in any of Claims 1 to 10; and
 - 10 (ii) a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor.
24. A method for making a cell according to any of Claims 11 to 13, which comprises the step of introducing: a nucleic acid sequence according to Claim 14; a nucleic acid construct according to any of Claims 15 to 18; a vector according to Claim 19 or 20; or a kit of vectors according to any Claims 21 to 23, into a cell.
- 15 25. A method according to Claim 24, in which the cell is from a sample isolated from a subject.
26. A pharmaceutical composition comprising a plurality of cells according to any of Claims 11 to 13.
27. A method for treating and/or preventing a disease, which comprises the step of
- 20 administering a pharmaceutical composition according to Claim 26 to a subject.
28. A method according to Claim 27, which comprises the following steps:
- (i) isolation of a cell-containing sample from a subject;
 - (ii) transduction or transfection of the cells with: a nucleic acid sequence according to Claim 14; a nucleic acid construct according to any of Claims 15 to 18; a vector
 - 25 according to Claim 19 or 20; or a kit of vectors according to any of Claims 21 to 23,; and
 - (iii) administering the cells from (ii) to a the subject.

29. A method according to Claim 28, in which the sample is a T-cell containing sample.
30. A method according to Claim 28 or 29, in which the disease is a cancer.
31. A pharmaceutical composition according to Claim 26 for use in treating and/or preventing a disease.
- 5 32. Use of a cell according to any of Claims 11 to 13 in the manufacture of a medicament for treating and/or preventing a disease.