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(54) Title: RECEPTOR

(57) Abstract: The present invention provides a chimeric receptor which comprises: a ligand-binding exodomain; and an endodomain which comprises: (i) a cytokine receptor endodomain; and (ii) an intracellular T cell signalling domain.

RECEPTOR

FIELD OF THE INVENTION

The present invention relates to a chimeric receptor (CR), and a cell which expresses such a chimeric receptor.

BACKGROUND TO THE INVENTION

Chimeric antigen receptors (CARs)

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 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 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 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.

There has been some success to date for the application of CAR T cells in the treatment of liquid tumours, such as leukemia and lymphoma. However, the use of CAR T cells for the treatment of solid tumours is more challenging, due to the immunosuppressive microenvironment which is hostile to T cells.

CAR T-cell persistence and activity can be enhanced by administration of cytokines, 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, which are not associated with the disadvantages mentioned above.

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 of a dual chimeric receptor system of the invention
The first CR has an endodomain which comprises a cytokine receptor endodomain (the common gamma chain) and an intracellular T cell signalling domain (CD3 zeta). The second CR has an endodomain which comprises a cytokine receptor endodomain (the IL2 receptor beta chain) and an intracellular T cell signalling domain (comprising both CD28 and OX40 co-stimulatory domains). The antigen-binding exodomains of the two chimeric receptors bind different epitopes on the same ligand. When the CRs bind the ligand, the cytokine endodomains on each molecule are brought into approximation, so that they can associate and lead to cytokine-like cell activation. Cell activation also occurs via the T-cell activating endodomains providing signal 1 and signal 2 to the cell. Note: Although only one chain is shown, the CRs in this system are homodimers.

Figure 3: Schematic diagram of an alternative dual CR system of the invention
In this system, the first and second CRs have a similar structure to the ones shown in Figure 2 in terms of endodomains etc. The difference is that the antigen-binding exodomains of the two chimeric receptors bind the same epitopes on the ligand. They may comprise identical antigen-binding portions. Where there is a tight synapse, the independent binding of an antigen by two chimeric receptors may bring the endodomains into close enough proximity for the cytokine endodomains to

asscoiate, leading to activation. Note: Although only one chain is shown, the CRs in this system are homodimers.

Figure 4: Schematic diagram of a CR:ZAP70 system of the invention
In this system, a single CR recognizes the cognate antigen and its endodomain comprises not only of T-cell signalling (which at a minimum would contain the CD3-Zeta endodomain), but also a cytokine receptor endodomain (for instance either that from common gamma chain, or from the IL2 receptor beta chain). This receptor is co-expressed with a fusion between ZAP70 SH2 domain and a complimentary cytokine receptor endodomain (for instance if the CR contains the common gamma chain, the ZAP70 SH2 domain might be fused to the IL2 receptor beta chain). Upon recognition of antigen, the CR CD3-Zeta endodomain ITAMS become phosphorylated and recruit the ZAP70 fusion protein. Now the two endodomains of the cytokine receptor are closely approximated and a cytokine signal transmitted.

Figure 5 : Amino acid sequence for a dual CR system as illustrated in Figure 2, showing the individual components. In this construct, both CARs recognise CD22. The first CR comprises a binder based on LT22 and an endodomain which comprises the IL2 receptor beta chain; the second CR comprises a binder based on RFB4 and an endodomain which comprises the IL2 receptor gamma chain and CD3 zeta endodomain.

Figure 6: Schematic diagram illustrating the chimeric receptor systems tested in the proliferation/survival assay described in Example 3. One chimeric receptor comprises an R11 scFv, whereas the other comprises an R12 scFv. R11 and R12 bind separate epitopes on the same antigen: ROR1.

Figure 7: Graph to show the fold increase of transduced cells when co-cultured with ligand-coated beads. R11-il2b-z-2A-R12-il2g-z is a construct encoding the 4th generation CAR system illustrated in Figure 6, which comprises both cytokine receptor endodomains and CD3 zeta endodomains; R11-z-2A-R12-z is a construct encoding an equivalent chimeric receptor system which lacks the cytokine receptor endodomains.

Figure 8: Graph to show the number of transduced cells after co-culture with ROR1-coated beads after 3 and 6 days.

Figure 9: Schematic diagram illustrating the chimeric receptor systems tested in the killing assay described in Example 4. One chimeric receptor comprises an R11 scFv, whereas the other comprises an R12 scFv. R11 and R12 bind separate epitopes on the same antigen: ROR1.

Figure 10: Graph to show cell target cell killing after 48 hours incubation at a 10:1 E:T ratio. R11-IL2B-Z-2A-R12-IL2G-z is a construct encoding the 4th generation CAR system illustrated in Figure 9, which comprises both cytokine receptor endodomains and CD3 zeta endodomains; R11-IL2B-2A-R12-IL2G is a construct encoding an equivalent chimeric receptor system which lacks the CD3 zeta endodomains.

Figure 11: Graph to show cell IFNγ secretion after 48 hours incubation at a 10:1 E:T ratio. R11-IL2RB-Z + R12-IL2RG-z denotes cells expressing the 4th generation CAR system illustrated in Figure 9, which comprises both cytokine receptor endodomains and CD3 zeta endodomains; R11-IL2RB + R12-IL2RG denotes cells expressing an equivalent chimeric receptor system which lacks the CD3 zeta endodomains.

Figure 12: Schematic diagram illustrating the chimeric receptor systems tested in the proliferation/survival assay described in Example 6. Both chimeric receptors comprises an R12 scFv, so they bind the same epitope on the same antigen: ROR1. The DNA sequence of the first R12 scFv was wobbled to prevent homologous recombination.

Figure 13: Graph to show the fold increase of transduced cells when co-cultured with ligand-coated beads. R12w-il2b-z-2A-R12-il2g-z is a construct encoding the 4th generation CAR system illustrated in Figure 12, which comprises both cytokine receptor endodomains and CD3 zeta endodomains; R12w-z-2A-R12-z is a construct encoding an equivalent chimeric receptor system which lacks the cytokine receptor endodomains.

Figure 14: Graph to show the number of transduced cells after co-culture with ROR1-coated beads after 3 and 6 days.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have developed a new type of chimeric receptor (CR) which grafts the binding specificity of, for example, an antibody, on to a combination

endodomain which comprises both cytokine receptor endodomain and intracellular T-cell signalling components. Ligation of the receptor provides both cytokine-type and T cell receptor-type activation and proliferation signals to the cell, causing enhanced activation and proliferation, than a conventional CAR.

Enhancement of engraftment, proliferation and survival is particularly useful in the treatment of solid tumours as it enables the CR-expressing cells to engraft and expand in a hostile tumour microenvironment.

Thus in a first aspect, the present invention provides a chimeric receptor comprising:

a ligand-binding exodomain; and

an endodomain which comprises:

(i) a cytokine receptor endodomain; and

(ii) an intracellular T cell signalling domain.

The ligand-binding exodomain may comprise a heavy chain variable domain (V_H) and/or a light chain variable domain (V_L).

The cytokine receptor endodomain may comprise or consist of a type I cytokine receptor endodomain α-, β-, or γ-chain. For example, the cytokine receptor endodomain may comprise or consist of:

(i) IL-2 receptor β-chain endodomain

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

(iii) common γ-chain receptor endodomain.

The intracellular T-cell signalling domain may comprise one or more of the following: CD3 zeta endodomain, CD28 endodomain, OX40 endodomain, 4-1BB endodomain, CD2 endodomain, CD27 endodomain, ICOS endodomain, CD40 endodomain.

The arrangement of the intracellular T-cell signalling domain(s) and the cytokine receptor endodomain(s) may be such that when the receptor is expressed at the surface of a cell, the intracellular T-cell signalling domain(s) is/are positioned distal to the membrane and the cytokine receptor endodomain(s) is/are positioned proximal to the membrane on the intracellular cell surface.

In a second aspect the present invention provides a chimeric receptor system.

In a first embodiment of the second aspect of the invention, the chimeric receptor system comprises at least two chimeric receptors according to the first aspect of the invention.

In this first embodiment, the chimeric receptor system comprises a first chimeric receptor which comprises a first cytokine receptor endodomain, and a second chimeric receptor which comprises a second cytokine receptor endodomain. The first cytokine receptor endodomain is complementary to the second cytokine receptor endodomain.

The first chimeric receptor and the second chimeric receptor may bind to different epitopes of the same antigen.

Alternatively, the first chimeric receptor and the second chimeric receptor may bind to the same epitope of the same antigen.

Alternatively, the ligand binding domain of the first chimeric receptor and the ligand binding domain of the second chimeric receptor may have complementary ligand-binding domains, such that together they are capable of ligand binding.

The term “complementary” indicates that the first and second cytokine endodomains associate leading to cell signalling.

The first cytokine receptor endodomain may be or comprise a type 1 cytokine receptor endodomain α- or β-chain, and the second cytokine receptor endodomain may be or comprise a type 1 cytokine receptor endodomain γ-chain, such that when the first chimeric receptor and the second chimeric receptor bind to the antigen, cytokine signalling through the α-/β-chain and γ-chain occurs.

The first chimeric receptor may comprise a CD3 zeta endodomain, and the second chimeric receptor may comprise one or more co-stimulatory domain(s) selected from CD28 endodomain, OX40 endodomain and 4-1BB endodomain.

Alternatively, both the first and second chimeric receptors may comprise an intracellular signalling domain such as the CD3 zeta endodomain.

In a second embodiment of the second aspect of the invention, the chimeric receptor system comprises a chimeric receptor according to the first aspect of the invention and an intracellular fusion protein.

In this second embodiment, the chimeric receptor comprises a first cytokine receptor endodomain, and the intracellular fusion protein comprises a second cytokine receptor endodomain.

The first cytokine receptor endodomain is complementary to the second cytokine receptor endodomain.

The chimeric receptor may comprise a type I cytokine receptor endodomain α- or β-chain, and the intracellular fusion protein may comprise a type I cytokine receptor endodomain γ-chain, or vice versa.

The chimeric receptor may comprise a CD3 zeta endodomain, and the intracellular fusion protein may comprise one or more co-stimulatory domain(s) selected from CD28 endodomain, OX40 endodomain and 4-1BB endodomain, or vice versa.

The chimeric receptor may comprise a CD3 zeta endodomain, and the intracellular fusion protein may lack an intracellular signalling domain.

The intracellular fusion protein may comprise a domain which binds to a phosphorylated CD3 zeta endodomain, such as a ZAP70 SH2 domain. When the chimeric receptor binds the target antigen, this leads to phosphorylation of the CD3 zeta endodomain. The ZAP70 SH2 domain of the intracellular fusion protein binds to the phosphorylated CD3 zeta endodomain, bringing the first and second cytokine receptor endodomains together.

In a third embodiment of the second aspect of the invention, the chimeric receptor system comprises a chimeric receptor according to the first aspect of the invention and a transmembrane protein.

The chimeric receptor comprises a first cytokine receptor endodomain, and the transmembrane protein comprises a second cytokine receptor endodomain. The first cytokine receptor endodomain is complementary to the second cytokine receptor endodomain.

The transmembrane protein may lack a ligand binding exodomain. The transmembrane protein may be tethered to the cell membrane, for example via a transmembrane domain or a myristoylation group.

The chimeric receptor may comprise a type I cytokine receptor endodomain α- or β-chain, and the transmembrane protein may comprise a type I cytokine receptor endodomain γ-chain, or vice versa.

The chimeric receptor may comprise a CD3 zeta endodomain, and the transmembrane protein may comprise one or more co-stimulatory domain(s) selected from CD28 endodomain, OX40 endodomain and 4-1BB endodomain.

The chimeric receptor may comprise a CD3 zeta endodomain, and the transmembrane protein may lack an intracellular signalling domain and/or a costimulatory domain.

The transmembrane protein may comprise a domain which binds to a phosphorylated CD3 zeta endodomain, such as a ZAP70 SH2 domain. When the chimeric receptor binds the target antigen, this leads to phosphorylation of the CD3 zeta endodomain. The ZAP70 SH2 domain of the transmembrane protein binds to the phosphorylated CD3 zeta endodomain, bringing the first and second cytokine receptor endodomains together.

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

In a first embodiment of the third aspect of the invention, the cell comprises a cell receptor system which comprises a first chimeric receptor and a second chimeric receptor.

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

The cell may alternatively comprise a first chimeric receptor and a second chimeric receptor which bind the same epitope on the same antigen.

The cell may alternatively comprise a first chimeric receptor and a second chimeric receptor which have complementary ligand-binding domains, such that together the ligand-binding domain of the first chimeric receptor and the ligand binding domain of the second chimeric receptor are capable of ligand binding.

The first chimeric receptor may comprise a first cytokine receptor endodomain and the second chimeric receptor may comprise a second cytokine receptor endodomain, and the first and second cytokine receptor endodomains may be capable of associating leading to cell signalling.

For example, the first chimeric receptor may comprise a type I cytokine receptor endodomain α- or β-chain, and the second chimeric receptor may comprise a type I cytokine receptor endodomain γ-chain, such that when the first chimeric receptor and the second chimeric receptor bind the antigen, combined signalling through the α-/β-chain and γ-chain occurs.

The first chimeric receptor may comprise a CD3 zeta endodomain; and the second chimeric receptor may comprise one or more co-stimulatory domain(s) selected from, for example, CD3 zeta endodomain, CD28 endodomain, OX40 endodomain, 4-1BB endodomain, CD2 endodomain, CD27 endodomain, ICOS endodomain and CD40 endodomain.

A cell according to the second aspect of the invention may also comprise a second receptor comprising:

a ligand-binding exodomain; and

an endodomain which comprises a cytokine receptor endodomain which is complementary to the cytokine receptor endodomain of the chimeric receptor;

which second receptor lacks an intracellular T cell signalling domain.

In a second embodiment of the third aspect of the invention, the cell comprises a chimeric receptor according to the first aspect of the invention and an intracellular fusion protein as defined above.

The intracellular fusion protein may comprise a ZAP70 SH2 domain.

In a third embodiment of the third aspect of the invention the cell comprises a chimeric receptor according to the first aspect of the invention and a transmembrane protein as defined above.

In a fourth aspect, the present application provides a nucleic acid sequence capable of encoding a chimeric receptor according to the first aspect of the invention.

In a fifth aspect there is provided a nucleic acid construct which encodes a chimeric receptor system according to the second aspect of the invention.

In a first embodiment of the fifth aspect of the invention, the nucleic acid construct comprises a first nucleic acid sequence encoding a first chimeric receptor and a second nucleic acid sequence encoding a second chimeric receptor.

The nucleic acid construct may have 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 chimeric receptor;

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

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

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

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

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

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

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

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

In the nucleic acid construct of the fifth aspect of the invention, endo 1 may comprise a nucleic acid sequence encoding a first chain of a cytokine receptor endodomain, and a nucleic acid sequence encoding a first intracellular T cell signalling domain; and endo 2 may comprise a nucleic acid sequence encoding a second chain of a cytokine receptor endodomain and a nucleic acid sequence encoding a second intracellular T cell signalling domain.

The coexpr 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.

In a second embodiment of the fifth aspect of the invention there is provided a nucleic acid construct which comprises a first nucleic acid sequence encoding a chimeric receptor according to the first aspect of the invention and a second nucleic acid sequence encoding an intracellular fusion protein.

The nucleic acid construct may have the structure:

AgB1-spacer1-TM1-endo1-coexpr-domain2-endo2

in which
AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the chimeric receptor;
spacer 1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo 1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the intracellular fusion protein
domain2 is a nucleic acid sequence encoding a second domain of the intracellular fusion protein;
endo 2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the intracellular fusion protein.

The second domain, "domain2", may encode a sequence capable of binding to a phosphorylated CD3 zeta domain. In this respect, "domain2" may be "ZAP70", a nucleic acid sequence encoding a ZAP70 SH2 domain.

In a third embodiment of the fifth aspect of the invention there is provided a nucleic acid construct which comprises a first nucleic acid sequence encoding a chimeric receptor and a second nucleic acid sequence encoding a transmembrane protein.

The nucleic acid construct may have the structure:

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

in which
AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the chimeric receptor;
spacer1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the transmembrane protein,
TM2 is a nucleic acid sequence encoding a transmembrane localisation sequence of the transmembrane domain,
endo2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the tranmembrane protein.

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

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

In a seventh aspect, there is provided a kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a first chimeric receptor as defined in the first aspect of the invention; and

ii) a vector comprising a nucleic acid sequence encoding a second chimeric receptor as defined in the first aspect of the invention.

There is also provided a kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a chimeric receptor as defined in the first aspect of the invention; and

ii) a vector comprising a nucleic acid sequence encoding a second receptor or an intracellular fusion protein as defined above.

There is also provided a kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a chimeric receptor as defined in the first aspect of the invention; and

ii) a vector comprising a nucleic acid sequence encoding a transmembrane protein as defined above.

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

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

In a ninth aspect there is provided a pharmaceutical composition comprising a plurality of cells according to the third aspect of the invention.

In a tenth 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 ninth 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 fourth aspect of the invention; a nucleic acid construct according to the fifth aspect of the invention; a vector according to the sixth aspect of the invention; or a kit of vectors according to the seventh 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 ninth 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 third aspect of the invention in the manufacture of a medicament for treating and/or preventing a disease.

DETAILED DESCRIPTION

CHIMERIC RECEPTOR (CR)

A chimeric receptor (CR) is a molecule which comprises a cytokine receptor endodomain and a heterologous ligand-binding exodomain. The endodomain of the chimeric receptor may also comprise an intracellular T cell signalling domain.

A chimeric receptor may therefore comprise:
(i) a ligand binding exodomain;
(ii) an optional spacer;
(iii) a transmembrane domain;
(iv) a cytokine-receptor endodomain; and
(v) an intracellular T-cell signalling domain.

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

The cytokine receptor 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.

The cytokine receptor 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).

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, IL13, 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

(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 (Kd ~ 10−11 M) on activated T cells and regulatory T cells.

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.

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.

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

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:
ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGG
ALGEGPGASPCNQHSPYWAPPCYTLKPET

SEQ ID No. 2: Endodomain derived from human IL-2Rβ:
NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAP
EISPLEVLERDKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEAC
QVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDDAYCTFPSRDDLLLFSPSL
LGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPP
PELVLREAGEEVPDAGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQ
GQDPTHLV

The term “derived from” means that the endodomain of the chimeric 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.

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.

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.

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.

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.

The chimeric 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.

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-7Rα:
KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEG
FLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACD
APILSSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQ
PILTSLGSNQEEAYVTMSSFYQNQ

MONOMERIC CHIMERIC RECEPTOR SYSTEMS

Chimeric antigen receptors are usually homodimers of two identical chains.

The chimeric receptor of the invention can be a homodimer, or a monomer which is brought into association with another chimeric receptor monomer in the presence of ligand.

In particular, the chimeric receptor may be a monomer which comprises:

(i) an exodomain;
(ii) a transmembrane domain;
(iii) a cytokine-receptor endodomain; and
(v) an intracellular T-cell signalling domain.

The exodomain may comprise a ligand-binding domain such as an scFv. A cell may comprise two monomeric CRs in which the ligand binding domain of the first CR and the ligand-binding domain of the second CR bind to different epitopes on the same ligand.

Alternatively, the exodomain may comprise a domain which, when brought together with the exodomain of another chimeric receptor, produces a functional ligand binding domain. For example, one monomeric chimeric receptor may comprise V_H and the second chimeric receptor comprises V_L of an antibody.

A monomeric cytokine receptor may also comprise one or more intracellular T cell signalling domain(s). For example, the receptor may comprise one or more of the following: CD3 zeta endodomain, CD28 endodomain, OX40 endodomain, 4-1BB endodomain, CD2 endodomain, CD27 endodomain, ICOS endodomain, CD40 endodomain.

DUAL CHIMERIC RECEPTOR SYSTEMS

Where a cell comprises two homodimeric or monomeric chimeric receptors, they may have “complementary” cytokine receptor endodomains. Complementary cytokine receptor endodomains are capable of associating with each other to induce cytokine-type signalling.

Examples of complementary cytokine receptor endodomains are given in the table below. In the dual CR system of the invention, one CR may comprise the first cytokine receptor endodomain and the other CR may comprise the second cytokine receptor endodomain

First cytokine receptor endodomain	Second cytokine receptor endodomain
IL2-receptor beta chain	Common gamma chain

IL7-receptor alpha chain	Common gamma chain

The dual chimeric receptor system of the invention comprises one or more intracellular T cell signalling domains. The intracellular T cell signalling domains may be "shared" between the two homodimeric or monomeric chimeric receptors, or one receptor may comprise intracellular T cell signalling domain(s) and the other one not. Some possible combinations are summarised in the following Table:

Intracellular T cell signalling domains in the first cytokine receptor	**Intracellular T cell signalling domains in the second cytokine receptor**
CD3 zeta	None
CD3 zeta and one or more co-stimulatory domains	None
CD3 zeta	CD3 zeta
CD3 zeta and one or more co-stimulatory domains	CD3 zeta and one or more co-stimulatory domains
CD3 zeta	CD3 zeta and one or more co-stimulatory domains
CD3 zeta	One or more co-stimulatory domains
CD3 zeta and one or more co-stimulatory domains	One or more co-stimulatory domains

For example, one receptor may comprise a CD3 zeta endodomain and the other receptor may comprise one or more co-stimulatory domains, such as CD3 zeta endodomain, CD28 endodomain, OX40 endodomain, 4-1BB endodomain, CD2 endodomain, CD27 endodomain, ICOS endodomain and/or CD40 endodomain.

ZAP70 CHIMERIC RECEPTOR SYSTEMS

In one embodiment of the invention, the chimeric receptor is expressed in the cell along with an intracellular fusion protein. The intracellular fusion protein comprises a cytokine receptor endodomain. The intracellular fusion protein may comprise a domain which binds to a phosphorylated CD3 zeta endodomain, such as a ZAP70 SH2 domain. This embodiment is illustrated schematically in Figure 4.

ZAP70 is a protein normally expressed near the surface membrane of T cells and natural killer cells. It is part of the T cell receptor (TCR), and plays a critical role in T-

cell signalling. Its molecular weight is 70 kDa, and is composed of 2 N-terminal SH2 domains and a C-terminal kinase domain. It is a member of the protein-tyrosine kinase family.

The earliest step in T cell activation is the recognition of a peptide MHC-complex on the target cell by the TCR. This initial event causes the close association of Lck kinase with the cytoplasmic tail of CD3-zeta in the TCR complex. Lck then phosphorylates tyrosine residues in the cytoplasmic tail of CD3-zeta which allows the recruitment of ZAP70. ZAP70 is an SH2 containing kinase that plays a pivotal role in T cell activation following engagement of the TCR. Tandem SH2 domains in ZAP70 bind to the phosphorylated CD3 resulting in ZAP70 being phosphorylated and activated by Lck or by other ZAP70 molecules in trans. Active ZAP70 is then able to phosphorylate downstream membrane proteins, key among them the linker of activated T cells (LAT) protein. LAT is a scaffold protein and its phosphorylation on multiple residues allows it to interact with several other SH2 domain-containing proteins including Grb2, PLC-g and Grap which recognize the phosphorylated peptides in LAT and transmit the T cell activation signal downstream ultimately resulting in a range of T cell responses.

An example ZAP70 protein is the human ZAP70 protein having the UniProtKB accession number P43403. This exemplified sequence is 619 amino acids in length and is shown as SEQ ID NO: 22.

ZAP70 amino acid sequence (SEQ ID NO: 22)

MPDPAAHLPFFYGSISRAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFH
HFPIERQLNGTYAIAGGKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPG
VFDCLRDAMVRDYVRQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLT
REEAERKLYSGAQTDGKFLLRPRKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEG
TKFDTLWQLVEYLKLKADGLIYCLKEACPNSSASNASGAAAPTLPAHPSTLTHPQRRI
DTLNSDGYTPEPARITSPDKPRPMPMDTSVYESPYSDPEELKDKKLFLKRDNLLIADI
ELGCGNFGSVRQGVYRMRKKQIDVAIKVLKQGTEKADTEEMMREAQIMHQLDNPYI
VRLIGVCQAEALMLVMEMAGGGPLHKFLVGKREEIPVSNVAELLHQVSMGMKYLEE
KNFVHRDLAARNVLLVNRHYAKISDFGLSKALGADDSYYTARSAGKWPLKWYAPEC
INFRKFSSRSDVWSYGVTMWEALSYGQKPYKKMKGPEVMAFIEQGKRMECPPECP
PELYALMSDCWIYKWEDRPDFLTVEQRMRACYYSLASKVEGPPGSTQKAEAACA

The ZAP70 sequence shown as SEQ ID No. 22 comprises tandem SH2 domains. SH2 1 comprises amino acids Nos 10-102 and SH2 2 comprises amino acid Nos 163-254 of this sequence. The ZAP70 SH2 domain may comprise SH2 1, SH2 2 or both SH2 domains.

The fusion protein may comprise tandem ZAP70 SH2 domains. For example, the fusion protein may comprise the sequence shown as SEQ ID NO: 23.

ZAP70 SH2 domain (SEQ ID NO: 23)

MPDPAAHLPFFYGSISRAEAEEHLKLAGMADGLFLLRQCLRSLGGYVLSLVHDVRFH
HFPIERQLNGTYAIAGGKAHCGPAELCEFYSRDPDGLPCNLRKPCNRPSGLEPQPG
VFDCLRDAMVRDYVRQTWKLEGEALEQAIISQAPQVEKLIATTAHERMPWYHSSLT
REEAERKLYSGAQTDGKFLLRPRKEQGTYALSLIYGKTVYHYLISQDKAGKYCIPEG
TKFDTLWQLVEYLKLKADGLIYCLKEACPNSSASNASGAAAPTLPAHPSTLTHP

The fusion protein may comprise a variant of SEQ ID NO: 23 having at least 80, 85, 90, 95, 98 or 99% sequence identity, provided that the variant sequence is a SH2 domain sequence having the required properties. In other words, the variant sequence must be capable of binding to the phosphorylated tyrosine residues in the cytoplasmic tail of CD3-zeta which allow the recruitment of ZAP70.

In certain embodiments, the fusion protein may comprise the ZAP70 SH2 domain and the ZAP70 kinase domain. For example, the fusion protein may comprise the sequence shown as SEQ ID NO: 22 or a variant thereof having at least 80, 85, 90, 95, 98 or 99% sequence identity.

The fusion protein also comprises a cytokine receptor endodomain. The cytokine receptor endodomain of the fusion protein may be “complementary” to the cytokine receptor endodomain of the chimeric receptor, as defined above. Complementary cytokine receptor endodomains are capable of associating with each other to induce cytokine-type signalling.

TRANSMEMBRANE PROTEIN

In another embodiment of the invention, the chimeric receptor is expressed in the cell along with a transmembrane protein. The chimeric receptor and the transmembrane protein comprise complementary cytokine receptor endodomains.

The transmembrane protein may be tethered to or associated with the cell membrane. For example, the transmembrane protein may comprise a transmembrane domain, which anchors the protein to the membrane of a cell. Alternatively the transmembrane protein may comprise a myristoyl group.

Myristoylation is a lipidation modification where a myristoyl group, derived from myristic acid, is covalently attached by an amide bond to the alpha-amino group of an N-terminal glycine residue.

The transmembrane protein may also comprise one or more co-stimulatory domains.

The transmembrane protein may lack a ligand-binding exodomain.

SPACER

The chimeric 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.

Where the cell of the present invention comprises two or more chimeric receptors, the spacers may be the same or different.

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.

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

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

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

GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGKKD

SEQ ID No. 5 (human CD8 stalk):
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

SEQ ID No. 6 (human IgG1 hinge):
AEPKSPDKTHTCPPCPKDPK

The spacer may be monomeric. Monomeric spacers may be generated by mutation of the cysteine residue(s) responsible for disulphide bond formation (Bridgeman et al (2010) J. Immunol. 184:6938-6949).

TRANSMEMBRANE DOMAIN

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

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-15R.

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

SEQ ID No. 8 - Transmembrane derived from human IL-2Rβ:
IPWLGHLLVGLSGAFGFIILVYLLI

SEQ ID No. 9 - Transmembrane derived from human IL-7Rα:
PILLTISILSFFSVALLVILACVLW

SEQ ID No. 10 - Transmembrane derived from human IL-15Rα:
AISTSTVLLCGLSAVSLLACYL

LIGAND-BINDING EXODOMAIN

The term "ligand binding domain" refers to the extracellular portion of the CR which is involved in ligand binding. The ligand-binding domain of a single chimeric receptor may be itself capable of binding the ligand (for example, if it is based on an scFv). Alternatively the ligand-binding domain may be capable of ligand binding when in association with another chimeric receptor (for example, where one CR comprises V_H and one CR comprises V_L of an antibody).

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 CR or a combination of complementary CR ligand-binding domains.

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 amino acid sequence shown in Figure 5 comprises two CARs, each having a CD22-binding ligand binding domain. One is based on the scFv LT22 and one is based on the scFv RFB4.

LIGAND

The CR or CR system of the present invention binds to a 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 CR may be a soluble ligand secreted by or shed from a tumour.

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

CELL SURFACE ANTIGEN

The CR or CR system 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 CR or CR system 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.

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	PSCA, PSMA
Bowel cancer	A33

PROSTATE-CANCER ASSOCIATED ANTIGENS

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

PSCA is a glycosylphosphatidylinositol-anchored cell membrane glycoprotein. It is is up-regulated in a large proportion of prostate cancers and is also detected in cancers 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 CR-expressing cell of the invention may comprise an antigen binding domain based on one of these antibodies.

PSMA is 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 CR of the invention may comprise an antigen binding domain based on one of these antibodies.

For example, the CR may comprise an scFv based on J591, having the sequence shown as SEQ ID No. 11.

SEQ ID No. 11 (J591 scFv)
EVQLQQSGPELKKPGTSVRISCKTSGYTFTEYTIHWVKQSHGKSLEWIGNINPNNG
GTTYNQKFEDKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGQGTTL
TVSSGGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDW
YQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTDFTLTITNVQSEDLADYFCQQY
NSYPLTFGAGTMLDLKR

SIGNAL PEPTIDE

The CR or transmembrane protein described herein may comprise a signal peptide so that when it/they is/are 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.

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.

The signal peptide may be at the amino terminus of the molecule. Numerous signal peptides are known in the art which are suitable for use with the CR on the invention.

CR ENDODOMAIN

The endodomain is the portion of a chimeric receptor or transmembrane protein which is located on the intracellular side of the membrane.

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. The same principle holds true for the chimeric receptor of the present invention.

Clustering of the chimeric receptors by kinetic segregation allows the cell signalling to occur via the intrcellular T-cell signalling domains

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.

The CR 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.

Where the cell comprises two or more chimeric receptors the intracellular signalling domains may be "shared" between the CR molecules. For example, one CR may comprise a CD3 zeta endodomain and another CR may comprise one or more co-stimulatory domains, such as from CD28, OX40 or 4-1BB.

Where the cell comprises a chimeric receptor and a transmembrane protein, the intracellular signalling domains may be "shared" between the CR and the transmembrane protein. For example, the CR may comprise a CD3 zeta endodomain and the transmembrane protein may comprise one or more co-stimulatory domains, such as from CD28, OX40 or 4-1BB.

The CR or transmembrane protein 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 one or more of the sequences shown as SEQ ID No. 12 to 20 or a variant thereof having at least 80% sequence identity.

SEQ ID No. 12 - CD3 Z endodomain
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

SEQ ID No. 13 - CD28 and CD3 Zeta endodomains
SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 14 - CD28, OX40 and CD3 Zeta endodomains
SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRDQRLPPDAHKPPG
GGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
LYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 15 - ICOS endodomain
CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL

SEQ ID No. 16 - CD27 endodomain
QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP

SEQ ID No. 17 - BTLA endodomain
RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCF
RMQEGSEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYASICVRS

SEQ ID No. 18 - CD30 endodomain
HRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGL
MSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIM
KADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVML
SVEEEGKEDPLPTAASGK

SEQ ID No. 19 - GITR endodomain
QLGLHIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGD
LWV

SEQ ID No. 20 - HVEM endodomain
CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRS
PNH

A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID No. 12 to 20, provided that the sequence provides an effective intracellular signalling domain.

NUCLEIC ACID

The present invention also provides a nucleic acid encoding a CR 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 CR;
spacer 1 is a nucleic acid sequence encoding the spacer of the CR;
TM1 is a a nucleic acid sequence encoding the transmembrane domain of the CR;
endo 1 is a nucleic acid sequence encoding the endodomain of the CR.

NUCLEIC ACID CONSTRUCT

The present invention further provides a nucleic acid construct which encodes a chimeric receptor system of the invention.

The nucleic acid construct may comprise a first nucleic acid sequence encoding a first CR as defined in connection with the first aspect of the invention; and a second nucleic acid sequence encoding a second CR as defined in connection with the first aspect of the invention.

The nucleic acid construct may have 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 chimeric receptor;
spacer 1 is a nucleic acid sequence encoding the spacer of the first chimeric receptor;

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

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

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

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

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

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

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

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 CRs are co-expressed at the cell surface.

The first and second CRs may bind distinct epitopes on the same antigen. Alternatively the first and second CRs may comprise complementary ligand-binding domains which, together, are capable of antigen binding.

The first and second CRs may have complementary cytokine receptor 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.

Alternatively the nucleic acid construct may comprise a first nucleic acid sequence encoding a chimeric receptor according to the first aspect of the invention and a second nucleic acid sequence encoding an intracellular fusion protein.

The nucleic acid construct may have the structure:

AgB1-spacer1-TM1-endo1-coexpr-domain2-endo2

in which

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

spacer 1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo 1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the intracellular fusion protein
domain2 is a nucleic acid sequence encoding a second domain of the intracellular fusion protein;
endo 2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the intracellular fusion protein.

The second domain, "domain2", may be "ZAP70", a nucleic acid sequence encoding a ZAP70 SH2 domain.

Alternatively the nucleic acid construct may comprise a first nucleic acid sequence encoding a chimeric receptor and a second nucleic acid sequence encoding a transmembrane protein.

The nucleic acid construct may have the structure:

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

in which
AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the chimeric receptor;
spacer1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the transmembrane protein,
TM2 is a nucleic acid sequence encoding a membrane localisation domain of the transmembrane domain,
endo2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the tranmembrane protein.

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 CRs. It may be a sequence encoding a cleavage site, such that the nucleic acid construct produces comprises two or more CRs, 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.

The cleavage site may be any sequence which enables the first and second CRs, 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 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 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.

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 endoprotease that can efficiently cleave precursor proteins at their paired basic amino acid processing sites. Examples of furin substrates include proparathyroid 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 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-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 expressed in the mammalian cell.

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 aptho- and cardioviruses is mediated by 2A "cleaving" at its own C-terminus. In apthoviruses, 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 (Donelly et al (2001) as above).

"2A-like" sequences have been found in picornaviruses other than aptho- 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 as:

SEQ ID No. 21: RAEGRGSLLTCGDVEENPGP.

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

VECTOR

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 CR(s) according to the first aspect of the invention. Such a vector may be used to introduce the nucleic acid sequence(s) into a host cell so that it expresses a CR 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.

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 CR(s) of the invention. The cell may comprise a CR system as defined above.

The cell may comprise one or more nucleic acid(s) or vector(s) of the present invention.

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

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.

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 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

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.

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

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).

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.

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

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 CR according to the first aspect of the invention, for example by treatment with an anti-CD3 monoclonal antibody.

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 CR.

The T or NK cells may then by 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.

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 unconnected donor (3rd party).

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

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

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 CR is PSA, PSMA or PSCA, the cancer may be prostate cancer.

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 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.

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

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

EXAMPLES

Example 1 – In vitro testing

T-cells are transduced with retroviral vector coding for standard CARs or CARs which transmit cytokine signals. Non-transduced and transduced T-cells are challenged with target cells expressing the CR cognate target. Activation of the cytokine pathway in response to target antigen can be directly detected by utilizing intracellular antibody staining and flow cytometry to measure the level of phosphorylation of known

mediators in the PI3 kinase, MAP Kinase and JAK-STAT pathways. Cytokine signalling can be indirectly determined by measuring T-cell proliferation, apoptosis and phenotype by flow-cytometry.

Example 2 – Generation of a "4th Generation" CAR system with antigen binding domains against distinct epitopes of a target antigen

A 4th generation CAR system was designed having scFvs which bind to different epitopes of the antigen ROR1 (Figure 6). The first chimeric receptor had an antigen binding domain comprising an R11 scFv, a human Fc spacer, an IL2 receptor β endomain and a CD3 zeta endodomain; the second chimeric receptor had an antigen binding domain comprising an R12 scFv, a CD8 stalk spacer, an IL2 receptor γ endodomain and a CD3 zeta endodomain. Two control receptor systems were also designed: one which lacked the cytokine receptor endodomains (Figure 6); and one which lacked the CD3 zeta domains (Figure 9), but which were otherwise identical to the system described above.

Example 3 – A 4th generation CAR system shows increased proliferation/survival than an equivalent CAR system lacking cytokine receptor endodomains

In order to investigate whether the presence of a chimeric receptor system comprising cytokine endodomains provides a proliferation/survival signal, the CTLL2 murine cytotoxic T cell line (ATCC® TIB-214™) was used which requires IL2 for growth. CTLL cells were transduced with a vector expressing one or other of the two chimeric receptor systems shown in Figure 6. Cell proliferation was assessed after 3 and 6 days of culture either with ROR1-coated beads or uncoated beads.

The results are shown in Figures 7 and 8. As shown in Figure 7, the 4th generation CAR which included cytokine receptor and CD3 zeta endodomains showed a greater fold-enrichment in transduced cells when co-cultured with ligand at both day 3 and day 6, than an equivalent chimeric receptor system which lacked cytokine receptor endodomains. As shown in Figure 8, the presence of ligand greatly increased cell survival/proliferation at both day 3 and day 6 for cells expressing the 4th generation CAR, whereas the presence of ligand had little effect on the proliferation/survival of cells expressing an equivalent chimeric receptor system lacking cytokine receptor endodomains.

Example 4 - A 4th generation CAR system shows increased target cell killing and IFNg release than an equivalent CAR system lacking CD3 zeta endodomains

Next it was investigated whether a 4th generation CAR which included cytokine receptor and CD3 zeta endodomains is capable of killing target cells. Healthy donor PBMCs were transduced with a vector expressing one or other of the two chimeric receptor systems shown in Figure 9. The transduced cells were co-cultured with either SupT1 cells or SupT1 target cells expressing ROR1 at a 10:1 E:T ratio for 48 hours. Killing of target cells was then analysed by FACS and an ELISA was used to assay IFNγ secretion.

As shown in Figure 10, the 4th generation CAR which included cytokine receptor and CD3 zeta endodomains was capable of killing ROR1-expressing target cells and killed much more efficiently than an equivalent chimeric receptor system which lacked CD3 zeta endodomains.

Co-culture of PBMCs expressing the 4th generation CAR with ROR1-expressing target cells lead to significant levels of IFNγ release, unlike PBMCs expressing an equivalent chimeric receptor system which lacks CD3 zeta endodomains (Figure 11).

Example 5 - Generation of a 4th Generation CAR system with antigen binding domains against the same epitope of a target antigen

A 4th generation CAR system was designed having scFvs which bind to the same epitope of the antigen ROR1 (Figure 12). The first chimeric receptor had an antigen binding domain comprising an R12 scFv, a human Fc spacer, an IL2 receptor β endomain and a CD3 zeta endodomain; the second chimeric receptor had an antigen binding domain comprising an R12 scFv, a CD8 stalk spacer, an IL2 receptor γ endodomain and a CD3 zeta endodomain. The DNA sequence of the R12ScFv of the first chimeric receptor was wobbled to avoid homologous recombination. A control receptor system was also designed which lacked the cytokine receptor endodomains but which was otherwise identical (also shown in Figure 12).

Example 6 - A 4th generation CAR system with antigen binding domains against the same epitope of a target antigen shows increased proliferation/survival than an equivalent CAR system lacking cytokine receptor endodomains

An equivalent survival/proliferation assay was conducted as described in Example 3 for the constructs developed in Example 5 and the results are shown in Figures 13 and 14. As shown in Figure 13, the 4th generation CAR which included cytokine receptor and CD3 zeta endodomains showed a greater fold-enrichment in transduced cells when co-cultured with ligand for 3 days than an equivalent chimeric receptor system which lacked cytokine receptor endodomains. As shown in Figure 8, the presence of ligand greatly increased cell survival/proliferation after three days of culture for cells expressing the 4th generation CAR, whereas the presence of ligand had little effect on the proliferation/survival of cells expressing an equivalent chimeric receptor system lacking cytokine receptor endodomains.

It is therefore possible to target an antigen using the chimeric receptor system of the present invention using two antigen binding domains which bind the same epitope of the target antigen. This potentially simplifies the design of the chimeric receptor system and avoids the need to find mutually exclusive epitopes for each target antigen.

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.

CLAIMS

1. A chimeric receptor comprising:
a ligand-binding exodomain; and
an endodomain which comprises:
(i) a cytokine receptor endodomain; and
(ii) an intracellular T cell signalling domain.

2. A chimeric receptor according to claim 1, wherein the ligand-binding exodomain comprises a heavy chain variable domain (V_H) and/or a light chain variable domain (V_L).

3. A chimeric receptor according to any preceding claim, wherein the cytokine receptor endodomain comprises or consists of a type I cytokine receptor endodomain α-, β-, or γ-chain.

4. A chimeric receptor according to claim 3, wherein the cytokine receptor endodomain comprises or consists of:
(i) IL-2 receptor β-chain endodomain
(ii) IL-7 receptor α-chain endodomain; or
(iii) common γ-chain receptor endodomain.

5. A chimeric receptor according to any preceding claim, wherein the intracellular T-cell signalling domain comprises one or more of the following: CD3 zeta endodomain, CD28 endodomain, OX40 endodomain, 4-1BB endodomain, CD2 endodomain, CD27 endodomain, ICOS endodomain, CD40 endodomain.

6. A chimeric receptor according to any preceding claim, wherein the arrangement of the intracellular T-cell signalling domain(s) and the cytokine receptor endodomain is such that when the receptor is expressed at the surface of a cell, the intracellular T-cell signalling domain(s) is/are positioned distal to the membrane and the cytokine receptor endodomain are positioned proximal to the membrane on the intracellular cell surface.

7. A chimeric receptor system which comprises first and second chimeric receptors according to any of claims 1 to 6;

wherein the first chimeric receptor comprises a first cytokine receptor endodomain, and the second chimeric receptor comprises a second cytokine receptor endodomain, and

and wherein the first cytokine receptor endodomain is complementary to the second cytokine receptor endodomain.

8. A chimeric receptor system according to claim 7, wherein the first chimeric receptor and the second chimeric receptor bind to different epitopes of the same antigen.

9. A chimeric receptor system according to claim 7, wherein the first chimeric receptor and the second chimeric receptor bind to the same epitope of the same antigen.

10. A chimeric receptor system according to claim 7, wherein the ligand binding domain of the first chimeric receptor and the ligand binding domain of the second chimeric receptor have complementary ligand-binding domains, such that together they are capable of ligand binding.

11. A chimeric receptor system according to any of claims 7 to 10, wherein the first and second cytokine endodomains associate leading to cell signalling.

12. A chimeric receptor system according to claim 11, wherein the first cytokine receptor endodomain is a type 1 cytokine receptor endodomain α- or β-chain, and the second cytokine receptor endodomains is a type 1 cytokine receptor endodomain γ-chain, such that when the first chimeric receptor and the second chimeric receptor bind to the antigen, cytokine signalling through the α-/β-chain and γ-chain occurs.

13. A chimeric receptor system according to any of claims 7 to 12, wherein the first chimeric receptor comprises a CD3 zeta endodomain, and the second chimeric receptor comprises one or more co-stimulatory domain(s) selected from CD28 endodomain, OX40 endodomain and 4-1BB endodomain.

14. A chimeric receptor system according to any of claims 7 to 12, wherein both the first and second chimeric receptors comprise CD3 zeta endodomains.

15. A chimeric receptor system comprising a chimeric receptor according to any of claims 1 to 6, and an intracellular fusion protein,

wherein the chimeric receptor comprises a first cytokine receptor endodomain, and the intracellular fusion protein comprises a second cytokine receptor endodomain,

and wherein the first cytokine receptor endodomain is complementary to the second cytokine receptor endodomain.

16. A chimeric receptor system according to claim 15, wherein the chimeric receptor comprises a type I cytokine receptor endodomain α- or β-chain, and the intracellular fusion protein comprises a type I cytokine receptor endodomain γ-chain.

17. A chimeric receptor system according to claim 15, wherein the chimeric receptor comprises a type I cytokine receptor endodomain γ-chain, and the intracellular fusion protein comprises a type I cytokine receptor endodomain α- or β-chain.

18. A chimeric receptor system according to claim 15 or 16, wherein the chimeric receptor comprises a CD3 zeta endodomain, and the intracellular fusion protein lacks an intracellular signalling domain.

19. A chimeric receptor system, according to any of claims 15 to 18, wherein the intracellular fusion protein comprises a ZAP70 SH2 domain.

20 A chimeric receptor system comprising a chimeric receptor according to any of claims 1 to 6, and a transmembrane protein,

wherein the chimeric receptor comprises a first cytokine receptor endodomain, and the transmembrane protein comprises a second cytokine receptor endodomain,

wherein the first cytokine receptor endodomain is complementary to the second cytokine receptor endodomains.

21. A chimeric receptor system according to claim 20, wherein the chimeric receptor comprises a type I cytokine receptor endodomain α- or β-chain and the transmembrane protein comprises a type I cytokine receptor endodomain γ-chain.

22. A chimeric receptor system according to claim 20, wherein the chimeric receptor comprises a type I cytokine receptor endodomain γ-chain and the

transmembrane protein comprises a type I cytokine receptor endodomain α- or β-chain.

23. A chimeric receptor system according to claim 20 or 21, wherein the chimeric receptor comprises a CD3 zeta endodomain, and the transmembrane protein lacks an intracellular signalling domain and a ligand binding exodomain.

24. A cell which comprises a chimeric receptor according to any ofclaims 1 to 6 or a chimeric receptor system according to any of claims 7 to 23.

25. A nucleic acid sequence encoding a chimeric receptor according to any of claims 1 to 6.

26. A nucleic acid construct encoding a chimeric receptor system according to any of claims 7 to 23.

27. A nucleic acid construct according to claim 26, which comprises a first nucleic acid sequence encoding a first chimeric receptor and a second nucleic acid sequence encoding a second chimeric receptor, 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 chimeric receptor;
spacer1 is a nucleic acid sequence encoding the spacer of the first chimeric receptor;
TM1 is a nucleic acid sequence encoding the transmembrane domain of the first chimeric receptor;
endo1 is a nucleic acid sequence encoding the endodomain of the first chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both chimeric receptors
AgB2 is a nucleic acid sequence encoding the antigen-binding domain of the second chimeric receptor;
spacer2 is a nucleic acid sequence encoding the spacer of the second chimeric receptor;
TM2 is a nucleic acid sequence encoding the transmembrane domain of the second chimeric receptor;

endo2 is a nucleic acid sequence encoding the endodomain of the second chimeric receptor

28. A nucleic acid construct according to claim 27, wherein endo1 comprises a nucleic acid sequence encoding a first chain of a cytokine receptor endodomain, and a nucleic acid sequence encoding a first intracellular T cell signalling domain; and endo2 comprises a nucleic acid sequence encoding a second chain of a cytokine receptor endodomain and a nucleic acid sequence encoding a second intracellular T cell signalling domain.

29. A nucleic acid construct according to claim 27 or 28, wherein coexpr encodes a sequence comprising a self-cleaving peptide.

30. A nucleic acid construct according to any of claims 27 to 29, wherein alternative codons are used in regions of sequence encoding the same or similar amino acid sequences, in order to avoid homologous recombination.

31. A nucleic acid construct according to claim 26, which comprises a first nucleic acid sequence encoding a chimeric receptor and a second nucleic acid sequence encoding an intracellular fusion protein, the nucleic acid construct having the structure:

AgB1-spacer1-TM1-endo1-coexpr-domain2-endo2

in which
AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the chimeric receptor;
spacer 1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo 1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the intracellular fusion protein;
domain2 is a nucleic acid sequence encoding a second domain for the intracellular fusion protein; and
endo 2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the intracellular fusion protein.

32. A nucleic acid construct according to claim 31, wherein domain2 is a nucleic acid sequence encoding a ZAP70 SH2 domain.

33. A nucleic acid construct according to claim 26, which comprises a first nucleic acid sequence encoding a chimeric receptor and a second nucleic acid sequence encoding a transmembrane protein, the nucleic acid construct having the structure:

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

in which
AgB1 is a nucleic acid sequence encoding the antigen-binding domain of the chimeric receptor;
spacer1 is a nucleic acid sequence encoding the spacer of the chimeric receptor;
TM1 is a nucleic acid sequence encoding the transmembrane domain of the chimeric receptor;
endo1 is a nucleic acid sequence encoding the endodomain of the chimeric receptor;
coexpr is a nucleic acid sequence enabling co-expression of both the chimeric receptor and the transmembrane protein,
TM2 is a nucleic acid sequence encoding a transmembrane domain of the transmembrane domain,
endo2 is a nucleic acid sequence encoding the cytokine receptor endodomain of the tranmembrane protein.

34. A vector comprising a nucleic acid sequence according to claim 25 or a nucleic acid construct according to any of claims 26 to 33.

35. A retroviral vector or a lentiviral vector or a transposon according to claim 34.

36. A kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a first chimeric receptor as defined in any of claims 1 to 6; and

ii) a vector comprising a nucleic acid sequence encoding a second chimeric receptor as defined in any of claims 1 to 6.

37. A kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a chimeric receptor as defined in any of claims 1 to 6; and

ii) a vector comprising a nucleic acid sequence encoding an intracellular fusion protein as defined in any of claims 15 to 19.

38. A kit which comprises:

i) a vector comprising a nucleic acid sequence encoding a chimeric receptor as defined in any of claims 1 to 6; and

ii) a vector comprising a nucleic acid sequence encoding a transmembrane protein as defined in any of claims 20 to 23.

39. A method for making a cell according to claim 24 , which comprises the step of introducing: a nucleic acid sequence according to claim 25; a nucleic acid construct according to any of claims 26 to 33; a vector according to claim 34 or 35; or a kit of vectors according to any of claims 36 to 38, into a cell.

40. A method according to claim 39, wherein the cell is from a sample isolated from a subject.

41. A pharmaceutical composition comprising a plurality of cells according to claim 24.

42. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to claim 41 to a subject.

43. A method according to claim 42, 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 25; a nucleic acid construct according to any of claims 26 to 33; a vector according to claim 34 or 35; or a kit of vectors according to any of claims 36 to 38; and

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

44. A method according to claim 43, wherein the sample is a T-cell containing sample.

45. A method according to any of claims 42 to 44, wherein the disease is a cancer.

46. A pharmaceutical composition according to claim 41 for use in treating and/or preventing a disease.

47. The use of a cell according to claim 24 in the manufacture of a medicament for treating and/or preventing a disease.

FIGURE 1

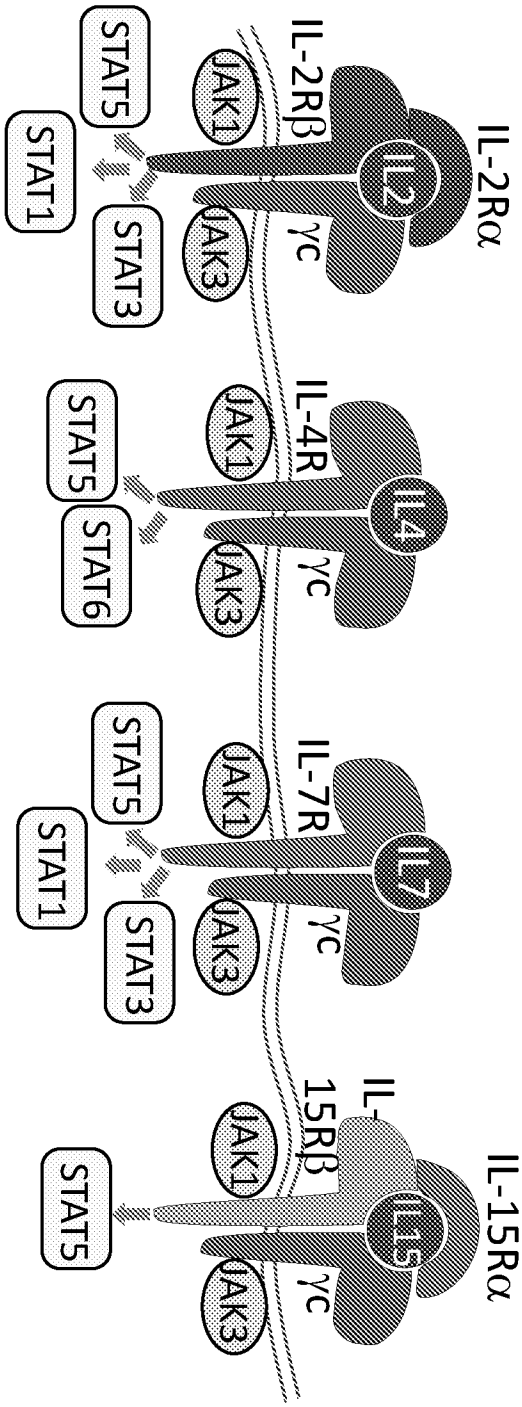

IL-2Rα
IL2
IL-2Rβ
γc
JAK1
JAK3
STAT5
STAT1
STAT3
IL-4R
IL4
STAT6
IL-7R
IL7
IL-15Rα
IL15
IL-15Rβ

FIGURE 2

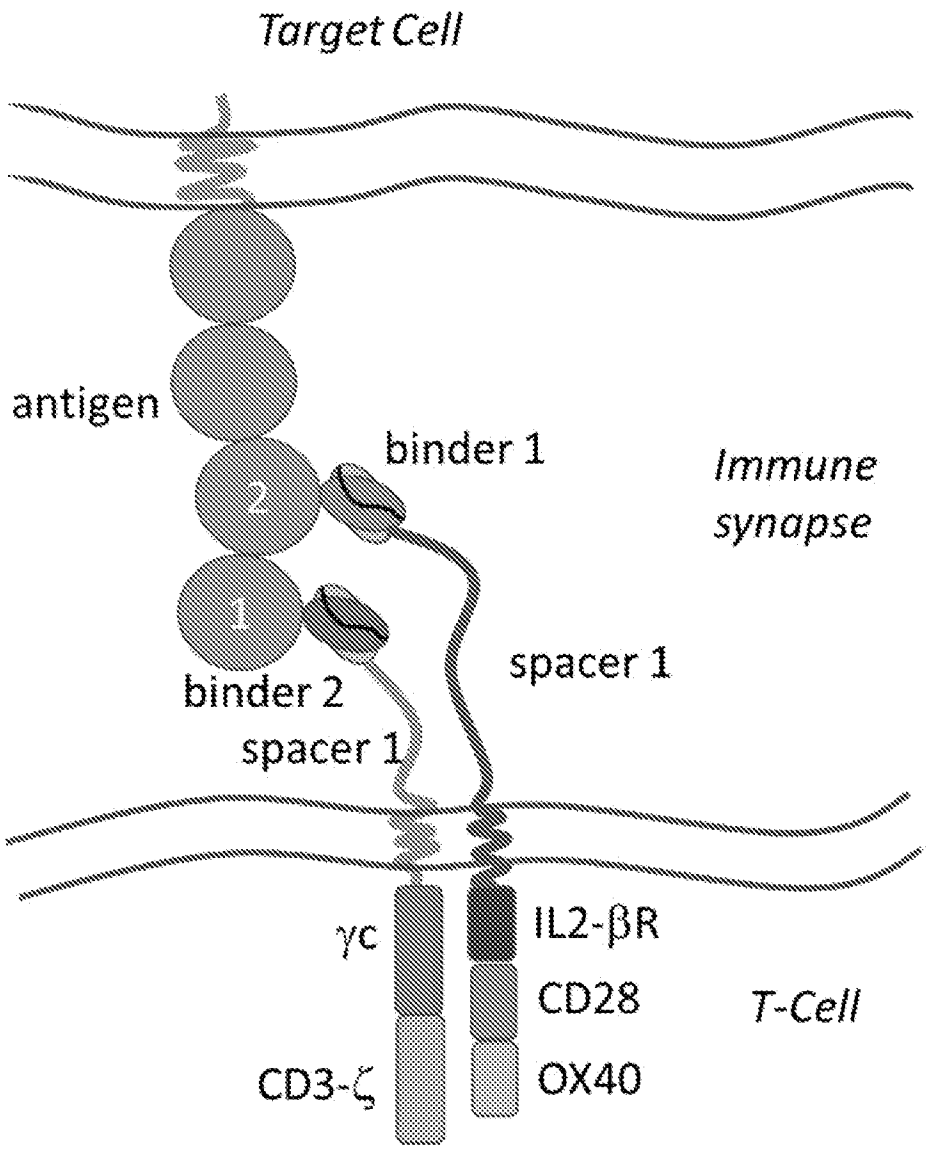

Target Cell
antigen
3
2
1
binder 1
binder 2
spacer 1
spacer 1
Immune
synapse
γc
IL2-βR
CD28
T-Cell
CD3-ζ
OX40

FIGURE 3

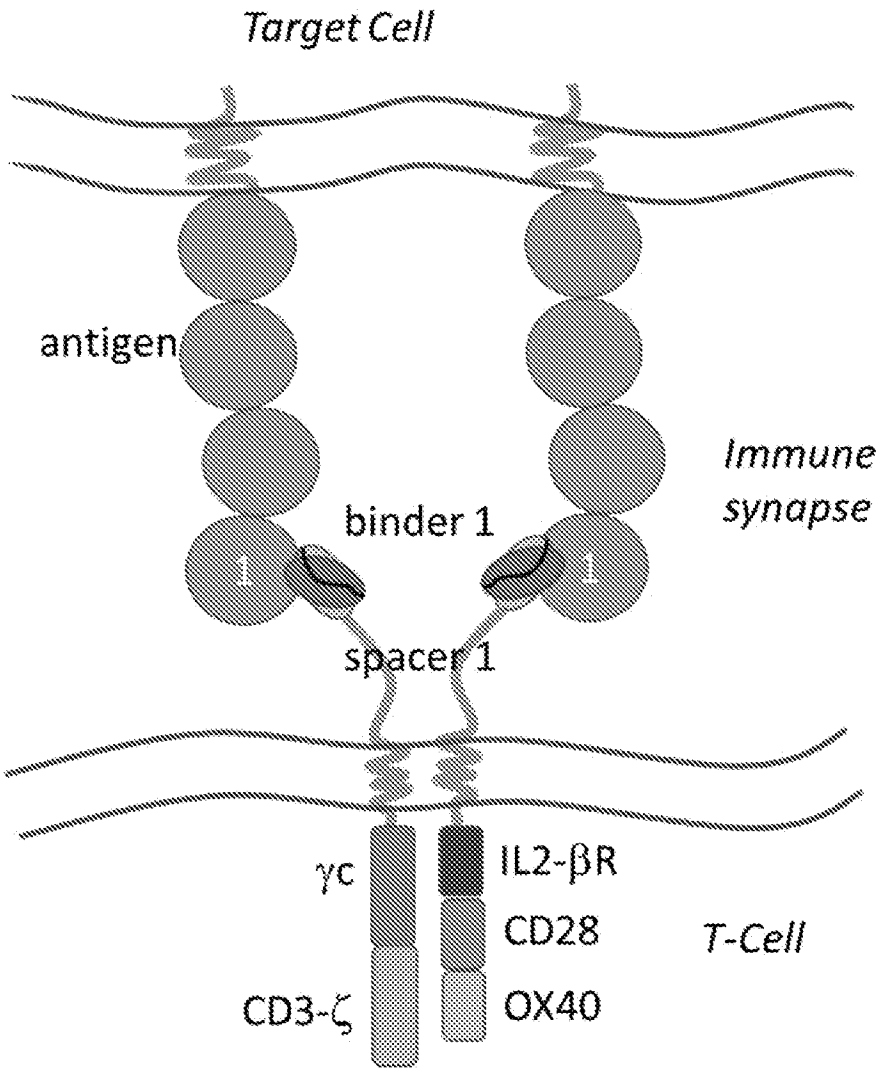

Target Cell
antigen
Immune synapse
binder 1
spacer 1
γc
IL2-βR
CD28
T-Cell
CD3-ζ
OX40

FIGURE 4

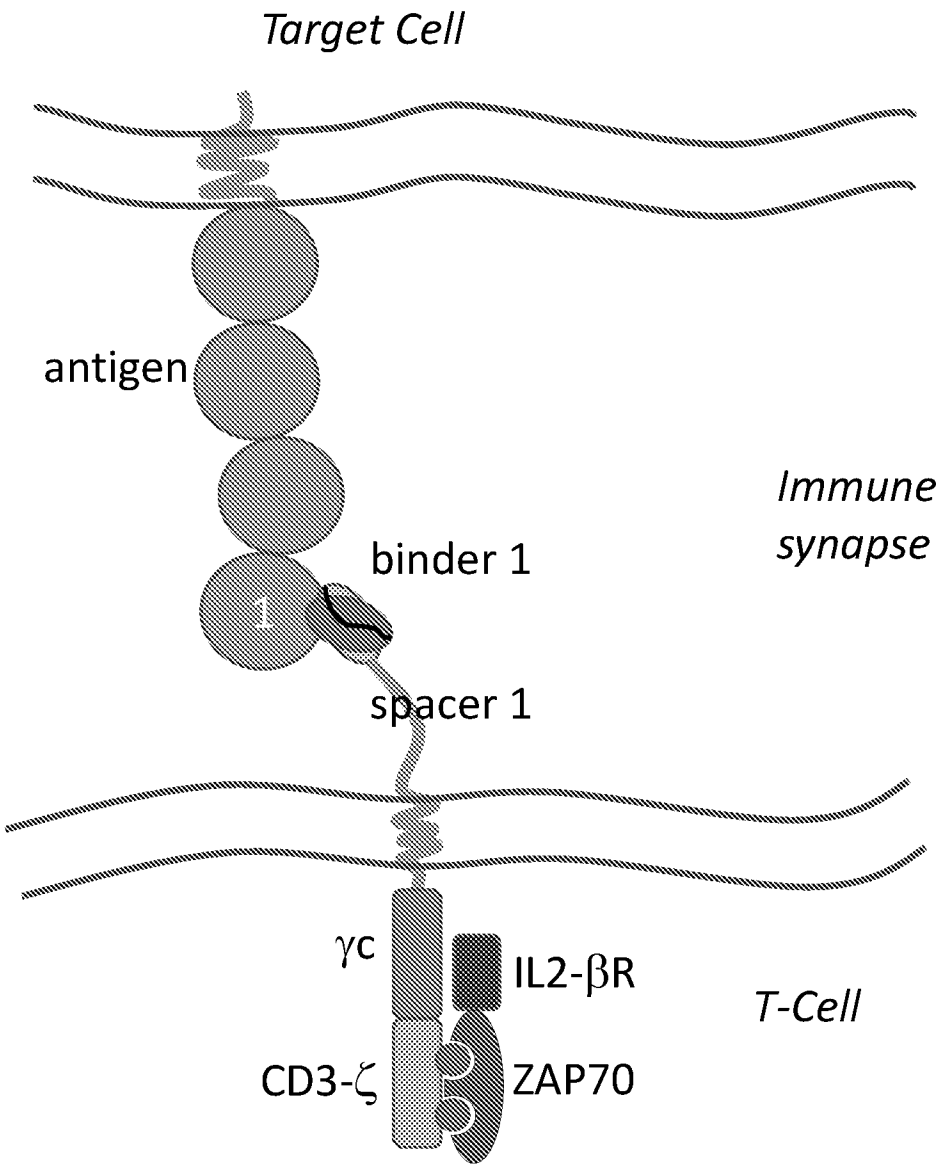

Target Cell
antigen
binder 1
1
spacer 1
Immune synapse
γc
IL2-βR
T-Cell
CD3-ζ
ZAP70

FIGURE 5

```
>aCD22huLT22-CD8STK-IL2RB-2A-aCD22huRFB4-HNG-IL2RG-Zeta
<------------------------- huLT22 scFv ----------------------------------------------
LPVTALLLPLALLLHAARPDIVMTQSPATLSVSPGERATLSCRSSQSLVHSNGNTYLHWYQQKPGQAPRLLIYKVSNRFSGVPARF

------------------------------ huLT22 scFv ----------------------------------------------
SGSGSGAEFTLTISSLQSEDFAVYYCSQSTHVPWTFGQGTRLEIKRSGGGGSGGGGSGGGGSEVQLVESGAEVKKPGSSVKVSCKA

---------------------------------- huLT22 scFv ----------------------------------------
SGYTFTNYWINWVRQAPGQGLEWMGNIYPSDSFTNYNQKFKDRVTITADKSTSTVYLELRNLRSDDTAVYYCTRDTQERSWYFDVW

---------><---------------- CD8 STK ------------------------><--- TM ------------><-----
GQGTLVTVSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIFWVVISVGSMGLIISLLCVYFWLERTMPR

---------------------------------- dIL2RG ---------------------------------------><-----
IPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGGALGEGPGASPCNQHSPYWAPPCYTLKPETRRVKFS

---------------------------------------- CD3-Zeta ----------------------------------------
RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQ

-- CD3-Zeta --------><--- FMD-2A -------><---------- huRFB4 scFv -----------------------
GLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPLPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASG

------------------------------- huRFB4 scFv ----------------------------------------------
FAFSIYDMSWVRQVPGKGLEWVSYISSGGGTTYYPDTVKGRFTISRDNSRNTLDLQMNSLRVEDTAVYYCARHSGYGSSYGVLFAY

---------------------------------- huRFB4 scFv ----------------------------------------
WGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDISNYLNWLQQKPGKAPKLLIYYTSILHSGVPS

---------- huRFB4 scFv --------------------------><------- IgG1 Hinge ---------------><
RFSGSGSGTEFTLTISSLQPEDFATYYCQQGNTLPWTFGQGTKLEIKRSPAEPKSPDKTHTCPPCPKDPKACDIYIWAPLAGTCGL

--- IgG1 Hinge ----->←---------------------- dIL2RB ----------------------------------
GHLLVGLSGAFGFIILVYLLINCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLER

---------------------------------- dIL2RB ----------------------------------------------
DKVTQLLLQQDKVPEPASLSSNHSLTSCFTNQGYFFFHLPDALEIEACQVYFTYDPYSEEDPDEGVAGAPTGSSPQPLQPLSGEDD

---------------------------------- dIL2RB ----------------------------------------------
AYCTFPSRDDLLLFSPSLLGGPSPPSTAPGGSGAGEERMPPSLQERVPRDWDPQPLGPPTPGVPDLVDFQPPPELVLREAGEEVPD

-------------------- dIL2RB ------------------->
AGPREGVSFPWSRPPGQGEFRALNARLPLNTDAYLSLQELQGQDPTHL
```

FIGURE 6

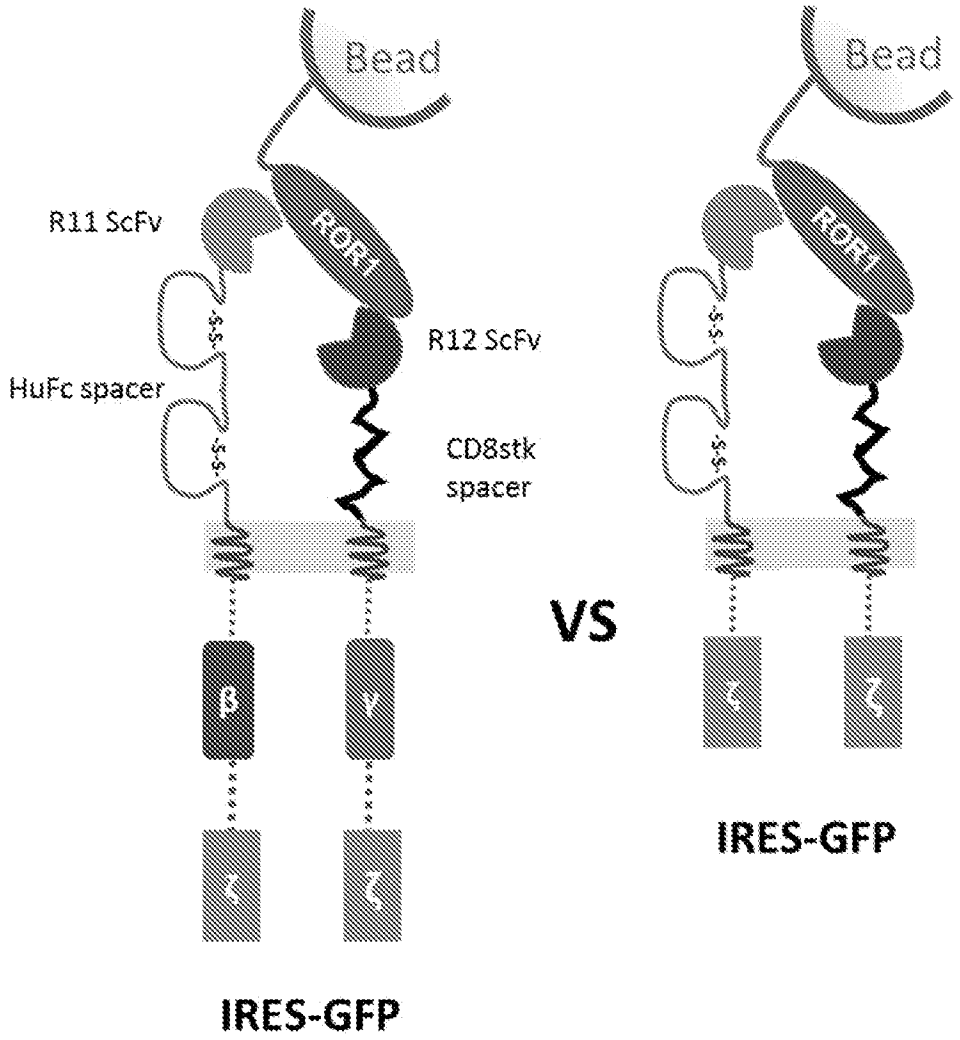


FIGURE 7

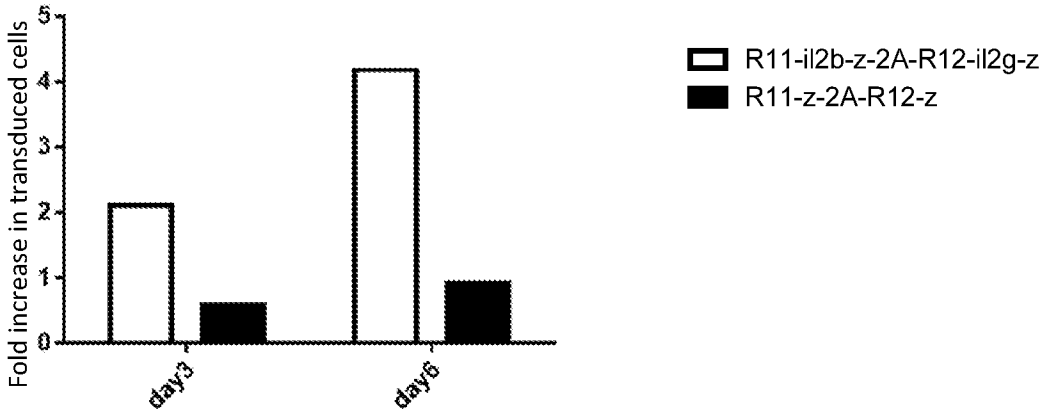


FIGURE 8

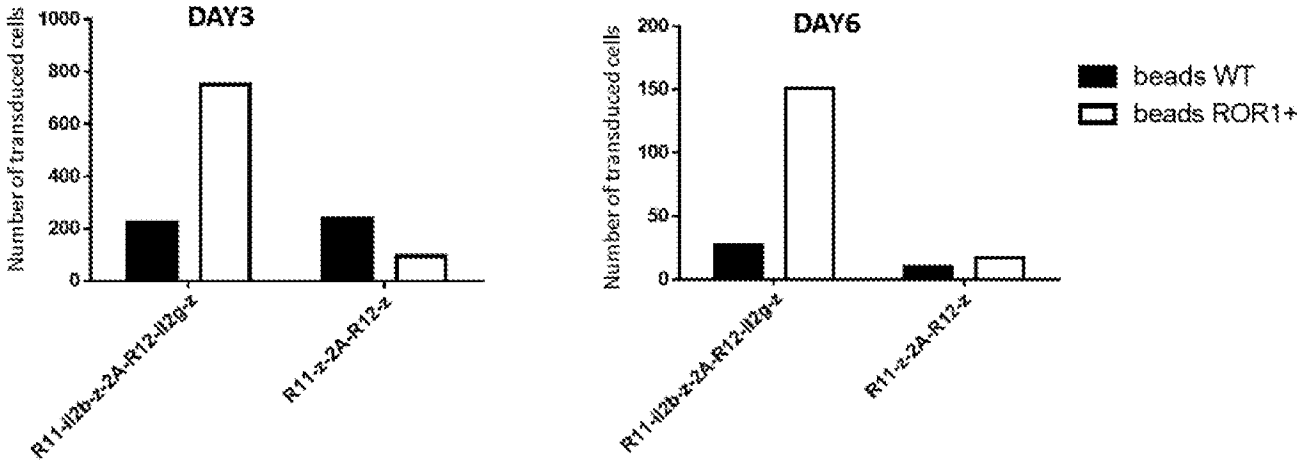


FIGURE 9

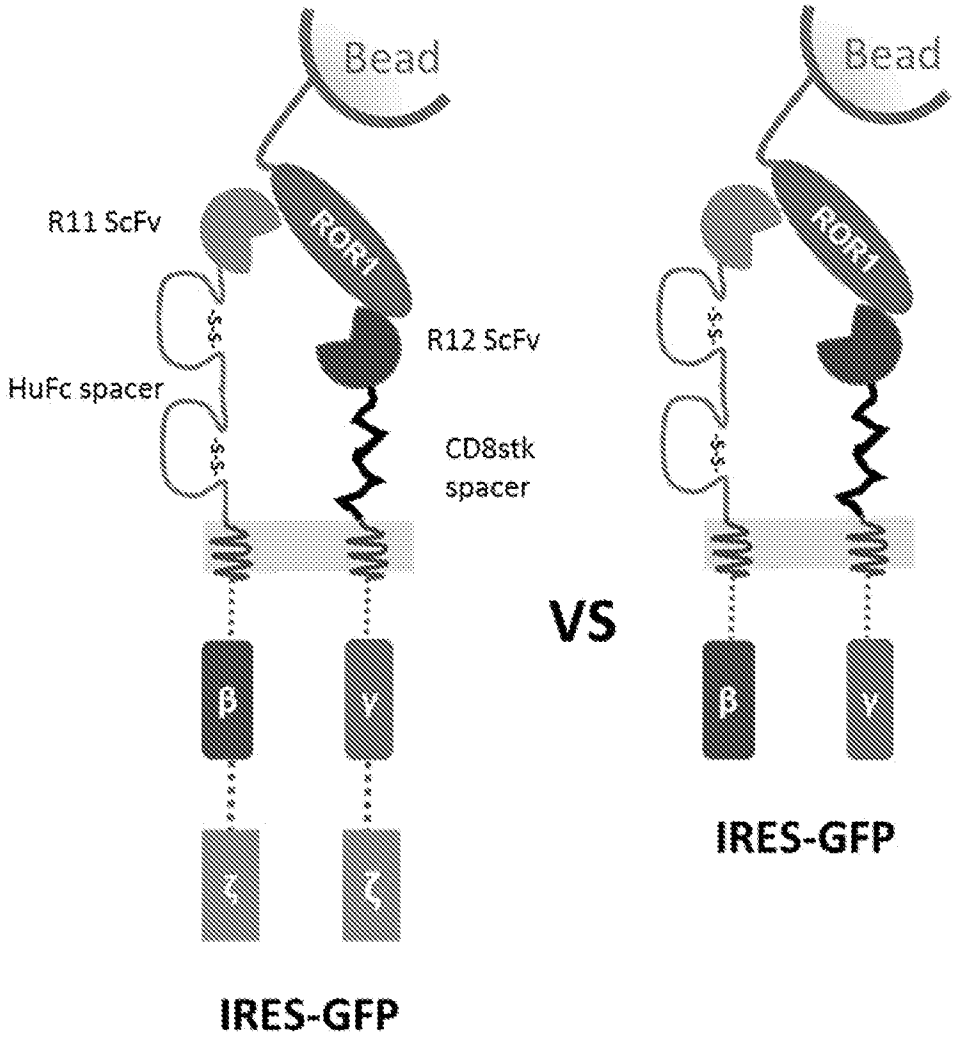


FIGURE 10

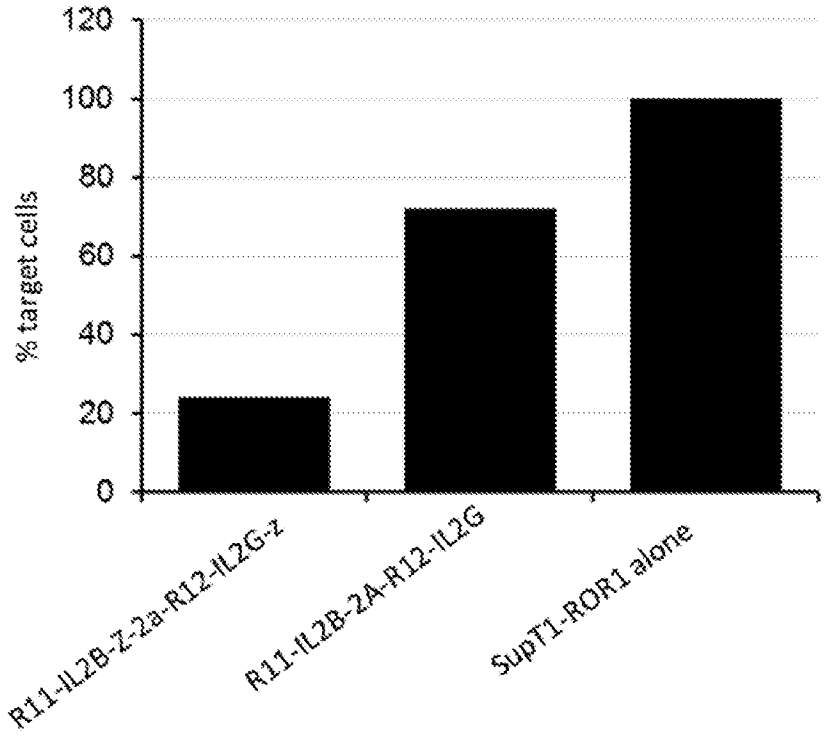


FIGURE 11

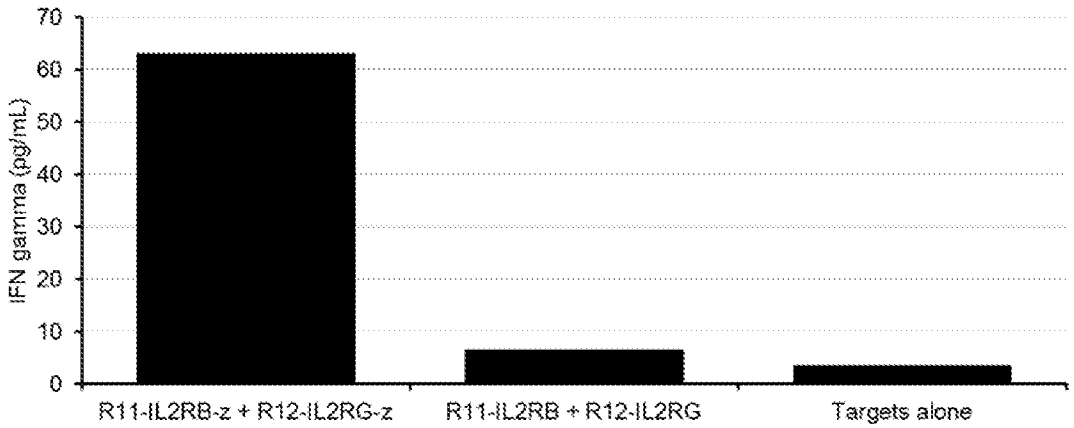


FIGURE 12

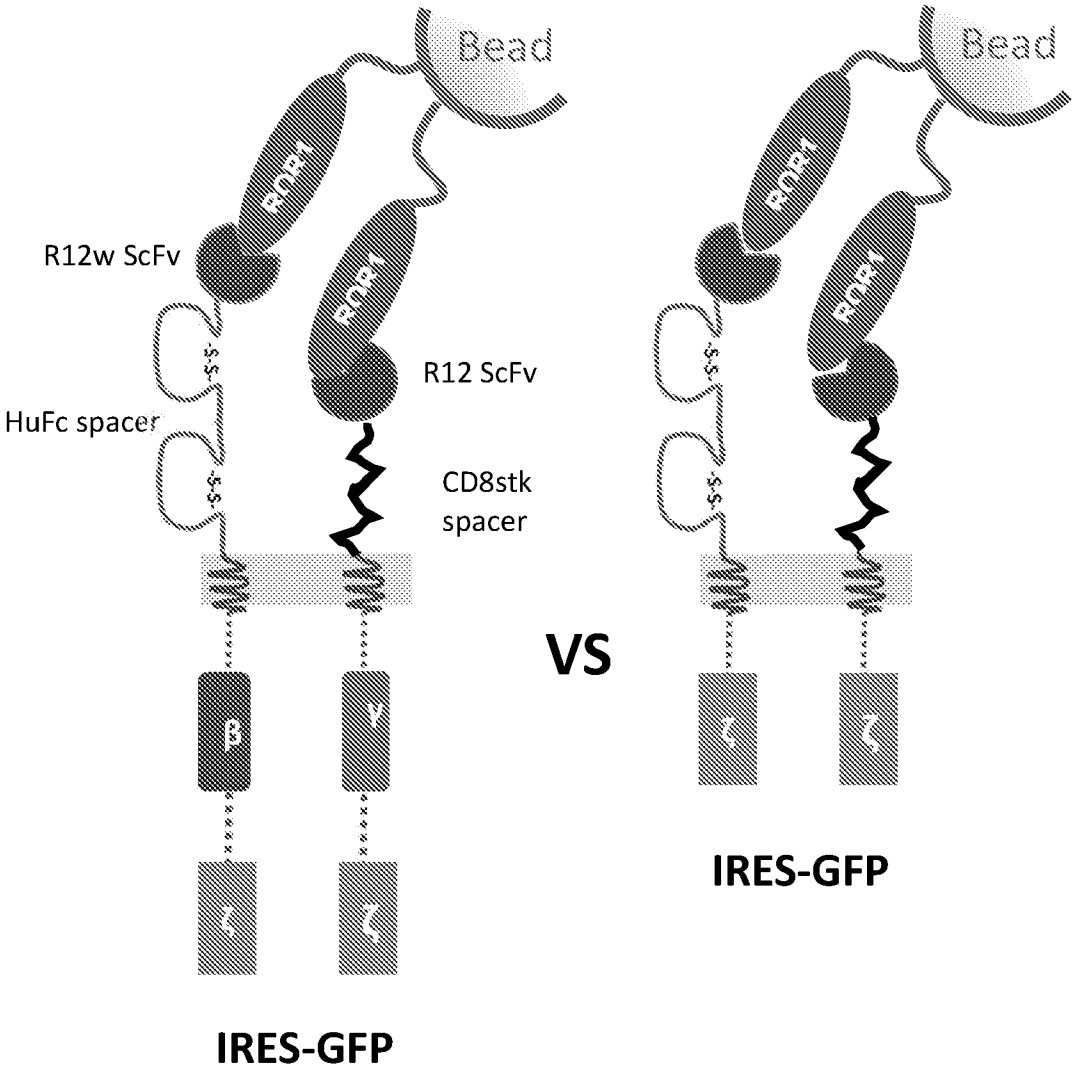

Bead
R12w ScFv
HuFc spacer
R12 ScFv
CD8stk spacer
VS
IRES-GFP
IRES-GFP

FIGURE 13

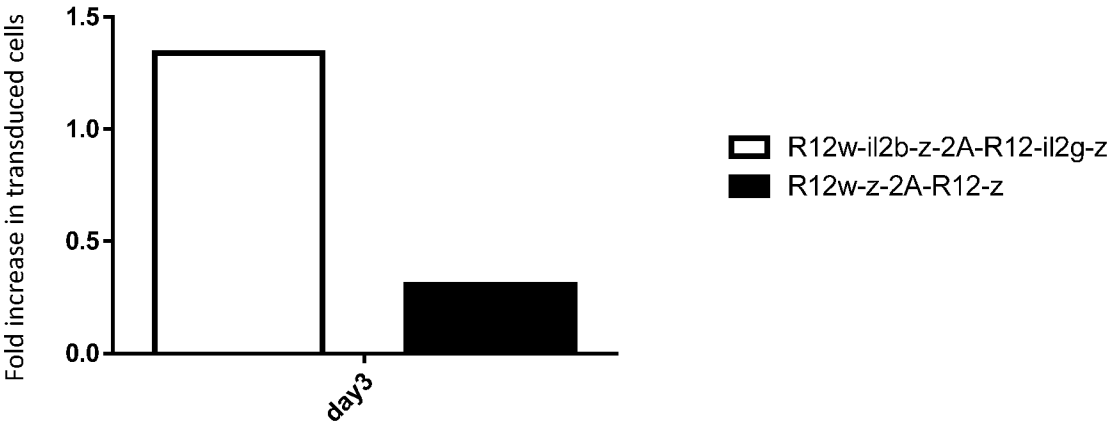
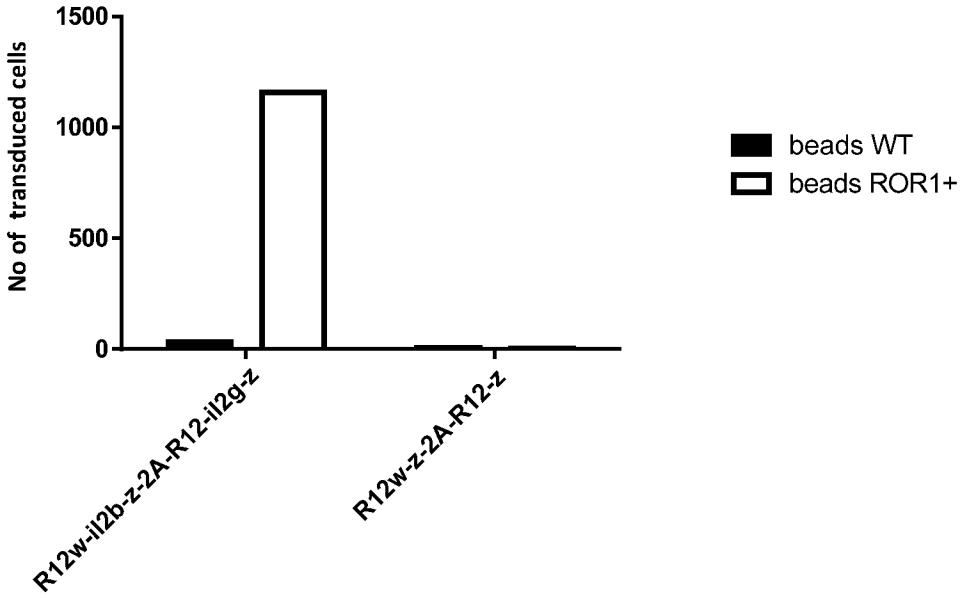


FIGURE 14



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/053290

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K14/55 C07K14/725 C12N5/0783 C07K14/715
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIN HUANG ET AL: "IGF1R- and ROR1-Specific CAR T Cells as a Potential Therapy for High Risk Sarcomas", PLOS ONE, vol. 10, no. 7, 14 July 2015 (2015-07-14), page e0133152, XP055339394, DOI: 10.1371/journal.pone.0133152 the whole document	1-3,5-47

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2017	03/02/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Manu, Dominique

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/053290

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. WILKIE ET AL: "Selective Expansion of Chimeric Antigen Receptor-targeted T-cells with Potent Effector Function using Interleukin-4", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 285, no. 33, 18 June 2010 (2010-06-18), pages 25538-25544, XP055125366, ISSN: 0021-9258, DOI: 10.1074/jbc.M110.127951 the whole document	1-3,5-47
X	WO 2012/138858 A1 (BAYLOR COLLEGE MEDICINE [US]; LEEN ANN MARIE [US]; VERA JUAN F [US]) 11 October 2012 (2012-10-11) the whole document	1-3,5-47
T	S J BAKER ET AL: "Hematopoietic cytokine receptor signaling", ONCOGENE, vol. 26, no. 47, 15 October 2007 (2007-10-15), pages 6724-6737, XP055339745, ISSN: 0950-9232, DOI: 10.1038/sj.onc.1210757 the whole document	
T	HULME MAIGAN A ET AL: "Central Role for Interleukin-2 in Type 1 Diabetes", DIABETES, AMERICAN DIABETES ASSOCIATION, US, vol. 61, no. 1, 1 January 2012 (2012-01-01), pages 14-22, XP009158774, ISSN: 0012-1797, DOI: 10.2337/DB11-1213 the whole document	
X,P	WO 2016/134284 A1 (UNIV OF FLORIDA RES FOUND INC [US]) 25 August 2016 (2016-08-25) the whole document	1-47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2016/053290

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2016/053290

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012138858 A1	11-10-2012	AU 2012240135 A1	24-10-2013
		AU 2016253549 A1	17-11-2016
		CA 2832569 A1	11-10-2012
		CN 103582699 A	12-02-2014
		EP 2694640 A1	12-02-2014
		JP 2014512183 A	22-05-2014
		NZ 616405 A	27-11-2015
		NZ 710810 A	30-09-2016
		US 2014050709 A1	20-02-2014
		WO 2012138858 A1	11-10-2012
WO 2016134284 A1	25-08-2016	NONE	