(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 May 2019 (31.05.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/102207 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 14/725* (2006.01)

(21) International Application Number:
PCT/GB2018/053390

(22) International Filing Date:
23 November 2018 (23.11.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
1719557.9 24 November 2017 (24.11.2017) GB

(71) Applicant: **AUTOLUS LIMITED** [GB/GB]; Forest House, 58 Wood Lane, London W12 7RZ (GB).

(72) Inventors: **PULÉ, Martin**; c/o Autolus Limited, Forest House, 58 Wood Lane, London W12 7RZ (GB). **RIGHI, Matteo**; c/o Autolus Limited, Forest House, 58 Wood Lane, London W12 7RZ (GB). **THOMAS, Simon**; c/o Autolus Limited, Forest House, 58 Wood Lane, London W12 7RZ (GB). **ONUOHA, Shimobi**; c/o Autolus Limited, Forest House, 58 Wood Lane, London W12 7RZ (GB). **CORDOBA, Shaun**; c/o Autolus Limited, Forest House, 58 Wood Lane, London W12 7RZ (GB).

(74) Agent: **D YOUNG & CO LLP**; 120 Holborn, London EC1N 2DY (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: POLYPEPTIDE

(57) Abstract: The present invention provides a chimeric polypeptide comprising: an antigen-binding domain which constitutively binds to an ectodomain of a first chain of a cytokine receptor; a transmembrane domain; and an endodomain from a second chain of the cytokine receptor which chimeric polypeptide, when expressed in a cell, binds to the endogenous first chain of the cytokine receptor causing constitutive cytokine signalling.

WO 2019/102207 A1

POLYPEPTIDE

FIELD OF THE INVENTION

The present invention relates to a chimeric polypeptide which, when expressed in a cell, causes constitutive cytokine signalling. The invention also relates to a cell which expresses such a chimeric polypeptide and optionally a chimeric antigen receptor or engineered T-cell receptor at the cell surface.

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.

CAR T cell engraftment and proliferation

Function of CAR T cells depends on survival and engraftment of the cells within the patients. In some settings, like ALL, survival of the CAR-T cells for up to 9 months appears important to prevent relapse and effect sustained remissions.

CAR T-cell persistence and survival can be enhanced by administration of cytokines. CAR-T cells have also been engineered to secrete cytokines *in situ*. However, there are advantages associated with both of these approaches. For example, systemic administration of cytokines can be toxic and 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).

An alternative approach is to engineer the cell to have a constitutive cytokine receptor. For example, WO2017/029512 describes a constitutively active cytokine-signalling chimeric transmembrane protein made by linking cytokine receptor endodomains to a "Fab" type exodomain (see Figure 2). This structure uses the natural dimerization components of antibodies, namely the dimerization domain from the heavy and light chain constant regions. The chimeric transmembrane protein has two chains; for example, a first polypeptide which comprises the antibody light κ chain and the IL2 receptor common γ chain as endodomain; and a second polypeptide which comprises the antibody heavy chain CH1 and an endodomain which comprises either: the IL2 receptor β chain (giving a constitutively active IL2-signalling molecule); or the IL7 receptor (giving a constitutively active IL7-signalling molecule).

When both polypeptides are expressed in a cell, the two chains associate and form the chimeric transmembrane protein at the cell surface, providing constitutive cytokine (for example constitutive IL-2 or IL-7) signalling.

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 illustrating the constitutively active cytokine-signalling chimeric transmembrane protein described in WO2017/029512. The chimeric transmembrane protein comprises a dimerization domain and a cytokine receptor endodomain. The complex has a "Fab" type architecture, as the dimerization domain comprises antibody-type heavy and light chain constant regions. Constant dimerization between these domains brings together the IL2 receptor common γ

chain with either the IL-2 receptor β chain or the IL-7 receptor α chain, leading to constitutive cytokine signalling.

Figure 3: Schematic diagram illustrating examples of constitutively active cytokine receptors of the invention. (A) The chimeric polypeptide comprises an anti-common gamma chain scFv ectodomain and an IL-7 endodomain. When expressed inside a cell, binding of the anti-γC scFv to engogenous gamma chain produces a constitutively active cytokine receptor. In this arrangement the cell receives only one type of cytokine signal, for example, IL-7; (B) The chimeric polypeptide comprises an anti-IL-receptor scFv ectodomain and an γC endodomain. When expressed inside a cell, binding of the anti-ILR scFv to an engogenous IL-receptor chain produces a constitutively active cytokine receptor. In this arrangement the cell receives may receive multiple cytokine signals, all of which signal through γC. The cytokine signal is regulated by physiological expression of cytokine receptor.

Figure 4: Construct diagrams for the two chimeric polypeptides made and tested in Example 1. (A) The construct coexpresses a marker gene (RQR8) and a chimeric polypeptide comprising an anti-γC scFv ectodomain and an IL-7 receptor endodomain. (B) The construct coexpresses a marker gene (RQR8) and a chimeric polypeptide comprising an anti-IL-7 receptor scFv ectodomain and a γC endodomain. "SP" - signal peptide; "RQR8" - the sort-suicide gene RQR8; "2A" - a FNDV derived 2A peptide cleavage site; "HA-Tag" - hemagglutinin tag; anti-yC/anti-IL7R scFv - scFv which specifically binds γC/IL7 receptor; "CD8STK" - spacer sequence from human CD8 stalk; "IL7R/γC" TM - transmembrane domain from IL7 receptor/gamma chain; "IL7R/γC endodomain" - endodomain from IL7 receptor or gamma chain.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have developed a chimeric polypeptide which, when expressed in a cell, causes constitutive cytokine signalling. The polypeptide specifically binds to an endogenous cytokine receptor in the cell, causing cytokine signalling even in the absence of cytokine.

In a first aspect, the present invention provides a chimeric polypeptide comprising:

an antigen-binding domain which constitutively binds to an ectodomain of a first chain of a cytokine receptor;

a transmembrane domain; and

an endodomain from a second chain of the cytokine receptor

which chimeric polypeptide, when expressed in a cell, binds to the endogenous first chain of the cytokine receptor causing constitutive cytokine signalling.

In a first embodiment of the first aspect of the invention, the first chain of the cytokine receptor is a type I cytokine receptor γ-chain; and the second chain of the cytokine receptor is type I cytokine receptor α-chain or β-chain.

In a second embodiment of the first aspect of the invention, the first chain of the cytokine receptor is a type I cytokine receptor α-chain or β-chain; and the second chain of the cytokine receptor is type I cytokine receptor γ-chain.

The type I cytokine receptor α-chain or β-chain may be selected from one of the following: IL-2 receptor, IL-4 receptor, IL-7 receptor, IL-9 receptor, IL-13 receptor and IL-15 receptor.

The type I cytokine receptor α-chain or β-chain may be an IL-7 receptor α-chain.

The antigen binding domain may comprise a dAb or scFv which binds the ectodomain of the first chain of the cytokine receptor.

In a second aspect, the present invention provides a constitutively-active cytokine receptor which comprises a chimeric polypeptide according to the first aspect of the invention in association with the first chain of the cytokine receptor.

In a third aspect, the present invention provides a cell which comprises a constitutively-active cytokine receptor which comprises a chimeric polypeptide according to the first aspect of the invention in association with the endogenous first chain of the cytokine receptor.

The cell may also comprise a chimeric antigen receptor or an engineered T-cell receptor.

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

In a fifth aspect, the present invention provides a nucleic acid construct which comprises a first nucleic acid sequence encoding a chimeric polypeptide according to

the first aspect of the invention; and a second nucleic acid encoding a chimeric antigen receptor or an engineered T-cell receptor.

In a sixth aspect, there is provided 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.

In a seventh 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; or a vector according to the sixth aspect of the invention, into a cell.

In an eighth aspect, there is provided a method for inducing constitutive cytokine signaling in a cell, which comprises the step of expressing a chimeric polypeptide according to the first aspect of the invention in the cell.

In a ninth aspect, there is provided a method for blocking deleterious cytokine signalling in a cell, which comprises the step of expressing a chimeric polypeptide according to the first aspect of the invention in the cell, wherein the antigen binding domain constitutively binds to common gamma chain, and wherein one or more deleterious cytokine(s) signal(s) through a cytokine receptor which comprises common gamma chain.

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

In an eleventh 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 tenth 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; or a vector according to the sixth aspect of the invention; and

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

The disease may be a cancer.

In a twetfth aspect, there is provided a pharmaceutical composition according to the tenth aspect of the invention for use in treating and/or preventing a disease.

In a thirteenth aspect, there is 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.

A cytokine receptor which comprises a chimeric polypeptide of the present invention has an advantage over the "Fab"-type constitutively active molecules described in WO2017/029512 because only one recombinant polypeptide chain needs to be expressed in the cell. The cytokine receptor of the present invention makes use of an endogenous cytokine receptor in the cell in order to complete the constitutively active cytokine signalling complex.

The fact that the chimeric polypeptide of the present invention "hijacks" an endogenous cytokine receptor gives an additional advantage in that other, external, cytokine signals, which would normally signal through the endogenous receptor, will be at least partially blocked. This can be beneficial for the cell, as high levels of certain cytokines can be deleterious. For example high levels of IL-2 can cause T cells to differentiate into regulatory T cells.

The arrangement shown in Figure 3a, which comprises an anti-γC scFv ectodomain, has the additional advantage that the cell is not engineered to encode common gamma chain. Retroviral expression of the common gamma chain has previously been associated with leukaemiagenesis in X-SCID patients.

The arrangement shown in Figure 3a avoids over-expression the common gamma chain. Unlike the Fab-type system, where the cell comprises both with wild type gamma chain and the transgenic gamma chain, in the system shown in Figure 3a, only the wild-type gamma chain is expressed in the cell. As the gamma chain is expressed physiologically by the cell it is naturally upregulated and down-regulated as needed.

DETAILED DESCRIPTION

CHIMERIC POLYPEPTIDE

A chimeric polypeptide is a molecule which comprises (i) an antigen-binding domain which constitutively binds to an ectodomain of a first chain of a cytokine receptor; and (ii) an endodomain from a second chain of the cytokine receptor

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 a cytokine receptor 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 same pathways may be initiated by the constitutively active cytokine receptor of the present invention.

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 polypeptide of the present invention comprises an endodomain which causes "cytokine-type" cell signalling when in the presence of an endogenous cytokine receptor chain.

The endodomain may be a cytokine receptor endodomain.

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

The endodomain may be derived from a type II cytokine receptor. Type II cytokine receptors include those that bind type I and type II interferons, and those that bind members of the interleukin-10 family (interleukin-10, interleukin-20 and interleukin-22).

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 polypeptide of the present invention may comprise an endodomain from the IL-2 receptor β-chain 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 polypeptide 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 polypeptide of the present invention may comprise an endodomain from 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

IL-15

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

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

SPACER

The chimeric polypeptide 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.

The spacer should be of an appropriate length so that the antigen-binding domain of the chimeric polypeptide is positioned at an appropriate distance from the cell membrane to specifically bind the endogenous cytokine receptor chain.

Where the cell of the present invention comprises a chimeric polypeptide and a chimeric antigen receptor (CAR), the spacer of the chimeric polypeptide and the CAR may be different, for example, having a different length. The spacer of the CAR may be longer than the spacer of the chimeric polypeptide.

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

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

TRANSMEMBRANE DOMAIN

The transmembrane domain is the sequence of a chimeric polypeptide 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

ANTIGEN-BINDING DOMAIN

The antigen-binding domain of the chimeric polypeptide of the present invention specifically binds to an ectodomain of a cytokine receptor. It may bind to the common gamma chain, or to an α- or β-chain, for example the IL-7α chain.

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.

Suitable antibodies from which antigen-binding domains may be derived, are known in the art. For example, US 6323027, which is incorporated by reference, describes antibodies which specifically bind to the common γ-chain.

The antigen binding domain may comprise an scFv which binds to the common γ-chain. The antigen binding domain may comprise the light chain CDRs shown as SEQ ID No. 11-13 and/or the heavy chain CDRs shown as SEQ ID No. 14-16.

SEQ ID No. 11 (CDRL1): KASQDVTTAVA
SEQ ID No. 12 (CDRL2): WASTRHT

SEQ ID No. 13 (CDRL3): QQHITPWT
SEQ ID No. 14 (CDRH1): SYGVH
SEQ ID No. 15 (CDRH2): VIWAGGSTBYNSALM
SEQ ID No. 16 (CDRH3): EGSTVDSMDY

The antigen binding domain may comprise the VL domain shown as SEQ ID No. 17 and/or the VH domain shown as SEQ ID No. 18.

SEQ ID No. 17 (VL):
DIVMTQSHKFMSTSVGDSITITCKASQDVTTAVAWYQQKPGQSPKLLIYWASTRHTG VPDRFTGSGSGTDYTLTISSVQAEDLALYYCQQHYITPWTFGGGTKLEI

SEQ ID No. 18 (VH):
LQESGPGLVAPQSQSLSITCTVDGFSLTSYGVHWVRQPPGKGLEWLGVIWAGGST NYNSALMSRLNINRDNSKSQIFLKMNSLQTDDTAIYYCAREGSTVDSMDYWGQGTT VT

CHIMERIC ANTIGEN RECEPTORS (CAR)

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

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

Early CAR designs had endodomains derived from the intracellular parts of either the γ chain of the FcεR1 or CD3ζ. Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3ζ results in second generation receptors which can transmit an activating and co-stimulatory

signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most potent co-stimulatory signal - namely immunological signal 2, which triggers T-cell proliferation. Some receptors have also been described which include TNF receptor family endodomains, such as the closely related OX40 and 41BB which transmit survival signals. Even more potent third generation CARs have now been described which have endodomains capable of transmitting activation, proliferation and survival signals.

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

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

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

SIGNAL PEPTIDE

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

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.

The signal peptide may comprise the sequence shown as SEQ ID No. 19, 20 or 21 or a variant thereof having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that the signal peptide still functions to cause cell surface expression of the CAR.

SEQ ID No. 19: MGTSLLCWMALCLLGADHADG

The signal peptide of SEQ ID No. 19 is compact and highly efficient and is derived from TCR beta chain. It is predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

SEQ ID No. 20: MSLPVTALLLPLALLLHAARP

The signal peptide of SEQ ID No. 20 is derived from IgG1.

SEQ ID No. 21: MAVPTQVLGLLLLWLTDARC

The signal peptide of SEQ ID No. 21 is derived from CD8a.

CAR ENDODOMAIN

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

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

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.

NUCLEIC ACID

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

NUCLEIC ACID CONSTRUCT

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

CPAgB-CPspacer-CPTM-CPendo-coexpr-CARAgB-CARspacer-CARTM-CARendo

or

CARAgB-CARspacer-CARTM-CARendo-coexpr-CPAgB-CPspacer-CPTM-CPendo

in which

CPAgB is a nucleic acid sequence encoding the antigen-binding domain of the chimeric polypeptide;

CPspacer is a nucleic acid sequence encoding the spacer of the chimeric polypeptide;

CPTM is a a nucleic acid sequence encoding the transmembrane domain of the chimeric polypeptide;

CPendo is a nucleic acid sequence encoding the endodomain of the chimeric polypeptide;

coexpr is a nucleic acid sequence enabling co-expression of both the chimeric polypeptide and the CAR

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

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

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

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

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

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

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

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

In the structure above, “coexpr” is a nucleic acid sequence enabling co-expression of both first and second CARs. It may be a sequence encoding a cleavage site, such that the nucleic acid construct produces a chimeric polypeptide and a CAR, joined by a cleavage site(s). The cleavage site may be self-cleaving, such that when the polypeptide is produced, it is immediately cleaved into individual peptides without the need for any external cleavage activity.

The cleavage site may be any sequence which enables the chimeric polypeptide and CAR to become separated.

The term “cleavage” is used herein for convenience, but the cleavage site may cause the peptides to separate into individual entities by a mechanism other than classical 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, for example be a furin cleavage site, a Tobacco Etch Virus (TEV) cleavage site or 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 the 2A-like sequence shown as SEQ ID No. 22 (RAEGRGSLLTCGDVEENPGP).

The present invention also provides a kit comprising a chimeric polypeptide according to the invention and one or more CAR(s) or TCR(s).

ENGINEERED T-CELL RECEPTOR (TCR)

The cell of the present invention may express a T-cell receptor (TCR), for example an engineered or non-endogenous TCR.

The TCR is a molecule found on the surface of T cells which is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules.

The TCR is a heterodimer composed of two different protein chains. In humans, in 95% of T cells the TCR consists of an alpha (α) chain and a beta (β) chain (encoded by TRA and TRB, respectively), whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains (encoded by TRG and TRD, respectively).

When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through signal transduction.

In contrast to conventional antibody-directed target antigens, antigens recognized by the TCR can include the entire array of potential intracellular proteins, which are processed and delivered to the cell surface as a peptide/MHC complex.

It is possible to engineer cells to express heterologous (i.e. non-native) TCR molecules by artificially introducing the TRA and TRB genes; or TRG and TRD genes into the cell using vector. For example the genes for engineered TCRs may be reintroduced into autologous T cells and transferred back into patients for T cell adoptive therapies.

The present invention also provides a cell which comprises a TCR and a chimeric polypeptide according to the first aspect of the invention.

VECTOR

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

CYTOKINE RECEPTOR

There is also provided a constitutively-active cytokine receptor which comprises a chimeric polypeptide of the invention in association with a cytokine receptor chain.

The chimeric polypeptide may associate with the first chain of a cytokine receptor. It may bind the ectodomain of the cytokine receptor chain via its antigen binding domain. The cytokine receptor chain which binds the chimeric polypeptide may be endogenous i.e. a polypeptide which is naturally encoded by the cell.

CELL

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

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

The cell may comprise a constitutively active cytokine receptor as defined above.

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 chimeric polypeptide-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, chimeric polypeptide-expressing cells are generated by introducing DNA or RNA coding for the chimeric polypeptide 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 chimeric polypeptide 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 chimeric polypeptide.

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.

INDUCING/BLOCKING CYTOKINE SIGNALLING

The present invention provides a method for inducing constitutive cytokine signaling in a cell, which comprises the step of expressing a chimeric polypeptide as defined above in the cell. The chimeric polypeptide binds to an endogenous cytokine receptor chain via the antigen binding domain, forming a constitutively active cytokine receptor at the cell surtace.

The present invention also provides a method for blocking deleterious cytokine signalling in a cell, by expressing a chimeric polypeptide of the invention in the cell. The chimeric polypeptide binds to a cytokine receptor chain, preventing that chain pairing with other endogenous cytokine receptor partner chains. For example, where

the , wherein the antigen binding domain constitutively binds to common gamma chain, it may reduce the concentration of one or more of the following receptors at the cell surface: IL-2 receptor, IL-4 receptor, IL-7 receptor, IL-9 receptor, IL-13 receptor and IL-15.

This may block deleterious cytokine signalling in the cell, for example excessive IL-2 mediated signalling.

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 chimeric polypeptide-expressing cell of the present invention for use in treating and/or preventing a disease.

The invention also relates to the use of a chimeric polypeptide-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.

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 – Design and testing of two chimeric polypeptides giving constitutively active cytokine-signalling

Two chimeric polypeptides were designed and tested using the constructs illustrated in Figure 4. The first construct encodes a chimeric polypeptide comprising an anti-γC scFv ectodomain and an IL-7 receptor endodomain. The second construct encodes a chimeric polypeptide comprising an anti-IL-7 receptor scFv ectodomain and a γC endodomain.

A nucleic acid encoding a marker (RQR8) is cloned in-frame with a nucleic acid sequences encoding one of these two polypeptides separated by a 2A-peptide encoding sequence.

The murine cell line 2E8 (ATCC TIB-239™) is dependent on IL7 for cell growth. In the absence of an IL-7 signal the cells undergo apoptosis. 2E8 cells are transduced with a vector expressing the chimeric polypeptides described above, together with RQR8, or left untransduced (WT). As a positive control, cells of all three types are co-cultured with 100 U/ml murine IL7. Cell proliferation is assessed after 3 and 7 days of culture.

The amino acid sequences for the two constructs tested are shown below.

Amino acid sequence of the components of the construct shown in Figure 4A

SEQ ID No. 23 - Signal Sequence from Murine heavy chain
MGWSCIILFLVATATGVHS

SEQ ID No. 24 - RQR8
MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAK
PTTTACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVRA

SEQ ID No. 25 - 2A
EGRGSLLTCGDVEENPG

SEQ ID No. 26 - Signal sequence from mouse kappa chain
METDTLILWVLLLLVPGSTG

SEQ ID No. 27 - anti-γC Light Chain variable region
DIVMTQSHKFMSTSVGDSITITCKASQDVTTAVAWYQQKPGQSPKLLIYWASTRHTG
VPDRFTGSGSGTDYTLTISSVQAEDLALYYCQQHYITPWTFGGGTKLEIKR

SEQ ID No. 28 - Linker
SGGGGSGGGGSGGGGS

SEQ ID No. 29 - anti-γC Heavy Chain Variable Region
QVQLQESGPGLVAPQSQSLSITCTVDGFSLTSYGVHWVRQPPGKGLEWLGVIWAG
GSTNYNSALMSRLNINRDNSKSQIFLKMNSLQTDDTAIYYCAREGSTVDSMDYWGQ
GTTVTVSS

SEQ ID No. 9 - IL7Ra transmembrane region
PILLTISILSFFSVALLVILACVLW

SEQ ID No. 3 - IL7Ra endodomain
KKRIKPIVWPSLPDHKKTLEHLCKKPRKNLNVSFNPESFLDCQIHRVDDIQARDEVEG
FLQDTFPQQLEESEKQRLGGDVQSPNCPSEDVVITPESFGRDSSLTCLAGNVSACD
APILSSSRSLDCRESGKNGPHVYQDLLLSLGTTNSTLPPPFSLQSGILTLNPVAQGQ
PILTSLGSNQEEAYVTMSSFYQNQ

Amino acid sequence of the components of the construct shown in Figure 4B

SEQ ID No. 23 - Signal Sequence from Murine heavy chain
MGWSCIILFLVATATGVHS

SEQ ID No. 24 - RQR8
MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAK
PTTTACPYSNPSLCSGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRPVVRA

SEQ ID No. 25 - 2A
EGRGSLLTCGDVEENPG

SEQ ID No. 26 - Signal sequence from mouse kappa chain
METDTLILWVLLLLVPGSTG

SEQ ID No. 30 - HA tag
YPYDVPDYA

SEQ ID No. 31 - anti-IL2receptor beta (CD122) specific variable light chain
MDFQVQIFSFLLISASVISRGQWLTQSPVIMSASPGEKVTMTCSAISSSYMYWYQQK
PGSSPRLLIYDTSNLVSGVPVRFSGSGSGTSYSLTISRMEAEDAATYYCQQWNTYP
YTFGGGTKLEIK

SEQ ID No. 28 - Linker
SGGGGSGGGGSGGGGS

SEQ ID No. 32 - anti-IL2receptor beta (CD122) specific variable heavy chain
MKLWLNWVFLLTLLHGIQCEVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWV
RQPPGKRLEWIAASRNKANDYTTEYSASVKGRFIVSRDTSQSILYLQMNALRAEDTA
IYYCARSYYRYDGMD YWGQGTSVTVSS

SEQ ID No. 5 - CD8stalk
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

SEQ ID No. 7 - IL2R common gamma chain TM
VVISVGSMGLIISLLCVYFWL

SEQ ID No. 1 - IL2R common gamma chain endodomain
ERTMPRIPTLKNLEDLVTEYHGNFSAWSGVSKGLAESLQPDYSERLCLVSEIPPKGG
ALGEGPGASPCNQHSPYWAPPCYTLKPET

Example 2 – *In vitro* testing

T-cells are transduced with either a CAR, or a construct which co-expresses a CAR with a chimeric polypeptide. Transduced T-cells are cultured in the presence or absence of exogenous IL2 and at different effector to target ratios. Proliferation of T-

cells and killing of target cells is determined. In this way, the contribution the chimeric polypeptide makes to proliferation and survival of T-cells can be measured.

Example 3 - *In vivo* testing

T-cells transduced with either a CAR, or a construct which co-expresses a CAR with a chimeric polypeptide are administered to tuour-bearing NSG mice. Mice within each cohort are sacrificed at different time-points and engraftment / expansion of T-cells at the tumour bed or within lymphoid tissues such as lymph nodes, spleen and bone-marrow measured by flow cytometry of said tissues.

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 polypeptide comprising:

an antigen-binding domain which constitutively binds to an ectodomain of a first chain of a cytokine receptor;

a transmembrane domain; and

an endodomain from a second chain of the cytokine receptor

which chimeric polypeptide, when expressed in a cell, binds to the endogenous first chain of the cytokine receptor causing constitutive cytokine signalling.

2. A chimeric polypeptide according to claim 1, wherein the first chain of the cytokine receptor is a type I cytokine receptor γ-chain; and the second chain of the cytokine receptor is type I cytokine receptor α-chain or β-chain.

3. A chimeric polypeptide according to claim 1, wherein the first chain of the cytokine receptor is a type I cytokine receptor α-chain or β-chain; and the second chain of the cytokine receptor is type I cytokine receptor γ-chain.

4. A chimeric polypeptide according to claim 2 or 3, wherein the type I cytokine receptor α-chain or β-chain is from one of the following: IL-2 receptor, IL-4 receptor, IL-7 receptor, IL-9 receptor, IL-13 receptor and IL-15 receptor.

5. A chimeric polypeptide according to claim 4, wherein the type I cytokine receptor α-chain or β-chain is IL-7 receptor α-chain.

6. A chimeric polypeptide according to any preceding claim where the antigen binding domain comprises a dAb or scFv which binds the ectodomain of the first chain of the cytokine receptor.

7. A constitutively-active cytokine receptor which comprises a chimeric polypeptide according to any preceding claim in association with the first chain of the cytokine receptor.

8. A cell which comprises a constitutively-active cytokine receptor which comprises a chimeric polypeptide according to any of claims 1 to 6 in association with the endogenous first chain of the cytokine receptor.

9. A cell according to claim 8, which also comprises a chimeric antigen receptor or an engineered T-cell receptor.

10. A nucleic acid sequence encoding a chimeric polypeptide according to any of claims 1 to 6.

11. A nucleic acid construct which comprises a first nucleic acid sequence encoding a chimeric polypeptide according to any of claims 1 to 6; and a second nucleic acid encoding a chimeric antigen receptor or an engineered T-cell receptor.

12. A vector comprising a nucleic acid sequence according to claim 10 or a nucleic acid construct according to claim 11.

13. A method for making a cell according claim 8 or 9, which comprises the step of introducing: a nucleic acid sequence according to claim 10; a nucleic acid construct according to claim 11;or a vector according to claim 12, into a cell.

14. A method for inducing constitutive cytokine signaling in a cell, which comprises the step of expressing a chimeric polypeptide according to any of claims 1 to 6 in the cell.

15. A method for blocking deleterious cytokine signalling in a cell, which comprises the step of expressing a chimeric polypeptide according to any of claims 1 to 6 in the cell, wherein the antigen binding domain constitutively binds to common gamma chain, and wherein one or more deleterious cytokine(s) signal(s) through a cytokine receptor which comprises common gamma chain.

16. A pharmaceutical composition comprising a plurality of cells according to claim 8 or 9.

17. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to claim 16 to a subject.

18. A method according to claim 17, 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 10; a nucleic acid construct according to claim 11; or a vector according to claim 12; and

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

19. A method according to claim 17 or 18, wherein the disease is a cancer.

20. A pharmaceutical composition according to claim 16 for use in treating and/or preventing a disease.

21. The use of a cell according to claim 8 or 9 in the manufacture of a medicament for treating and/or preventing a disease.

1/4

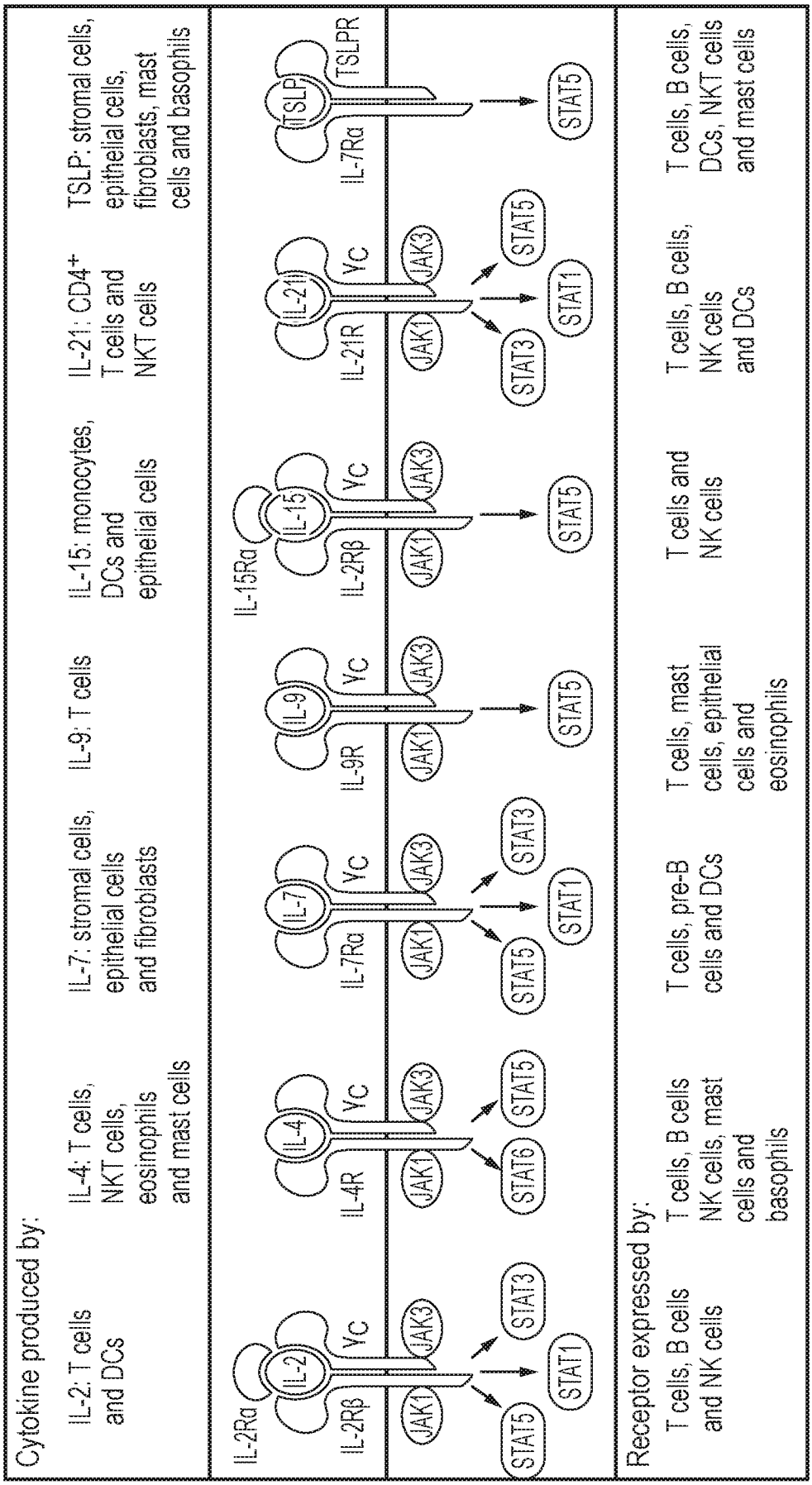
Cytokine produced by:
IL-2: T cells and DCs
IL-4: T cells, NKT cells, eosinophils and mast cells
IL-7: stromal cells, epithelial cells and fibroblasts
IL-9: T cells
IL-15: monocytes, DCs and epithelial cells
IL-21: CD4+ T cells and NKT cells
TSLP: stromal cells, epithelial cells, fibroblasts, mast cells and basophils
IL-2Rα
IL-2
IL-2Rβ
γc
JAK1
JAK3
STAT5
STAT3
STAT1
IL-4
IL-4R
STAT6
IL-7
IL-7Rα
IL-9
IL-9R
IL-15Rα
IL-15
IL-2Rβ
IL-21
IL-21R
TSLP
TSLPR
IL-7Rα
Receptor expressed by:
T cells, B cells and NK cells
T cells, B cells NK cells, mast cells and basophils
T cells, pre-B cells and DCs
T cells, mast cells, epithelial cells and eosinophils
T cells and NK cells
T cells, B cells, NK cells and DCs
T cells, B cells, DCs, NKT cells and mast cells

FIG. 1

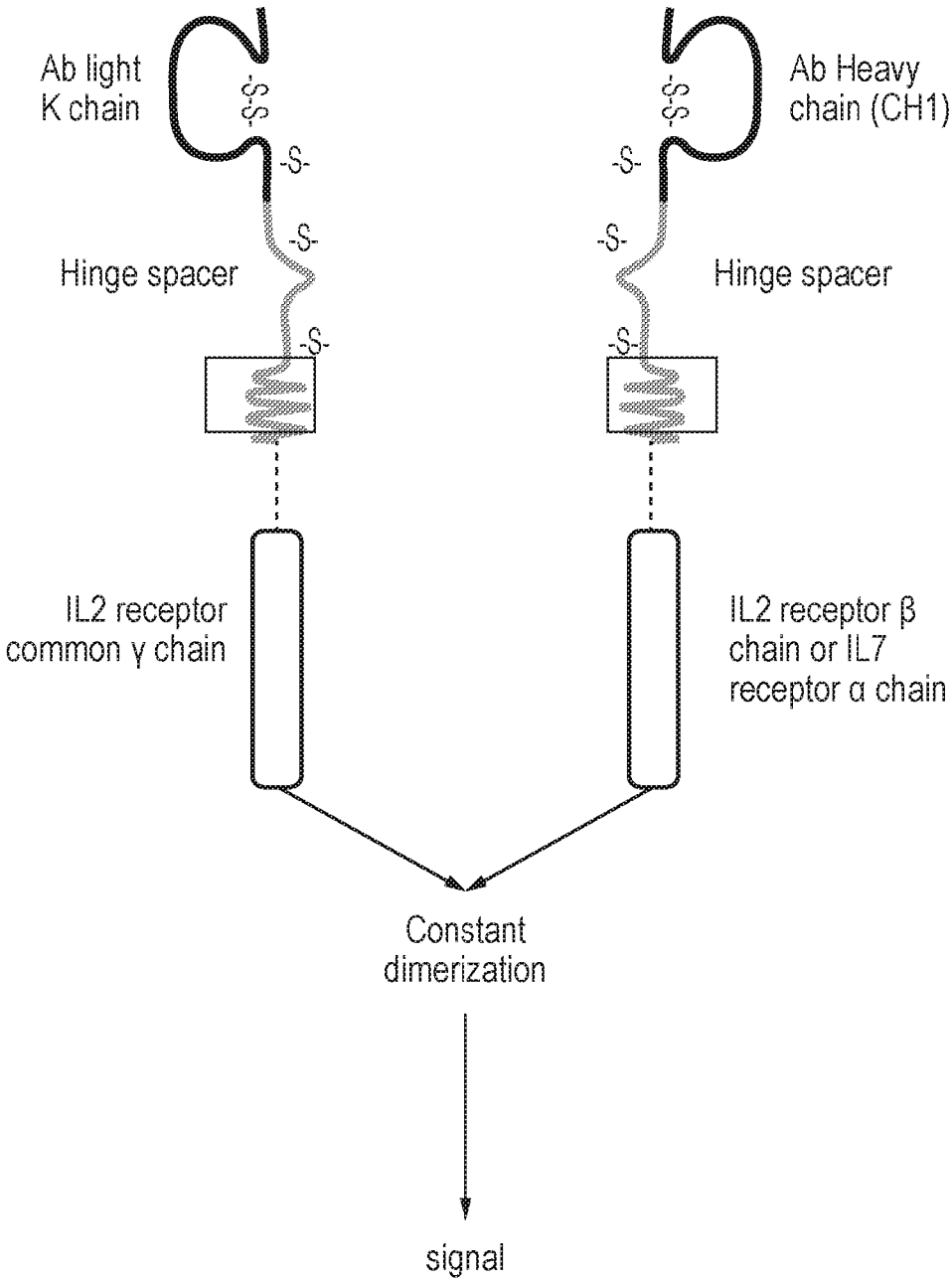
Ab light
K chain
-S-S-
-S-
-S-
Hinge spacer
-S-
IL2 receptor
common γ chain
Ab Heavy
chain (CH1)
-S-S-
-S-
-S-
Hinge spacer
-S-
IL2 receptor β
chain or IL7
receptor α chain
Constant
dimerization
signal

FIG. 2

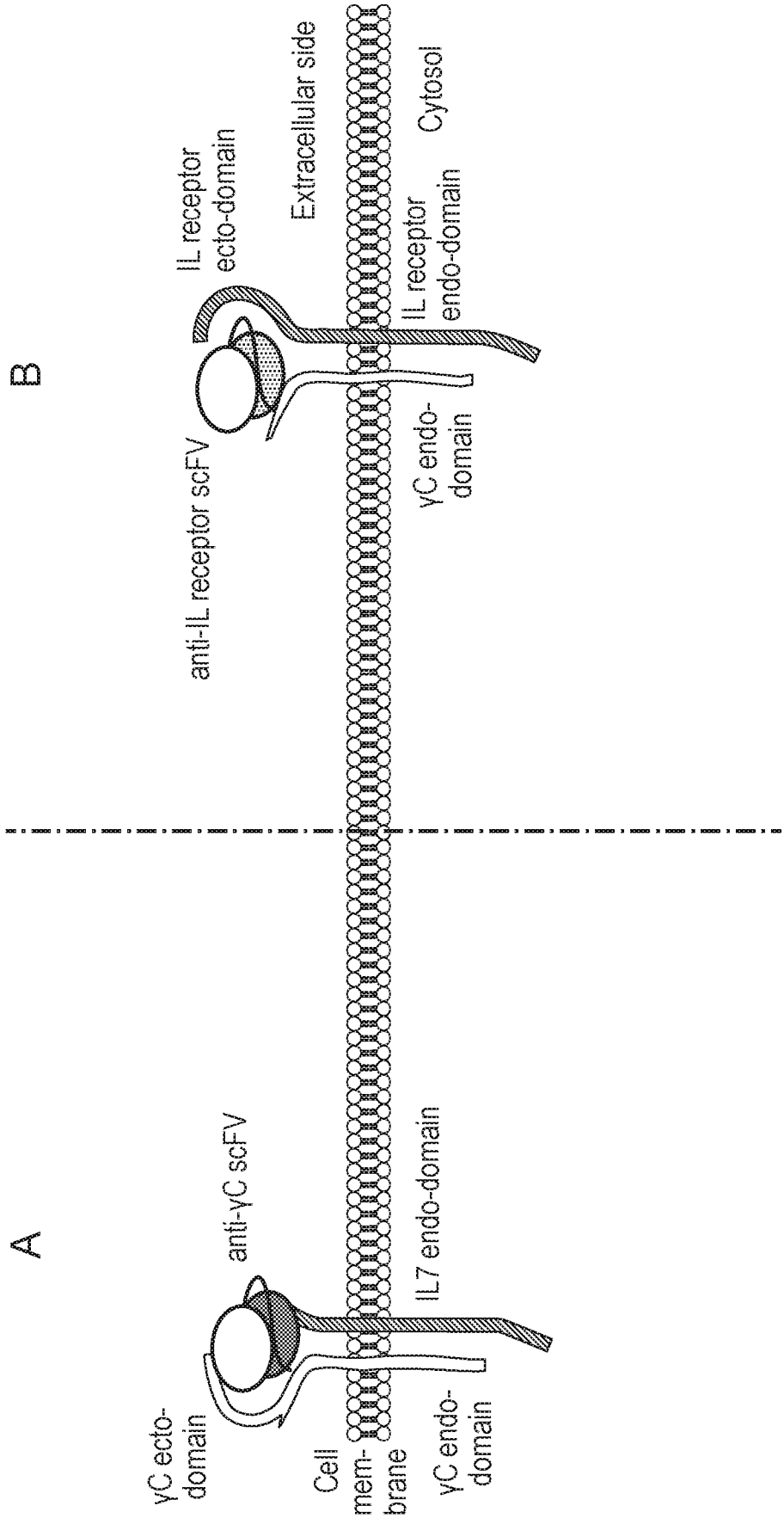
A
B
anti-γC scFV
IL7 endo-domain
γC ecto-domain
Cell mem-brane
γC endo-domain
anti-IL receptor scFV
IL receptor ecto-domain
Extracellular side
Cytosol
IL receptor endo-domain
γC endo-domain

FIG. 3

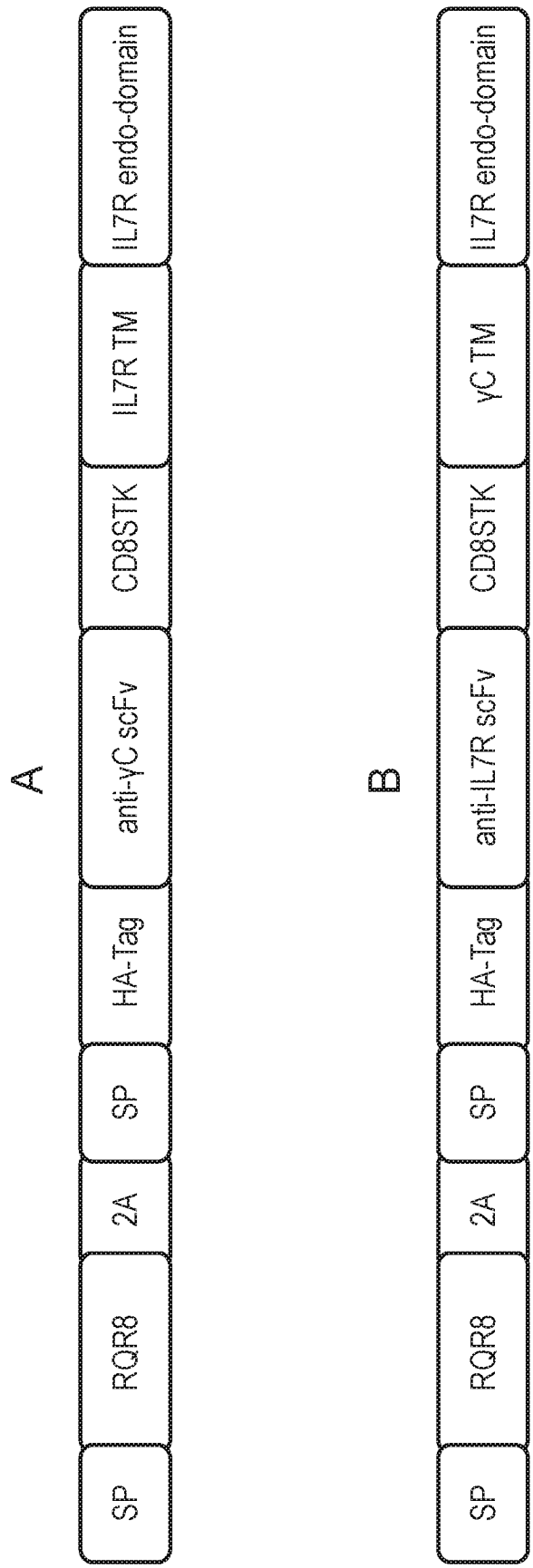
A
SP
RQR8
2A
SP
HA-Tag
anti-γC scFv
CD8STK
IL7R TM
IL7R endo-domain
B
SP
RQR8
2A
SP
HA-Tag
anti-IL7R scFv
CD8STK
γC TM
IL7R endo-domain

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/053390

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K14/725
ADD.

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

B. FIELDS SEARCHED

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

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, Sequence Search, 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	WO 2017/029512 A1 (AUTOLUS LTD [GB]) 23 February 2017 (2017-02-23) figure 1; examples 1-4 -----	1-21
X	THOMAS SHUM ET AL: "Constitutive Signaling from an Engineered IL7 Receptor Promotes Durable Tumor Elimination by Tumor-Redirected T Cells", CANCER DISCOVERY, vol. 7, no. 11, 1 November 2017 (2017-11-01), pages 1238-1247, XP055515623, US ISSN: 2159-8274, DOI: 10.1158/2159-8290.CD-17-0538 page 1239; figure 1 page 1244 ----- -/--	1-21

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 2019	12/02/2019

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	Vollbach, Silke

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/053390

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOGO T ET AL: "Selective expansion of genetically modified T cells using an antibody/interleukin-2 receptor chimera", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 337, no. 1, 20 August 2008 (2008-08-20), pages 16-23, XP023172874, ISSN: 0022-1759, DOI: 10.1016/J.JIM.2008.05.003 [retrieved on 2008-06-10] the whole document -----	1-21

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2018/053390

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017029512 A1	23-02-2017	AU 2016308618 A1	08-03-2018
		BR 112018003308 A2	18-09-2018
		CA 2995785 A1	23-02-2017
		CL 2018000415 A1	20-07-2018
		CN 108350047 A	31-07-2018
		EP 3337814 A1	27-06-2018
		EP 3438123 A1	06-02-2019
		JP 2018523485 A	23-08-2018
		KR 20180054625 A	24-05-2018
		US 2018244797 A1	30-08-2018
		US 2019016820 A1	17-01-2019
		WO 2017029512 A1	23-02-2017