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(54) **Title:** SIGNALLING SYSTEM

(57) **Abstract:** The present invention relates to a chimeric antigen-receptor (CAR) signalling system comprising; (i) a targeting component comprising an antigen-binding domain, a transmembrane domain and a first heterodimerization domain; and (ii) an intracellular signalling component comprising a signalling domain and a second heterodimerization domain; wherein spontaneous heterodimerization between the first and second heterodimerization domains causes the targeting component and signalling component to form a functional CAR complex.



SIGNALLING SYSTEM

FIELD OF THE INVENTION

5 The present invention relates to an antigen receptor signalling system.

BACKGROUND TO THE INVENTION

10 Traditionally, antigen-specific T-cells have been generated by selective expansion of peripheral blood T-cells natively specific for the target antigen. However, it is difficult and quite often impossible to select and expand large numbers of T-cells specific for most cancer antigens. Gene-therapy with integrating vectors affords a solution to this problem as transgenic expression of Chimeric Antigen Receptor (CAR) allows generation of large numbers of T-cells specific to any surface antigen by *ex vivo* viral vector transduction of a
15 bulk population of peripheral blood T-cells.

Chimeric antigen receptors are proteins which graft the specificity of an antigen binder, such as 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
20 spacer, a transmembrane domain all connected to a compound endodomain which transmits T-cell survival and activation signals (see Figure 1A).

The most common forms of these molecules are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies which recognize a target antigen, fused via a
25 spacer and a trans-membrane domain to a signalling 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
30 treatment of various cancers.

A number of toxicities have been reported from CAR studies, and additional theoretical toxicities exist. Such toxicities include immunological toxicity caused by sustained intense activation of the CAR T-cells resulting in a macrophage activation syndrome (MAS) and "On-
35 target off-tumour" toxicity i.e. recognition of the target antigen on normal tissues.

MAS is presumed to be caused by persistent antigen-driven activation and proliferation of T-cells which in turn release copious inflammatory cytokines leading to hyper-activation of macrophages and a feed-forward cycle of immune activation. A large spike in serum IL-6 is characteristic and the syndrome can result in a severe systemic illness requiring ICU admission.

On-target off-tumour toxicity has been reported with other CARs, for example a group of patients treated with a CAR against the renal cell carcinoma antigen CAIX developed unexpected and treatment limiting biliary toxicity. Two fatalities have been reported with CAR studies: one patient died of a respiratory distress syndrome which occurred immediately post-infusion of a large dose of 3rd generation anti-ERBB2 CAR T-cells; a further patient died in a different study after a possible cytokine storm following treatment of CLL with a second generation anti-CD19 CAR.

These toxicities are very difficult to predict even with detailed animal studies or non-human primate work. Crucially, unlike small molecules and biologics, CAR T-cells do not have a half-life and one cannot cease administration and wait for the agent to breakdown/become excreted. CAR T-cells are autonomous and can engraft and proliferate. Toxicity can therefore be progressive and fulminant.

A key aspect of these toxicities is immune over-activation i.e. over-activation of CAR T-cells.

Suicide genes are genetically expressed elements which can conditionally destroy cells which express them. Examples include Herpes-simplex virus thymidine kinase, which renders cells susceptible to Ganciclovir; inducible Caspase 9, which renders cells susceptible to a small molecular homodimerizer and CD20 and RQR8, which renders cells susceptible to Rituximab.

This technology adds a certain amount of safety to CAR T-cell therapy, however there are limitations. Firstly, it is a binary approach wherein all the CAR T-cells are destroyed upon addition of the suicide entity. In addition, medicinal therapeutics often have a therapeutic window. With a suicide gene the potency of the product cannot be tuned such that efficacy with tolerable toxicity can be achieved. Secondly, it is not clear whether a suicide gene would help with some of the immune-toxicities described above: for instance by the time a macrophage activation syndrome had been triggered, it may well no longer need the CAR T-cells to perpetuate and the suicide gene would no longer be helpful. The more acute

cytokine release syndromes probably occur too quickly for the suicide gene to work. Finally, suicide genes are not “fail-safe”, i.e. the default status is for the CAR T-cells to be active.

There is thus a need for alternative methods for controlling CAR T-cells that are not associated with the disadvantages and problems mentioned above.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have found that it is possible to separate the antigen-recognition and signalling components of a CAR to produce a system in which signalling only occurs when the antigen-recognition and signalling components dimerize and form a functional CAR complex.

Thus in a first aspect the present invention relates to a chimeric antigen-receptor (CAR) signalling system comprising;

- (i) a targeting component comprising an antigen-binding domain, a transmembrane domain and a first heterodimerization domain; and
- (ii) an intracellular signalling component comprising a signalling domain and a second heterodimerization domain;

wherein spontaneous heterodimerization between the first and second heterodimerization domains causes the targeting component and signalling component to form a functional CAR complex.

The first and second heterodimerization domains are capable of spontaneous dimerization with each other. Heterodimerization occurs with the first and second heterodimerization domains alone, without the need for any separate molecule acting as an “inducer” of dimerization.

The first and second heterodimerization domains may comprise leucine zipper domains, DDD1 and AD1 domains, Barnase and Barnstar domains or human pancreatic RNase and S-peptide domains.

The CAR signalling system may comprise multiple targeting components, each recognizing a different antigen.

The first heterodimerization domains of the multiple targeting components may differ in affinity for the second heterodimerization domain such that each antigen propagates different signalling strengths.

- 5 The signalling domain of the signalling component may comprise a single endodomain selected from CD3 zeta endodomain, CD28 endodomain, 41BB endodomain, OX40 endodomain, an ICOS endodomain, a CD27 endodomain, a BTLA endodomain, a CD30 endodomain, a GITR endodomain and an HVEM endodomain.
- 10 The signalling domain of the signalling component may comprise at least one of CD3 zeta endodomain, CD28 endodomain, 41BB endodomain and OX40 endodomain.

The CAR signalling system may comprise a plurality of signalling components, each comprising a signalling domain and a second heterodimerization domain, wherein the
15 second heterodimerization domains each recognise the same first heterodimerization domain but the signalling domains comprise different signalling endodomains.

The CAR signalling system may comprise a plurality of signalling components which comprise a plurality of second heterodimerization domains, each of which independently
20 recognise the first heterodimerization domains with different affinities.

In the CAR signalling system of the first aspect of the invention, the targeting component may comprise more than two heterodimerisation domains, such that a single targeting component is capable of heterodimerising with more than two signalling components. For
25 example, the targeting component may comprise from 3 to 12 heterodimerisation domains. The targeting component may comprise 4, 6, 8 or 10 heterodimerization domains.

Each signalling component may comprise a plurality of signalling domains, which may be the same or different. For example, each signalling component may comprise two, three or four
30 copies of the same signalling domain, such as CD3 zeta.

In a second aspect, the present invention provides a targeting component suitable for use in the CAR signalling system according to the first aspect of the invention which comprises an antigen-binding domain, a transmembrane domain and a heterodimerization domain.
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The targeting component may comprise a plurality of heterodimerization domains, such as more than two heterodimerisation domains.

In a third aspect, the present invention comprises a signalling component suitable for use in the CAR signalling system according to the first aspect of the invention which comprises a signalling domain and a heterodimerization domain.

5 The signalling component may comprise a plurality of signalling domains.

In a fourth aspect, the present invention provides a nucleic acid encoding the targeting component according to the second aspect of the invention.

10 In a fifth aspect, the present invention provides a nucleic acid encoding the signalling component according to the third aspect of the invention.

In a sixth aspect, the present invention provides a nucleic acid construct encoding a CAR signalling system according to the first aspect of the invention, wherein the nucleic acid
15 construct comprises the following structure:

$$A - X - B$$

in which

A and B are nucleic acid sequences encoding a targeting component or a signalling component as defined in the second or third aspect of the present invention; and

20 X is a nucleic acid sequence which encodes a cleavage site, such that A is cleaved from B after translation.

The nucleic acid construct may have one of the following structures:

25 a) AbD – spacer – TM – endo – Het1 – coexpr – Het2 – SD

b) AbD – spacer – TM – endo – Het1 – coexpr – SD – Het2

c) Het2 – SD – coexpr – AbD – spacer – TM – endo – Het1 or

30

d) SD – Het2 – coexpr – AbD – spacer – TM – endo – Het1

in which

AbD is a nucleic acid sequence encoding the antigen-binding domain of the targeting
35 molecule;

spacer is a nucleic acid sequence encoding a spacer of the targeting molecule;

TM is a nucleic acid sequence encoding the transmembrane domain of the targeting molecule;

endo is a nucleic acid sequence encoding the endodomain of the targeting molecule;

Het1 is a nucleic acid sequence encoding the heterodimerization motif of the targeting molecule;

coexpr is a nucleic acid sequence encoding a cleavage site;

Het2 is a nucleic acid sequence encoding the heterodimerization motif of the signalling molecule; and

SD is nucleic acid sequence encoding the signalling domain of the signalling molecule.

The nucleic acid construct may encode multiple targeting components and/or multiple signalling components as defined in the first aspect of the present invention, wherein the multiple targeting components or multiple signalling components are separated by cleavage sites, such that the multiple targeting components or multiple signalling components are cleaved after translation.

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

The vector may be a retroviral vector or a lentiviral vector or a transposon.

In an eighth aspect, the present invention provides a cell which comprises a CAR signalling system according to the first aspect of the present invention.

In a ninth aspect, the present invention provides a cell which expresses at least one targeting component according to the second aspect of the invention and at least one signalling component according to the third aspect of the invention.

The cell may comprise a nucleic acid according to fourth or fifth aspect of the invention or a nucleic acid construct according to the sixth aspect of the present invention or a vector according to the seventh aspect of the present invention.

The cell may be a T cell or NK cell.

In tenth aspect, the present invention provides a pharmaceutical composition comprising a plurality of cells according to the ninth aspect of the invention.

In an eleventh aspect, the present invention provides a pharmaceutical composition according to the tenth aspect of the invention for use in treating and/or preventing a disease.

- 5 In a twelfth aspect, the present invention relates to 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:

- 10 (i) isolation of a T cell or NK cell containing sample from a subject;
(ii) transduction or transfection of the T cells or NK cells with a nucleic acid according to the fourth or fifth aspect of the invention or a nucleic acid construct according to the sixth aspect of the present invention or a vector according to the seventh aspects of the present invention; and
15 (iii) administering the T cells or NK cells from (ii) to the subject.

In a thirteenth aspect, the present invention relates to the use of a pharmaceutical composition according to the tenth aspect of the present invention in the manufacture of a medicament for the treatment and/or prevention of a disease.

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The disease may be cancer.

In a fourteenth aspect, the present invention provides a kit which comprises a nucleic acid according to the fourth or fifth aspect of the invention or a nucleic acid construct according to the sixth aspect of the present invention or a vector according to the seventh aspect of the present invention.

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The kit may comprise a cell according to the ninth aspect of the present invention.

30 In a sixteenth aspect, the present invention provides a method for making a cell according to the ninth aspect of the invention, which comprises the step of introducing a nucleic acid according to the fourth or fifth aspect of the invention or a nucleic acid construct according to any of the sixth aspect of the present invention or a vector according to the seventh aspects of the present invention, into the cell.

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

The CAR signalling system of the present invention provides several advantages compared to classic CAR molecules. For example, steric hindrance of second messengers can be avoided because the signalling domains may be independent of each other (i.e. provided on separate signalling components).

Further, it is possible to include a plurality of different signalling molecules and different signalling domain(s) may be provided on separate signalling components. As such each signalling domain may be proximal to the membrane. This proximity to the membrane is optimal for signalling. This is in contrast to a classic CAR, in which the signalling domain becomes crowded and the most distal element may be too far from the membrane.

It is possible to amplify the T-cell activation signal by recruiting multiple signalling components for each antigen recognition molecule. This is especially useful for the detection of low density antigens.

In addition, by altering the affinity of the heterodimerization domain on the signalling component, it is possible to "tune" the mix of co-stimulatory signals the T-cell receives. For instance, it might be advantageous for the T-cell to receive a high level of OX40 signal, intermediate amounts of Zeta signal and low amounts of CD28. Such a signal may lead, for example, to a slow but steady rejection of a tumour and thus avoid immune-toxicity and encourage T-cell persistence.

It is also possible to target multiple antigens more conveniently since the signalling components are shared. In this case, two targeting components may be co-expressed along with the signalling components. In the case of targeting more than one antigen, if one antigen is more densely expressed than another, the heterodimerization domains on the respective targeting domains can be altered such that the signalling components interact with each targeting component in order to create a balanced signal for recognition of each antigen. In this way, a very versatile and tunable CAR signalling system can be constructed.

DESCRIPTION OF THE FIGURES

Figure 1 - a) Schematic diagram illustrating a classical CAR. (b) to (d): Different generations and permutations of CAR endodomains: (b) initial designs transmitted ITAM signals alone through Fc ϵ R1- γ or CD3 ζ endodomain, while later designs transmitted additional (c) one or (d) two co-stimulatory signals in the same compound endodomain.

Figure 2 – Concept of a zipCAR. The targeting component is a type I transmembrane protein whose ectodomain comprises a target recognition domain and a spacer, and whose endodomain comprises a heterodimerization motif (HD1). The signalling component is an intracellular protein and comprises a heterodimerization motif (HD2) fused to a signalling domain. While the two components are separate proteins, they assemble after expression to form a functional CAR.

Figure 3 – (a) A Leucine zipper based zipCAR comprising Acid/Basic Leucine zippers. In this case, the homodimeric targeting component will recruit two signalling components. (b) A DDD1/AD1 based ZipCAR. In this case, since the DDD1/AD1 interaction is trimeric, the homodimeric targeting domain will recruit four signalling domains.

Figure 4 – zipCAR constructs. (a) A bicistronic construct to test an Acid/Base Leucine zipper zipCAR. (b) A control construct for the Leucine zipper zipCAR which is identical to construct (a) apart from the leucine zipper of the signalling component has been deleted. (c) A bicistronic construct to test the DDD1/AD1 based zipCAR. (d) A control construct for the DDD1/AD1 zipCAR which is identical to construct (c) apart from deletion of the AD1 segment from the signalling component.

Figure 5 – Expression and function of the Acid/Base Leucine Zipper ZipCAR. (a) Expression of a non-transduced BW5 cell line, control leucine zipper zipCAR, and leucine zipper zipCAR is shown as staining of BW5 cells with recombinant CD33-Fc. (b) IL2 release of leucine zipper zipCAR and control leucine zipper zipCAR in response to cognate antigen at different effector to target ratios.

Figure 6 – Expression and function of the DDD1/AD1 ZipCAR. (a) Expression of a non-transduced BW5 cell line, control leucine zipper zipCAR, and leucine zipper zipCAR is shown as staining of BW5 cells with recombinant CD33-Fc. (b) IL2 release of DDD1/AD1 zipCAR and control DDD1/AD1 zipCAR in response to cognate antigen at different effector to target ratios.

Figure 7 – zipCAR with multiple signalling components. The multiple signalling components interact with the targeting component(s) such that a multitude of signals can be transmitted.

Figure 8 – ZipCAR with multiple signalling components with altered heterodimerization motifs. Multiple signalling components are expressed which interact with the targeting component(s). The heterodimerization domains of the different signalling components (HD2)

are have differing affinities for the heterodimerization domains of the targeting components (HD1). In this way a multitude of signals can be transmitted in trans where the average intensity of each signal can be tuned.

5 **Figure 9** – ZipCAR with multiple targeting components. Multiple different targeting components are expressed and each targeting component recognizes a different antigen. The signalling components are capable of interacting with each targeting component and hence a cell expressing such a CAR signalling system is capable of recognizing each of the antigens recognized by the targeting components.

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Figure 10 – ZipCAR with multiple targeting components with different heterodimerization domains. Multiple different targeting components are expressed and each targeting component recognizes a different antigen. The heterodimerization domain of each targeting component (HD1) differs such that each targeting component recruits signalling components
15 with different kinetics. Consequently, binding of different cognate antigens results in different signalling intensities.

Figure 11 – Amino acid sequences of the constructs depicted in Figure 4.

20 **Figure 12** – Schematic illustration of a “superCAR” ZipCAR with multiple heterodimerisation domains. An anti-CD19 targeting component was engineered to express eight heterodimerisation domains. The effect on –cell activation was tested with various signalling components: HA tag alone (control); HA tag, heterodimerisation domain and one TCRzeta; HA tag, heterodimerisation domain and two TCRzeta domains; HA tag, heterodimerisation
25 domain and three TCRzeta domains.

Figure 13 - IL2 release of DDD1/AD1 superCAR ZipCAR shown in Figure 12 with various signalling components in response to cognate antigen at different levels of target antigen expression

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DETAILED DESCRIPTION OF THE INVENTION

CHIMERIC ANTIGEN RECEPTORS (CARs)

35 Classical CARs, which are shown schematically in Figure 1, 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 spacer domain may be necessary to isolate the binder from the membrane and to allow it a suitable orientation. A common spacer domain used is the Fc of IgG1. More compact
5 spacers can suffice e.g. the stalk from CD8 α and even just the IgG1 hinge alone, depending on the antigen. 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
10 of the Fc ϵ R1 or CD3 ζ . Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3 ζ results in second generation receptors which can
15 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.
20 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
25 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.

In a first aspect, the present invention relates to a CAR system in which the antigen-
30 recognizing/antigen binding domain and transmembrane domain are provided on a first molecule (termed herein 'targeting component'), which localizes to the cell membrane. The intracellular signalling domain is provided on a second, intracellular molecule (termed herein 'signalling component').

35 Importantly, the targeting component comprises a first heterodimerization domain and the signalling component comprises a second heterodimerization domain. The first and second heterodimerization domains are capable of dimerization with one another. Binding of the

first and second heterodimerization domains causes co-localization of the targeting component and the signalling component at the cell surface. This forms a functional CAR complex at the cell surface and thus, when antigen binds to the antigen binding domain of the targeting component, there is signalling through the signalling component.

Herein 'co-localization' or 'heterodimerization' of the targeting and signalling components is analogous to ligation/recruitment of the signalling component to the targeting component via binding of the first heterodimerization domain of the targeting component and the second heterodimerization domain of the signalling component.

Signalling through the signalling component may be determined by a variety of methods known in the art. Such methods include assaying signal transduction, for example assaying levels of specific protein tyrosine kinases (PTKs), breakdown of phosphatidylinositol 4,5-bisphosphate (PIP2), activation of protein kinase C (PKC) and elevation of intracellular calcium ion concentration. Functional readouts, such as clonal expansion of T cells, upregulation of activation markers on the cell surface, differentiation into effector cells and induction of cytotoxicity or cytokine secretion may also be utilised. As an illustration, in the present examples the inventors determined levels of interleukin-2 (IL-2) produced by T-cells expressing a targeting component and signalling component of the CAR system according to the present invention upon binding of antigen to the receptor component in the presence of varying concentrations of an agent.

FIRST AND SECOND HETERODIMERIZATION DOMAINS

The first and second heterodimerization domains of the present CAR system may be any combination of domains which interact resulting in co-localization of the targeting component and signalling component at the cell membrane.

As such, the first heterodimerization domain and second heterodimerization domain may be capable of specifically binding to one another.

The first and second heterodimerization domains are capable of spontaneous dimerization with each other. Heterodimerization occurs with the first and second heterodimerization domains alone, without the need for any separate molecule acting as an "inducer" of dimerization.

The signalling system of the present invention is not limited by the arrangement of a specific pair of heterodimerization domains. The targeting component may comprise either domain from a pair of heterodimerizing domains so long as the signalling component comprises the corresponding, complementary domain which enables the targeting component and the signalling component to co-localize at the cell membrane.

The heterodimerization domains for use in the present CAR system are not limited to those which interact at a 1:1 ratio. For example, heterodimerization domains may interact to form multimers (e.g. trimers or tetramers). The domains may interact in a manner which co-localises a single first heterodimerization domain with multiple (e.g. 2 or 3) second heterodimerization domains. Herein it may be advantageous to have a signalling domain which comprises the second heterodimerization domain, such that multiple signalling components can co-localise with a single targeting component. This may be advantageous, for example, when a high level of signalling is required upon binding of antigen to the targeting component.

The targeting component may comprise a plurality of heterodimerization domains, so that it interacts with a plurality of signalling components. For example, the targeting component may comprise more than two heterodimerization domains, such a 3 to 10 heterodimerization domains. Figure 12 shows a targeting component which comprises 8 heterodimerization domains.

For convenience, the term heterodimerization domain is used herein for all domains which mediate co-localization the targeting and signalling components.

A large variety of appropriate heterodimerization domains are known in the art, examples of which are provided herein.

The first and second heterodimerization domains may be leucine zippers.

Leucine zippers are well known in the art (see *Hakoshima; Encyclopedia of Life Sciences; 2005*, for example). The leucine zipper is a super-secondary structure that functions as a dimerization domain. Its presence generates adhesion forces in parallel alpha helices. A single leucine zipper consists of multiple leucine residues at approximately 7-residue intervals, which forms an amphipathic alpha helix with a hydrophobic region running along one side. This hydrophobic region provides an area for dimerization, allowing the motifs to

"zip" together. Leucine zippers are typically 20 to 40 amino acids in length, for example approximately 30 amino acids.

The first and/or second heterodimerization domain may comprise the sequence shown as SEQ ID NO: 1 or 2. The first heterodimerization domain may comprise the sequence shown as SEQ ID NO: 1 and the second heterodimerization domain may comprise the sequence shown as SEQ ID NO: 2, or vice versa.

SEQ ID NO: 1: QLEKELQALEKENAQLWELQALEKELAQ

SEQ ID NO: 2: QLEKKLQALKKKNAQLKWLQALKKKLAQ

In certain embodiments, the first and second heterodimerization domains may be acidic (e.g. SEQ ID NO: 1) or basic (e.g. SEQ ID NO: 2) leucine zippers. In particular, where the first heterodimerization domain is an acidic leucine zipper, the second heterodimerization is a basic leucine zipper and vice versa.

The first and second heterodimerization domains may be dimerization and docking domain (DDD1) and anchoring domain (AD1). These domains and the interaction between them is known in the art (Rossi *et al.*; PNAS; 2006; 103(18); 6841-6846).

DDD1 is a short alpha helical structure derived from Protein Kinase A (PKA). AD1 is a short alpha helical structure derived from A-kinase anchor proteins (AKAPs).

The DDD1 domain may comprise the sequence shown as SEQ ID NO: 3.

SEQ ID NO: 3: SHIQIPPGLTELLQGYTVEVLRQQPPDLVEFAVEYFTRLREARA

The AD1 domain may comprise the sequence shown as SEQ ID NO: 4

SEQ ID NO: 4: VQIEYLAKQIVDNAIQQA

Since the DDD1/AD1 interaction is trimeric, an AD1 domain present on the targeting component will recruit four signalling domains comprising a DDD1 domain. Thus in a particular embodiment, the targeting component comprises an AD1 domain and the receptor component comprises a DDD1 domain.

The heterodimerization domains may be derived from the Bacterial Ribonuclease (Barnase) and Barnstar peptides.

Barnase is the *Bacillus amyloliquefaciens* ribonuclease protein. It is composed on 110 amino acids. Barnstar functions to inhibit the nuclease activity of Barnase and therefore binds Barnstar with a very high affinity (an on-rate of $10^8 \text{s}^{-1} \text{M}^{-1}$).

The heterodimerization domains may be derived from Human Pancreatic RNases and S-peptide.

Human Pancreatic RNase are pyrimidine-specific endonucleases. S-peptide is the enzymatically inactive proteolytic fragment of RNase A, which lacks the RNA binding site.

The present invention also encompasses variants of the heterodimerization sequences described herein which retain the ability to dimerize with the corresponding heterodimerization domain. The heterodimerization domain may be a variant having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that domain still functions to cause co-localization of the targeting component and signalling component at the cell membrane.

A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to a sequence of a heterodimerization domain described herein, provided that the sequence still functions to cause co-localization of the targeting component and signalling component at the cell membrane.

TARGETING COMPONENT

The term targeting component is analogous to 'receptor component'.

The present invention provides a targeting component comprising an antigen-binding domain, an optional spacer domain, a transmembrane domain and a first heterodimerization domain. When expressed in a cell, the targeting component localises to the cell membrane. Here, the antigen-binding domain of the molecule is orientated on the extracellular side of the membrane and the first heterodimerization domain is localised to the intracellular side of the membrane.

The targeting component therefore provides the antigen-binding function of the CAR system of the present invention.

ANTIGEN BINDING DOMAIN

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The antigen-binding domain is the portion of a classical CAR which recognizes antigen. In the signalling system of the present invention the antigen-binding is located within the targeting component.

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

Various tumour associated antigens (TAA) are known, as shown in the following 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.

20

Table 1

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

TRANSMEMBRANE DOMAIN

The transmembrane domain is the sequence of a classical CAR that spans the membrane. In the signalling system of the present invention the transmembrane domain is located in the targeting component. It may comprise a hydrophobic alpha helix. The transmembrane domain may be derived from CD28, which gives good receptor stability.

SIGNAL PEPTIDE

The targeting component of the CAR system of the present invention may comprise a signal peptide so that when the targeting component 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: 5, 6 or 7 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: 5: MGTSLLCWMALCCLLGADHADG

The signal peptide of SEQ ID NO: 5 is compact and highly efficient. It is predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

SEQ ID NO: 6: MSLPVTALLPLALLLHAARP

The signal peptide of SEQ ID NO: 6 is derived from IgG1.

SEQ ID NO: 7: MAVPTQVLGLLLLWLTDARC

The signal peptide of SEQ ID NO: 7 is derived from CD8.

SPACER DOMAIN

5

The CAR system described herein may comprise a spacer sequence to connect the antigen-binding domain with the transmembrane domain in the targeting component. A flexible spacer allows the antigen-binding domain to orient in different directions to facilitate binding.

10

The spacer sequence may, for example, comprise an IgG1 Fc region, an IgG1 hinge or a human CD8 stalk or the mouse CD8 stalk. The spacer 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.

15

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

SEQ ID NO: 8 (hinge-CH₂CH₃ of human IgG1)

AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFN
 20 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
 KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL
 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKKD

SEQ ID NO: 9 (human CD8 stalk):

25 TTTTAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

SEQ ID NO: 10 (human IgG1 hinge):

AEPKSPDKTHTCPPCPKDPK

30 SEQ ID NO: 11 (CD2 ectodomain)

KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSKDKKIAQFRKEKETFKEDTYKLF
 KNGTLKIKHLKTDDQDIYKVSIDYDTKGKNVLEKIFDLKIQERVSKPKISWTCINTTLTCEVMNG
 TDPELNLYQDGKHLKLSQRVITHKWTTSLSAFKCTAGNKVSKESSVEPVSCPEKGLD

35 SEQ ID NO: 12 (CD34 ectodomain)

SLDNNGTATPELPTQGTFNSVSTNVSYQETTTTPSTLGSTSLHPVSQHGNEATTNITETTVKF
 TSTSVITSVYGNTNSSVQSQTSVISTVFTTPANVSTPETTLKPSLSPGNVSDLSTTSTSLATS

PTKPYTSSSPILSDIKAEIKCSGIREVKLTQGICLEQNKTS SCAEFKKDRGEGLARVLCGEEQ
 ADADAGAQVCSLLLAQSEVRPQCLLLVLNRTEISSKLQLMKKHQSDLKKLGILDFTEQDVA
 SHQSYSQKT

5 TARGETING COMPONENT COMPRISING A PLURALITY OF FIRST HETERODIMERIZATION DOMAINS

The targeting component may comprise a plurality of first heterodimerization domains and thus be capable of recruiting more than one signalling component.

10

The plurality of first heterodimerization domains may be present in a single intracellular domain of the receptor component.

15 The targeting component may comprise an appropriate number of transmembrane domains such that each first binding domain is orientated on the intracellular side of the cell membrane. For example the targeting component may comprise 3, 5, 7, 9, 11, or more transmembrane domains. In this way, a single targeting component may recruit multiple signalling components amplifying signalling in response to antigen.

20 The first heterodimerization domains may each be variants which have a different affinity for the second heterodimerization domain of the signalling component.

MULTIPLE TARGETING COMPONENTS

25 In another embodiment of the invention, the CAR system may comprise two or more targeting components each recognizing different antigens but comprising of the same intracellular heterodimerization domain. Such a CAR system would be capable of recognizing multiple antigens (Figures 9 and 10). This might be useful for instance in avoiding tumour escape.

30

In a further related aspect of the invention, the first heterodimerization domains of the targeting components differ in residues which dictate their affinity for the second heterodimerization domain of the signalling component. In this way, a CAR system can be tuned such that signalling in response to one antigen is greater or less than the response to another (Figure 10). This might be useful for instance when targeting two tumour antigens simultaneously but one is expressed at a higher density than the other. Response to this antigen could be tuned down to avoid toxicity caused by over-stimulation.

35

Methods suitable for altering the amino acid residues of the first or second heterodimerization domain such that the binding affinity between the two domains is altered are known in the art and include substitution, addition and removal of amino acids using both targeted and random mutagenesis. Methods for determining the binding affinity between a first heterodimerization domain and a second heterodimerization domain are also well known in the art and include bioinformatics prediction of protein-protein interactions, affinity electrophoresis, surface plasma resonance, bio-layer interferometry, dual polarisation interferometry, static light scattering and dynamic light scattering.

INTRACELLULAR SIGNALLING COMPONENT

The present invention also provides a signalling component comprising a signalling domain and a second heterodimerization domain. The signalling component is a soluble molecule and thus localises to the cytoplasm when it is expressed in a cell, for example a T cell.

No signalling occurs through the signalling domain of the signalling component unless it is co-localised with the targeting component provided by the present invention.

INTRACELLULAR SIGNALLING DOMAIN

The intracellular signalling domain is the signal-transmission portion of a classical CAR. In the signalling system of the present invention, the intracellular signalling domain (signalling domain) is part of the signalling component. When expressed in a cell, for example a T cell, the membrane-bound, targeting component and the intracellular signalling component are brought into proximity via the interaction between the first and second heterodimerization domains. After antigen recognition, receptors cluster, native CD45 and CD148 are excluded from the synapse and a signal is transmitted to the cell.

As such, the signalling domain of the signalling component is analogous to the endodomain of a classical CAR molecule.

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 (illustrated in Figure 1B).

The signalling component described herein comprises a signalling domain; it 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 (Figure 1).

The signalling component 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 signalling component of a CAR system according to the present invention may comprise the sequence shown as SEQ ID NO: 13 to 21 or a variant thereof having at least 80% sequence identity.

SEQ ID NO: 13 - CD3 Z endodomain

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 14 - CD28 and CD3 Zeta endodomains

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID NO: 15 - CD28, OX40 and CD3 Zeta endodomains

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 16 - ICOS endodomain

CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL

SEQ ID No. 17 - CD27 endodomain

QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP

SEQ ID No. 18 - BTLA endodomain

RRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQE
GSEVYSNPCLEENKPGIVYASLNHHSVIGPNSRLARNVKEAPTEYASICVRS

SEQ ID No. 19 - CD30 endodomain

5 HRRACRKIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQP
LMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNKNIEKIYIMKADTVIVGT
VKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPT
AASGK

10 SEQ ID No. 20 - GTR endodomain

QLGLHIWQLRSQCMWPRETQLLLEVPSTEDARSCQFPEEERGERSAEEKGRLGDLWW

SEQ ID No. 21 - HVEM endodomain

CVKRRKPRGDVVKVIVSVQRKRQEAEGEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH

15

A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID NO: 13 to 21 provided that the sequence provides an effective intracellular signalling domain.

20 The intracellular signalling component may comprise a plurality of signalling domains, for example, 2 to 5 signalling domains, which may be the same or different. In particular the signalling component may comprise 2 or 3 copies of the same intracellular signalling domain.

25 MULTIPLE SIGNALLING COMPONENTS

The signalling system according to the first aspect of the present invention may comprise a plurality of signalling components, each comprising a signalling domain and a second heterodimerization domain, wherein each second binding domain is bound by the same first
30 binding domain of the receptor component but the signalling domains comprise different endodomains (Figures 7 and 8). In this way, multiple different endodomains can be activated simultaneously. This is advantageous over a compound signalling domain since each signalling domain remains unencumbered from other signalling domains.

35 If each signalling component comprises a second heterodimerization domain which differs in residues which alter their affinity to the first heterodimerization domain of the receptor component, the signalling components comprising different signalling domains will ligate to

the first binding domain with differing kinetics (Figure 8). This allows greater control over the signalling in response to antigen-binding by the receptor component as different signalling components are recruited to the receptor component in varying kinetics/dynamics. This is advantageous since rather than a fixed equal ratio of signal transmitted by a compound endodomain, an optimal T-cell activation signal may require different proportions of different immunological signals.

NUCLEIC ACID

The present invention further provides a nucleic acid encoding the targeting component of the second aspect and a nucleic acid encoding a signalling component of the third aspect.

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.

NUCLEIC ACID CONSTRUCT

The present invention also provides a nucleic acid construct which encodes both the targeting component and the signalling component.

- 5 The nucleic acid construct may produce a polypeptide which comprises the targeting component and the signalling component joined by a cleavage site. The cleavage site may be self-cleaving, such that when the polypeptide is produced, it is immediately cleaved into the receptor component and the signalling component without the need for any external cleavage activity.

10

For example, the present invention provides a nucleic acid construct encoding a CAR signalling system according to the first aspect of the invention, wherein the nucleic acid sequence comprises the following structure:

A - X - B

- 15 in which A and B are nucleic acid sequences encoding a targeting component or a signalling component as defined by the second and third aspects of the invention; and X is a nucleic acid sequence which encodes a cleavage site, such that A is cleaved from B after translation.

- 20 The cleavage site may be any sequence which enables the polypeptide comprising targeting component and the signalling component to become separated.

- The term "cleavage" is used herein for convenience, but the cleavage site may cause the targeting component and the signalling component 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 targeting component and the signalling component, causes the targeting component and the signalling component to be expressed as separate entities.
- 25
- 30

The cleavage site may be a furin cleavage site.

35

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 parathyroid hormone, transforming growth factor beta 1 precursor, proalbumin, pro-beta-secretase, membrane type-1 matrix metalloproteinase, beta subunit of pro-nerve growth factor and von Willebrand factor. Furin cleaves proteins just downstream of a basic amino acid target sequence (canonically, Arg-X-(Arg/Lys)-Arg') and is enriched in the Golgi 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 be 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 targeting component and the signalling component and the self-cleaving peptide is produced, it is immediately "cleaved" or separated into distinct and discrete first and second polypeptides without the need for any external cleavage activity.

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

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

YHADYYKQRLIHDVEMNPGP (SEQ ID No. 22)

HYAGYFADLLIHDINETNPGP (SEQ ID No. 23)

QCTNYALLKLAGDVESNPGP (SEQ ID No. 24)

5 ATNFSLLKQAGDVEENPGP (SEQ ID No. 25)

AARQMLLLLSGDVETNPGP (SEQ ID No. 26)

RAEGRGSLTCTGDVEENPGP (SEQ ID No. 27)

TRAEIEDELIRAGIESNPGP (SEQ ID No. 28)

TRAEIEDELIRADIESNPGP (SEQ ID No. 29)

10 AKFQIDKILISGDVELNPGP (SEQ ID No. 30)

SSIIRTKMLVSGDVEENPGP (SEQ ID No. 31)

CDAQRQKLLLSGDIEQNPGP (SEQ ID No. 32)

YPIDFGGFLVKADSEFNPGP (SEQ ID No. 33)

15 The cleavage site may comprise the 2A-like sequence shown as SEQ ID No. 27 (RAEGRGSLTCTGDVEENPGP).

The present invention also provides a kit comprising a nucleic acid encoding the targeting component of the second aspect of the invention, and/or a nucleic acid encoding a signalling component of the third aspect of the invention.

VECTOR

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

30 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.

35 CELL

The present invention also relates to a cell, such as an immune cell comprising the CAR system according to the first aspect of the invention.

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

5

The cell may comprise a receptor component and a signalling component of the present invention.

10

The cell may comprise at least one targeting component of the present invention. For example the cell may comprise one, two, three, four, five, up to a plurality of targeting components of the present invention.

15

The cell may comprise at least one receptor component of the present invention. For example the cell may comprise one, two, three, four, five, up to a plurality of receptor components of the present invention.

20

T cell may be T cells or T lymphocytes which 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.

25

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.

30

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.

35

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 CAR cells of the invention may be any of the cell types mentioned above.

T or NK cells expressing the molecules of the CAR system 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 expressing the molecules of the CAR system 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, CAR cells are generated by introducing DNA or RNA coding for the targeting component and signalling component by one of many means including transduction with a viral vector, transfection with DNA or RNA.

The CAR 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 CAR system 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 the targeting component and/or signalling component of the CAR system according to the second and third aspects of the invention.

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

The present invention also provides a kit which comprises a T or NK cell comprising the CAR system according to the first aspect of the invention.

PHARMACEUTICAL COMPOSITION

The present invention also relates to a pharmaceutical composition containing a plurality of cells expressing the components of the CAR system of the first aspect of 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 CAR cell of the present invention for use in treating and/or preventing a disease.

5 The invention also relates to the use of a CAR 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 an infection, such as a viral infection.

10 The methods of the invention may also be for the control of pathogenic immune responses, for example in autoimmune diseases, allergies and graft-vs-host rejection.

The methods may be for the treatment of a cancerous disease, such as bladder cancer, breast cancer, colon cancer, endometrial cancer, kidney cancer (renal cell), leukaemia, lung
15 cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer and thyroid cancer.

The CAR cells of the present invention may be capable of killing target cells, such as cancer cells. The target cell may be recognisable by expression of a TAA, for example the
20 expression of a TAA provided above in Table 1.

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

25 The CAR 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
30 way to limit the scope of the invention.

EXAMPLES

Example 1 – Production of zipCARs

35

Two different zipCARs where constructed (see Figure 3).

Leucine zipper zipCAR

One zipCAR was constructed such that the targeting component recognized CD33 through an anti-CD33 scFv, and had an endodomain which comprised the Leucine zipper base, P1. This endodomain was linked to the transmembrane domain via a short intracellular linker sequence derived from CD4.

This targeting component was co-expressed with a signalling domain which comprised the leucine zipper acid P1 as a heterodimerization domain fused via a short serine-glycine linker to a signalling domain comprising the CD3-Zeta endodomain.

Both components were co-expressed in the same retroviral vector using the self-cleaving FMD-2A peptide (see Figure 4).

DDD1/AD1 zipCAR

The DDD1/AD1 zipCAR was constructed in an analogous fashion to the leucine zipper zipCAR. The endodomain of the targeting component comprised DDD1 and the signalling component was a fusion between AD1 and the CD3-Zeta endodomain (see Figure 4).

For each zipCAR, the targeting component and signalling component are independent of each other following initial translation and self-cleavage by the FMD-2A peptide. The targeting component is diverted to the cell surface via its signal peptide and anchored in the membrane via its trans-membrane domain, whilst the signalling component remains in solution in the cytosol. The two components then associate via their respective heterodimerization domains.

Control constructs were generated for each zipCAR system. These were identical to the corresponding zipCAR constructs except that the heterodimerization domains of the signalling components were deleted (see Figure 4). Hence, no intracellular association could occur.

The amino acid sequence of each of the described constructs is shown in Figure 11.

The murine T-cell line BW5 was transduced with each zipCAR and respective control constructs. Expression of the CAR targeting domain could be detected by staining the T-cells with recombinant CD33-Fc fusion, staining with an appropriate secondary antibody and analysing the T-cells by flow cytometry (see Figures 5 and 6).

The T-cells were then challenged with beads coated with the cognate antigen (CD33) at different ratios of beads to T-cells. IL-2 release was measured after this antigen challenge. ZipCARs responded to the antigen while control constructs did not (see Figures 5 and 6).

5 Example 2 – Production of superCAR ZipCARs

A DDD1/AD1 ZipCAR was constructed, in which the targeting component comprised an scFv against CD19, an IgGFc spacer, and eight heterodimerisation domains (Figure 12). The targeting component was tested in combination with various different signalling
10 components having 0, 1, 2 or 3 copies of the TCR zeta signalling domain. As DD1 binds AD1 in a 2:1 stoichiometry, these signalling domains give 0, 16, 32 and 48 copies of the TCR zeta domain respectively for each 8-heterodimerization-domain targeting component. As a control, an equivalent classical non-ZipCAR format CAR was constructed, having two copies of TCR zeta (Figure 12: IgG_Zeta).

15 The murine T-cell line BW5 was transduced with each zipCAR and the control construct and challenged with SupT1 cells expressing the cognate antigen (CD19) at different concentrations: low, mid and high. These SupT1 cells were engineered to express CD19 at different levels by the use of suboptimal signal peptides and/or the introduction of
20 cytoplasmic retention motifs derived from Tyrp-1 (inserted proximal to the membrane) or glycoprotein E3-19k from adenovirus (inserted on the C-terminus). IL-2 release was measured after antigen challenge.

The results are shown in Figure 13. It was found that superCAR ZipCARs comprising 16 or
25 32 copies of TCR zeta per targeting component (IgGx4AD1+DDD1-Z and IgGx4AD1+Z-DDD1-Z respectively) gave a greater response to antigen than the equivalent classical CAR comprising two copies of TCR zeta per molecule.

All publications mentioned in the above specification are herein incorporated by reference.
30 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
35 for carrying out the invention which are obvious to those skilled in molecular biology, cell biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A chimeric antigen-receptor (CAR) signalling system comprising;
(i) a targeting component comprising an antigen-binding domain, a
transmembrane domain and a first heterodimerization domain; and
(ii) an intracellular signalling component comprising a signalling domain and a
second heterodimerization domain;

wherein spontaneous heterodimerization between the first and second heterodimerization domains causes the targeting component and signalling component to form a functional CAR complex.

2. A CAR signalling system according to claim 1 wherein the first and second heterodimerization domains comprise leucine zipper domains.

3. A CAR signalling system according to claim 1 wherein the first and second heterodimerization domains comprise DDD1 and AD1 domains.

4. A CAR signalling system according to claim 1 wherein the first and second heterodimerization domains comprise Barnase and Barnstar domains.

5. A CAR signalling system according to claim 1 wherein the first and second heterodimerization domains comprise human pancreatic RNase and S-peptide domains.

6. A CAR signalling system according to any preceding claim, which comprises multiple targeting components, each recognizing a different antigen.

7. A CAR signalling system according to claim 6, wherein the first heterodimerization domains of the multiple targeting components differ in affinity for the second heterodimerization domain such that each antigen propagates different signalling strengths.

8. A CAR signalling system according to any preceding claim, wherein the signalling domain of the signalling component comprises a single endodomain selected from CD3 zeta endodomain, CD28 endodomain, 41BB endodomain, OX40 endodomain, an ICOS endodomain, a CD27 endodomain, a BTLA endodomain, a CD30 endodomain, a GITR endodomain and an HVEM endodomain.

9. A CAR signalling system according to any preceding claim, wherein the signalling domain of the signalling component comprises at least one of CD3 zeta endodomain, CD28 endodomain, 41BB endodomain and OX40 endodomain.

10. A CAR signalling system according to any preceding claim which comprises a plurality of signalling components, each comprising a signalling domain and a second heterodimerization domain, wherein the second heterodimerization domains each recognise the same first heterodimerization domain but the signalling domains comprise different signalling endodomains.

11. A CAR signalling system according to any preceding claim, which comprises a plurality of signalling components which comprise a plurality of second heterodimerization domains, each of which independently recognise the first heterodimerization domains with different affinities.

12. A CAR signalling system according to any preceding claim, wherein the targeting component comprises more than two heterodimerisation domains, such that a single targeting component is capable of heterodimerising with more than two signalling components.

13. A CAR signalling system according to claim 12, wherein each signalling component comprises a plurality of signalling domains.

14. A targeting component suitable for use in the CAR signalling system according to any of claims 1 to 13 which comprises an antigen-binding domain, a transmembrane domain and a heterodimerization domain.

15. A targeting component according to claim 14 which comprises more than two heterodimerisation domains.

16. A signalling component suitable for use in the CAR signalling system according to any of claims 1 to 13 which comprises a signalling endodomain and a heterodimerization domain.

17. A signalling component according to claim 16, which comprises a plurality of signalling endodomains.

18. A nucleic acid encoding a targeting component according to claim 14 or 15.

19. A nucleic acid encoding a signalling component according to claim 16 or 17.

5 20. A nucleic acid construct encoding a CAR signalling system according to any of claims 1 to 3 wherein the nucleic acid construct comprises the following structure

$$A - X - B$$

in which

A is a nucleic acid sequences encoding a targeting component as defined in claim 14 or 15;

10 and B is a nucleic acid sequence encoding a signalling component as defined claim 16 or 17; and

X is a nucleic acid sequence which encodes a cleavage site, such that A is cleaved from B after translation.

15 21. A nucleic acid construct according to claim 20, which has one of the following structures:

a) AbD – spacer –TM - endo – Het1– coexpr – Het2 – SD

20 b) AbD – spacer –TM - endo – Het1– coexpr – SD - Het2

c) Het2 – SD - coexpr – AbD – spacer –TM - endo – Het1 or

d) SD - Het2- coexpr – AbD – spacer –TM - endo – Het1

25

in which

AbD is a nucleic acid sequence encoding the antigen-binding domain of the targeting molecule;

spacer is a nucleic acid sequence encoding a spacer of the targeting molecule;

30 TM is a nucleic acid sequence encoding the transmembrane domain of the targeting molecule;

endo is a nucleic acid sequence encoding the endodomain of the targeting molecule;

Het1 is a nucleic acid sequence encoding the heterodimerization motif of the targeting molecule;

35 coexpr is a nucleic acid sequence encoding a cleavage site;

Het2 is a nucleic acid sequence encoding the heterodimerization motif of the signalling molecule; and

SD is nucleic acid sequence encoding the intracellular signalling domain of the signalling molecule.

22. A nucleic acid construct according to claim 20 which encodes multiple CAR signalling system targeting components according to claim 6 and/or multiple CAR signalling system signalling components according to claim 10, wherein the multiple targeting components or multiple signalling components are separated by cleavage sites, such that the multiple targeting components or multiple signalling components are cleaved after translation.

23. A vector comprising a nucleic acid according to claim 18 or 19 or a nucleic acid construct according to any of claims 20 to 22.

24. A vector according to claim 23 which is a retroviral vector or a lentiviral vector or a transposon.

25. A cell which comprises a CAR signalling system according to any of claims 1 to 11.

26. A cell which expresses at least one targeting component according to claim 14 or 15 and at least one signalling component according to claim 16 or 17.

27. A cell according to claim 26 which comprises a nucleic acid according to claim 18 or 19 or a nucleic acid construct according to any of claims 20 to 22 or a vector according to claim 23 or 24.

28. A cell according to any one claims 25 to 27 which is a T cell or NK cell.

29. A pharmaceutical composition comprising a plurality of cells according to any of claims 25 to 28.

30. A pharmaceutical composition according to claim 29 for use in treating and/or preventing a disease.

31. A method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to claim 29 to a subject.

32. A method according to claim 31, which comprises the following steps:

(i) isolation of a T cell or NK cell containing sample from a subject;

- (ii) transduction or transfection of the T cells or NK cells with a nucleic acid construct according to any of claims 20 to 22 or a vector according to claim 23 or 24; and
- (iii) administering the T cells or NK cells from (ii) to the subject.

5 33. The use of a pharmaceutical composition according to claim 29 in the manufacture of a medicament for the treatment and/or prevention of a disease.

34. A pharmaceutical composition for use according to claim 30 or a method according to claim 31 or 32 or a use according to claim 33, wherein the disease is cancer.

10

35. A kit which comprises a nucleic acid according to claim 18 or 19, a nucleic acid construct according to any of claims 20 to 22 or a vector according to claim 23 or 24.

36. A kit which comprises a cell according to any of claims 25 to 28.

15

37. A method for making a cell according to any of claims 25 to 28, which comprises the step of introducing: a nucleic acid construct according to any of claims 20 to 22 or a vector according to claim 23 or 24, into a cell.

20 38. A method according to claim 37, wherein the cell is from a sample isolated from a subject.

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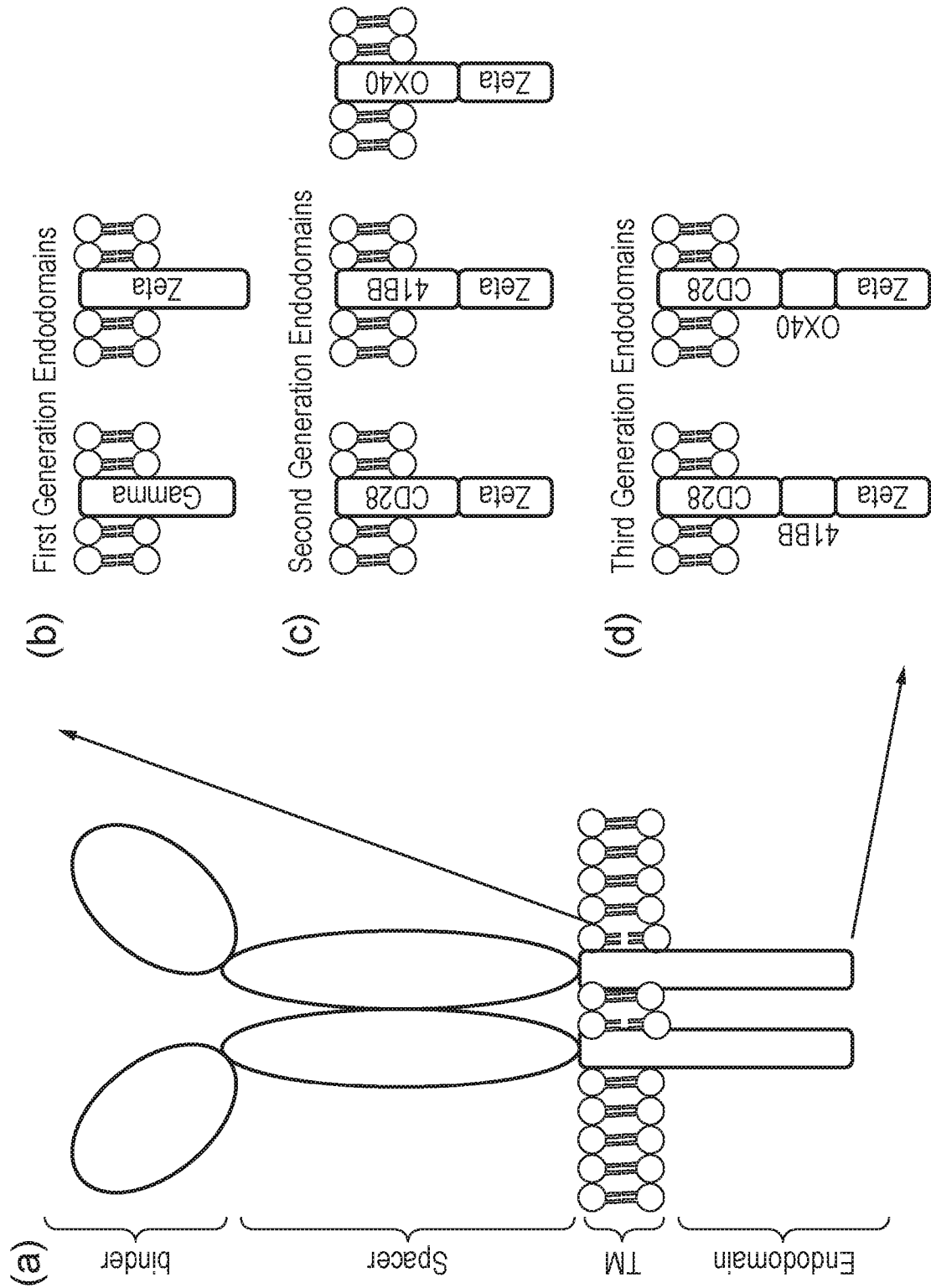


FIG. 1

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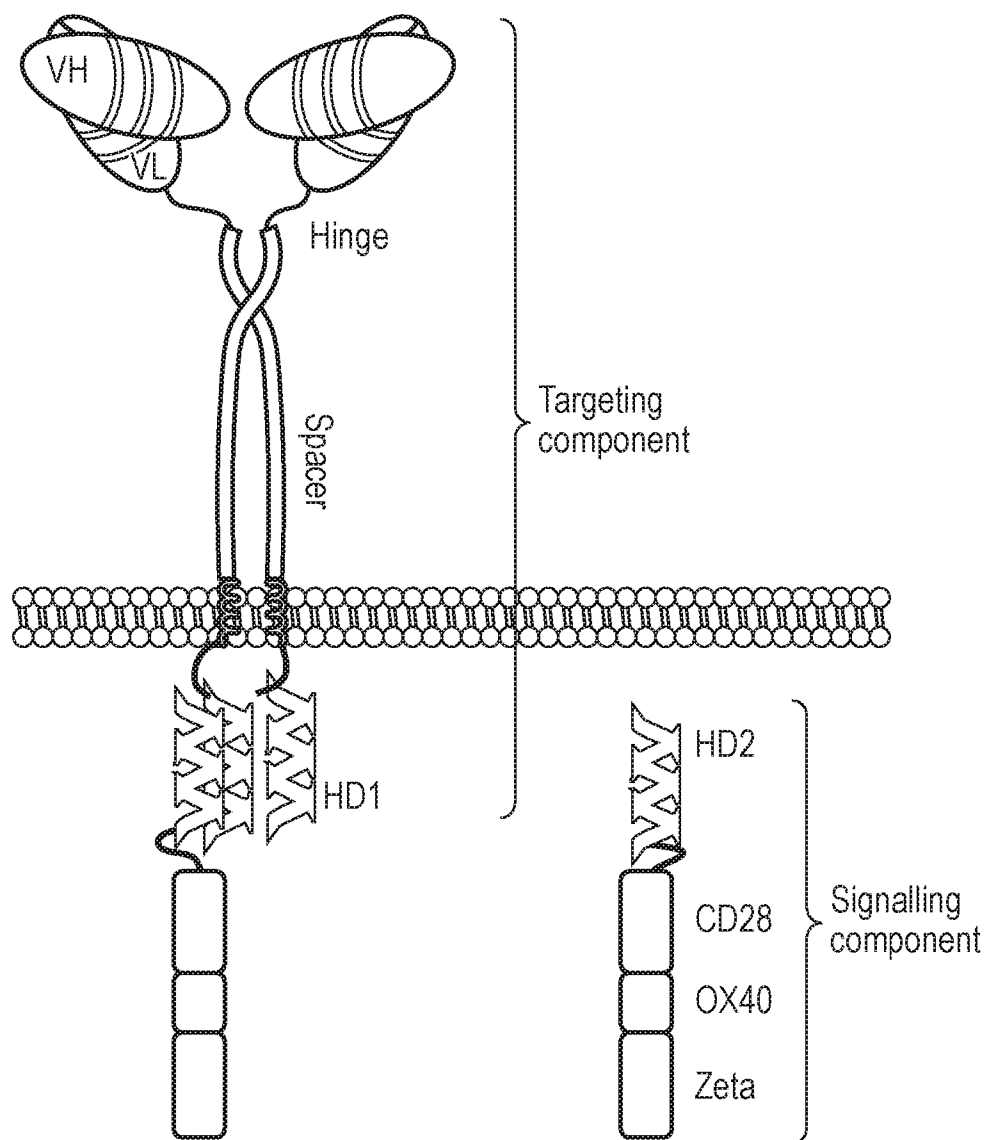
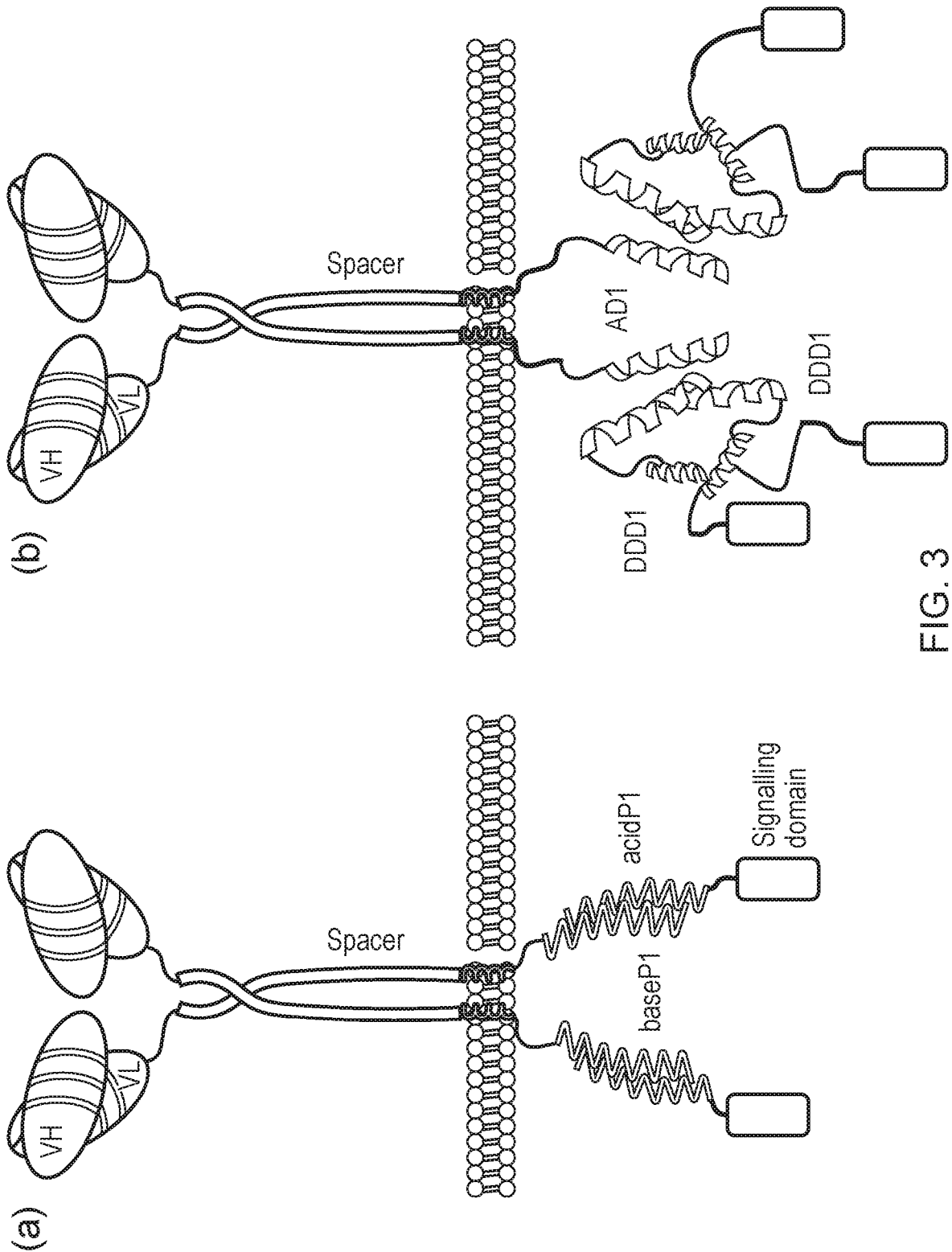


FIG. 2

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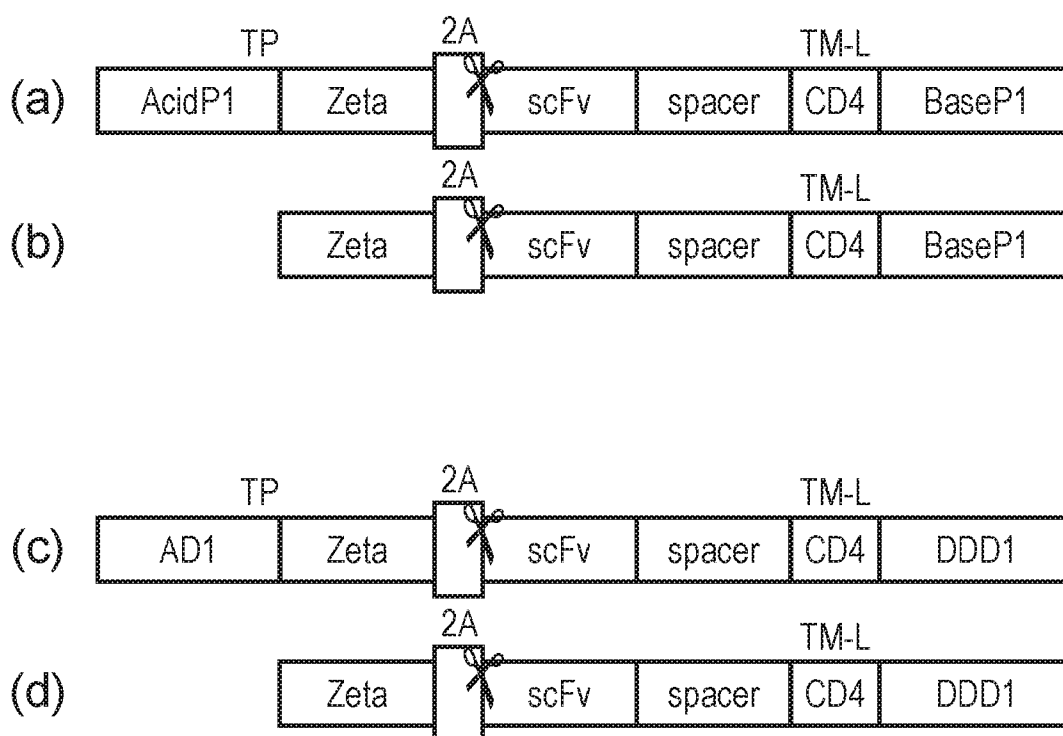
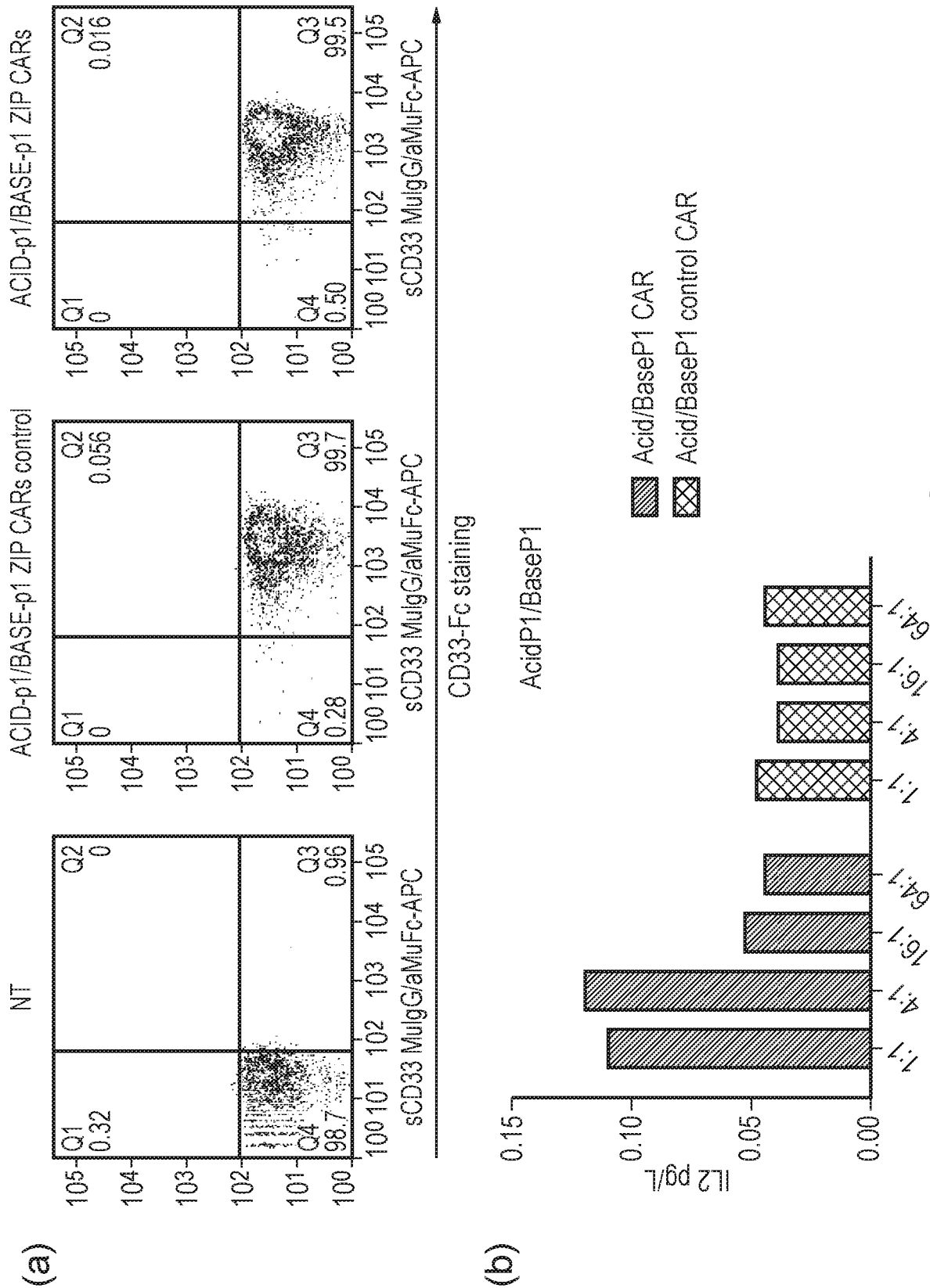


FIG. 4



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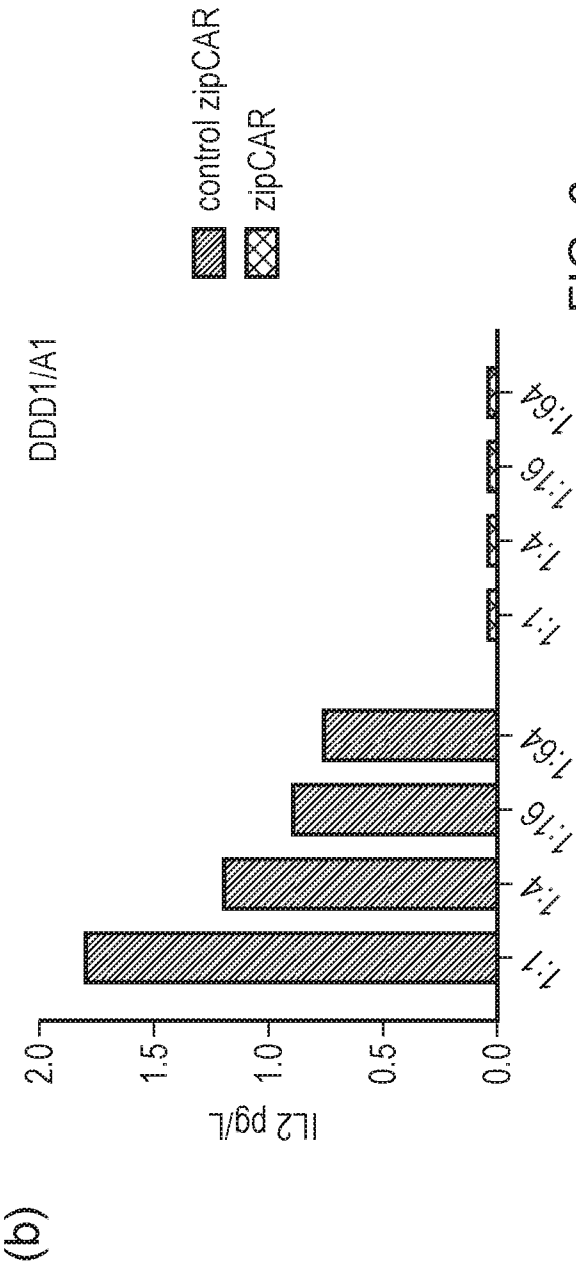
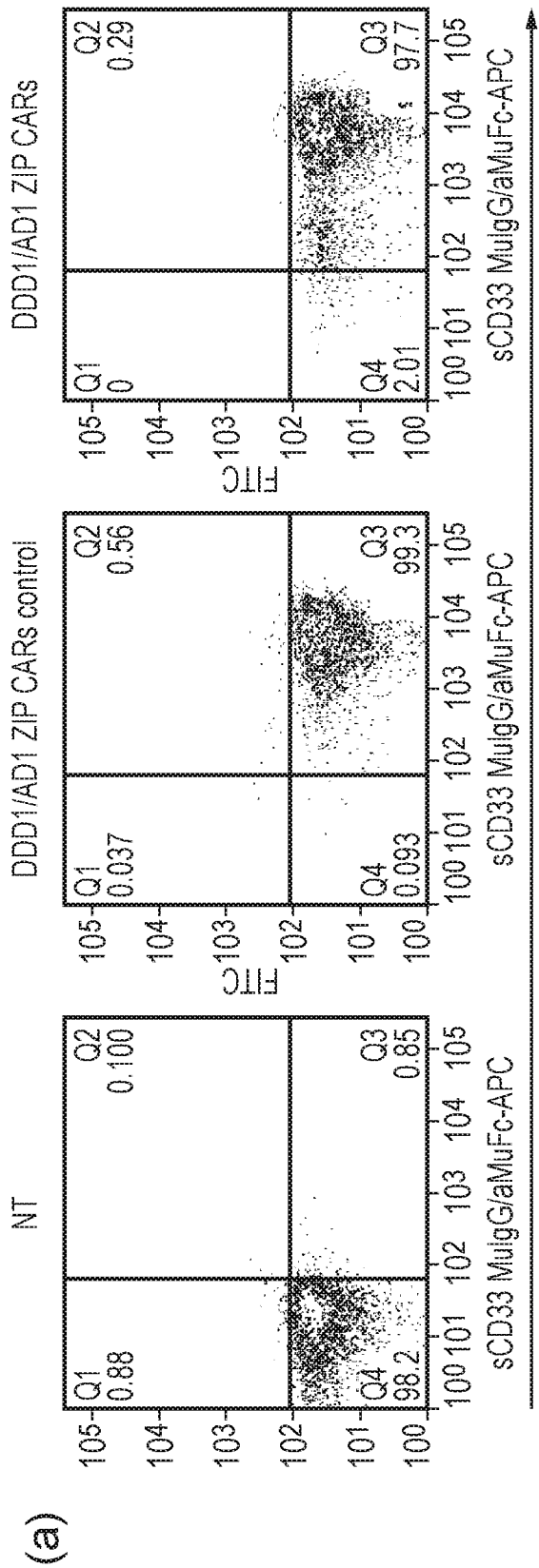


FIG. 6

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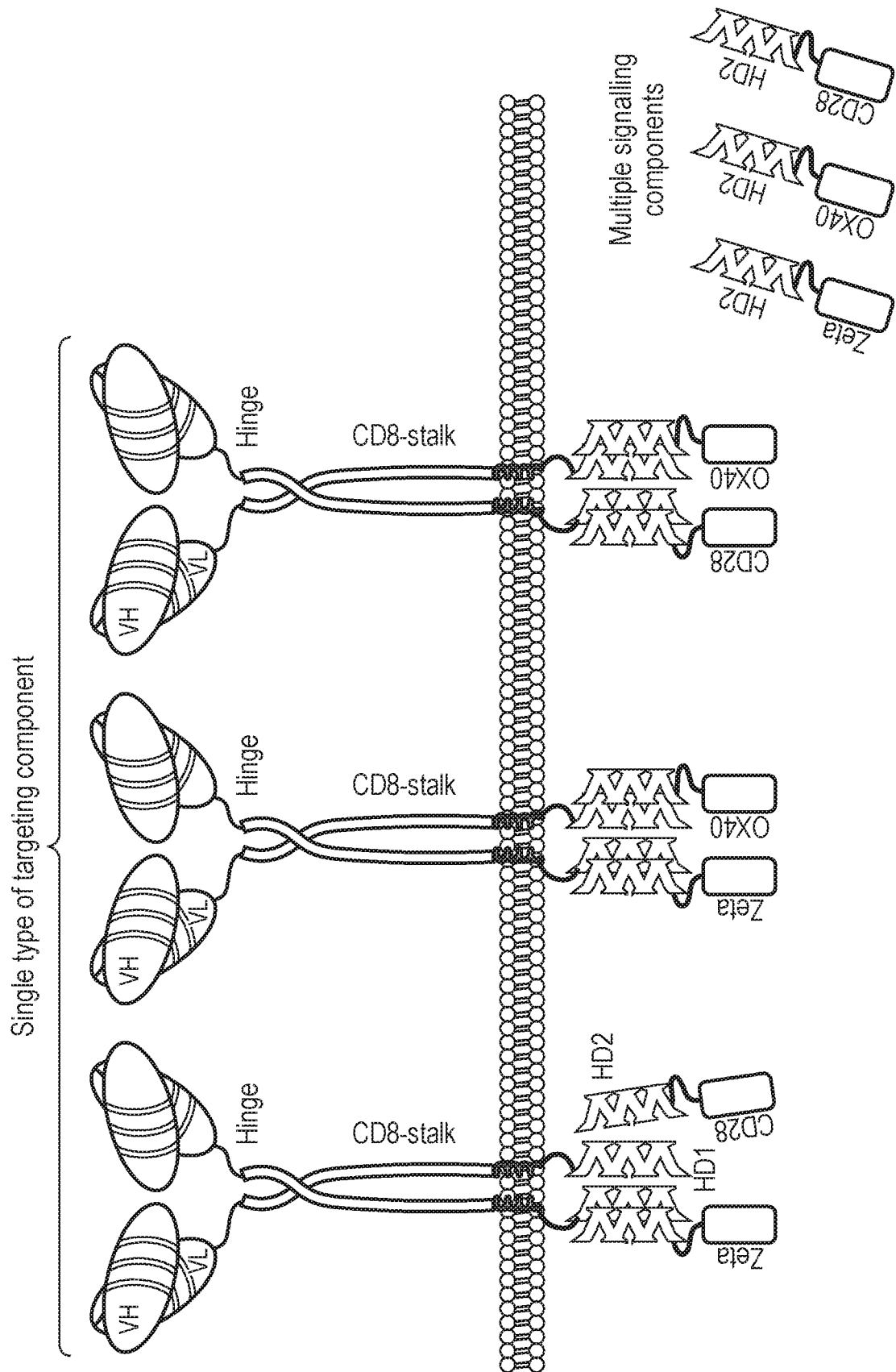


FIG. 7

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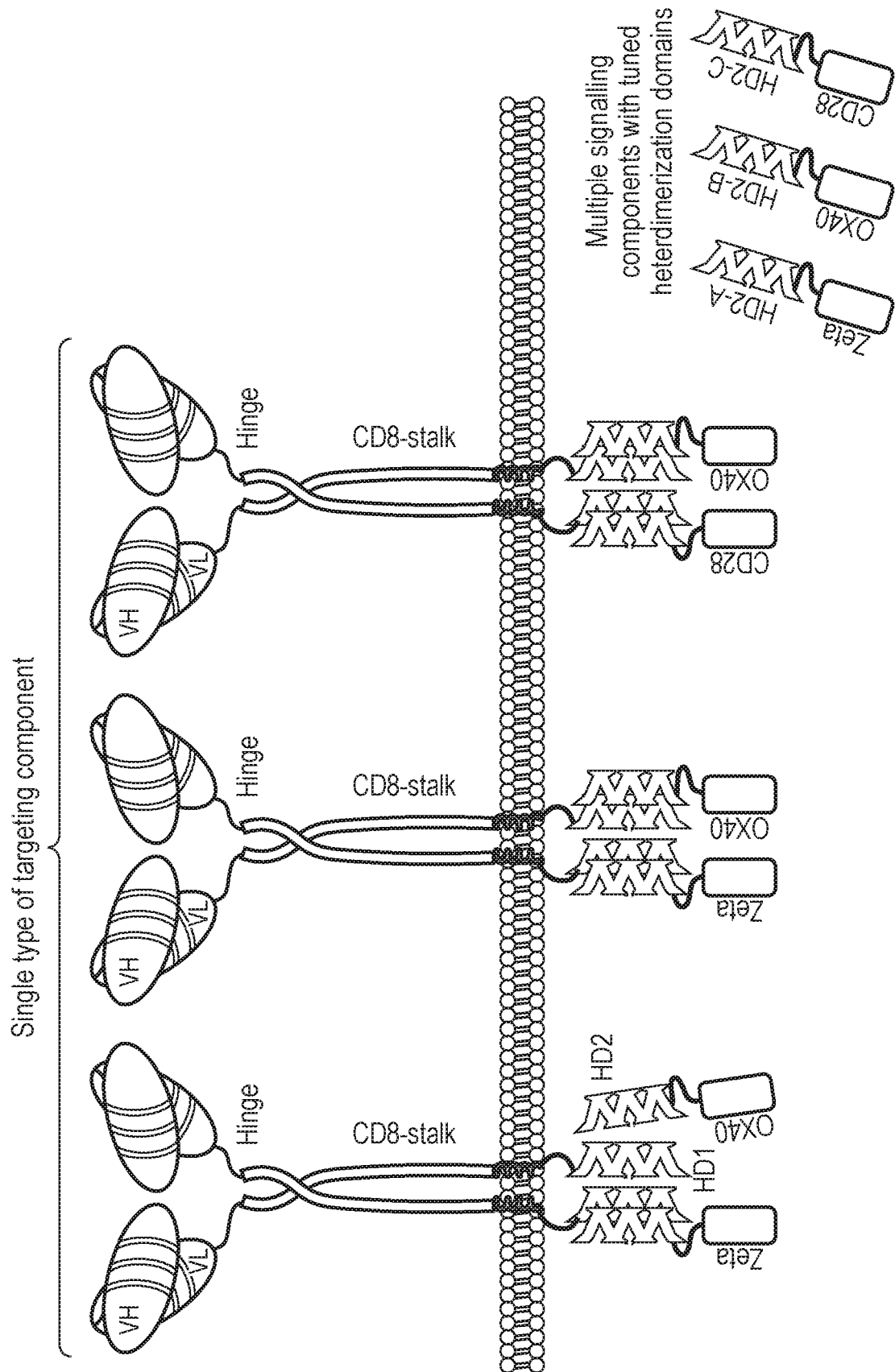


FIG. 8

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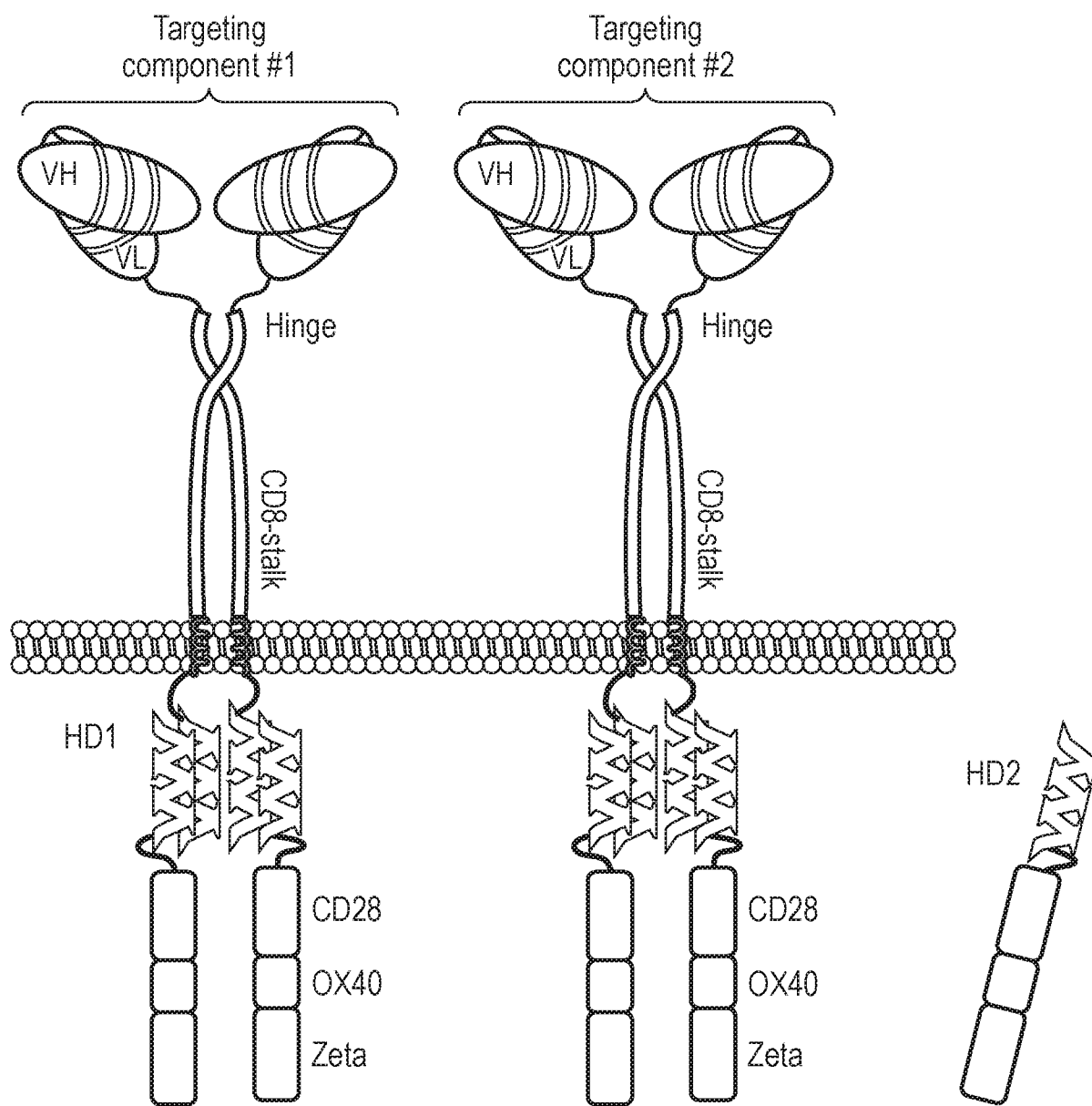


FIG. 9

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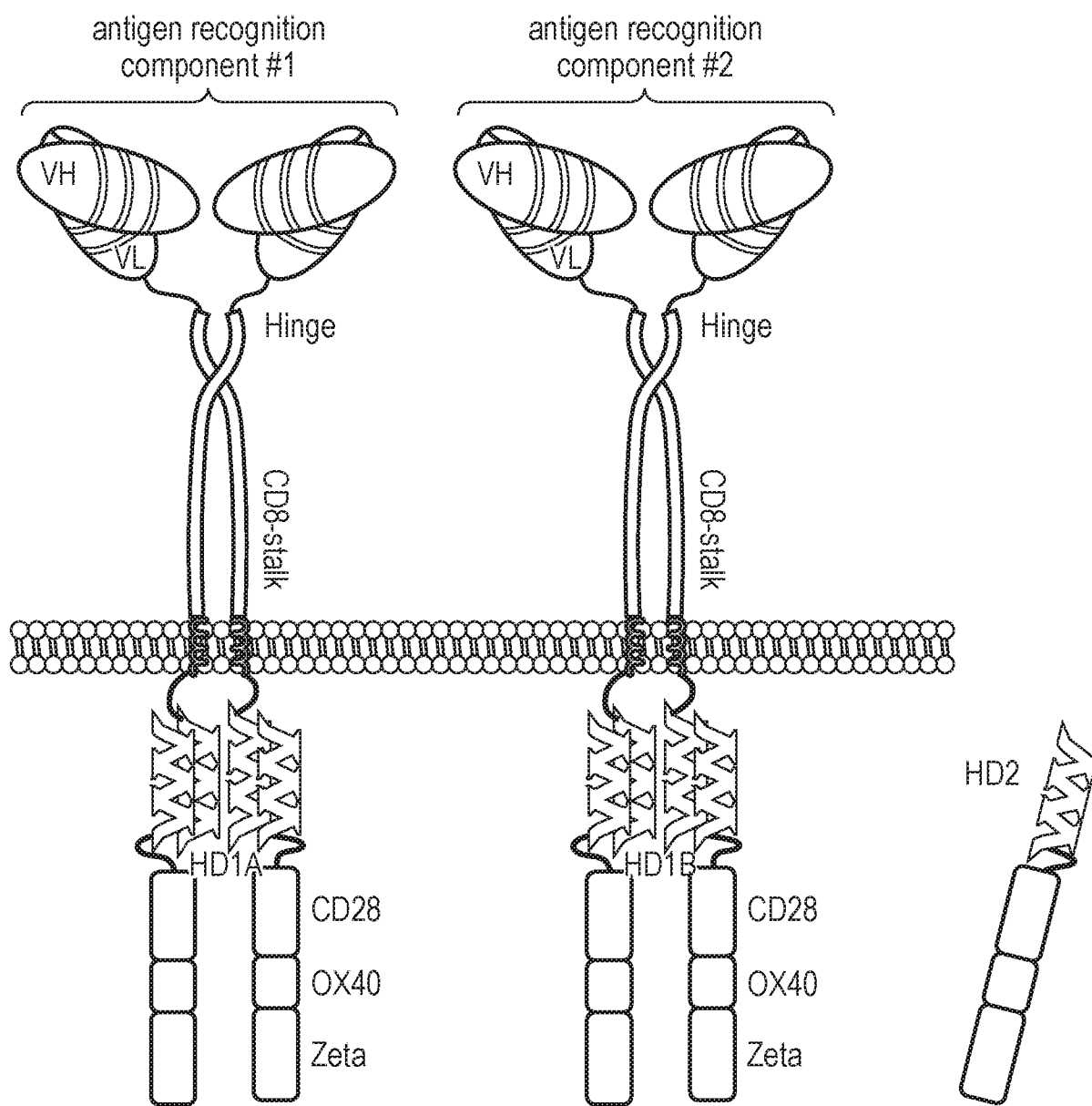


FIG. 10

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>Acid/Base ZipCAR (SEQ ID NO: 34)

AQLEKELQALEKENAQLEWELQALEKELAQSGGGGSADAPAYQQGQNQLYNELNLRREYDVLDKRRGRDPENGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPRAEGRGSLITCGDVEENPGPMAPTQVLGLL
LLWLTARCDIQMTQSPSSLASVGDVITITCRASEIYFNLVWYQQKPGKAPKLLIYDNRLLADGVPSRFSGSGGTQYTLTISS
LQPEDEATYQCQHYKNYPLTFGQGTKLEIKRSGGGSGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWI
RQAPGKGLEWSSISLNGGSTYYRDSVKGRFTISRDNASTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGGTTLTVSSMPC
PAPPVAGPSVFLFPPKPKDTLMIAARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKMALIVLGGVAGLLLFIGLGIFFMALIVLGGVAGLLL
FIGLGIFFCVRCHRRRRSGGSAQLEKQLQALKKNAQLKWKLQALKKKLAQ

>Acid/Base ZipCAR Control (SEQ ID NO: 35)

ADAPAYQQGQNQLYNELNLRREYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKHDGLYQGL
STATKDTYDALHMQALPPRAEGRGSLITCGDVEENPGPMAPTQVLGLLWLTARCDIQMTQSPSSLASVGDVITITCRASE
DIYFNLVWYQQKPGKAPKLLIYDNRLLADGVPSRFSGSGGTQYTLTISSIQPEDFATYQCQHYKNYPLTFGQGTKLEIKRSGGGG
SGGGSGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISR
NAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGGTTLTVSSMPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKPCPAPPVAGPSV
FLFPPKPKDTLMIAARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKMALIVLGGVAGLLLFIGLGIFFMALIVLGGVAGLLLFIGLGIFFCVRCHRRRRSGGSAQLEKQLQALKKK
NAQLKWKLQALKKKLAQ

FIG. 11

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>DDD1/AD1 ZipCAR (SEQ ID NO: 36)

VQIEYLAKQIVDNAIQQASGGGSADAPAYQQQNQLYNELNLRREYDVLKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAE
 AYSEIGMKGERRRGKHDGLYQGLSTATKDTYDALHMQALPPRAEGRGSLTTCGDVEENPGPMVPTQVLGLLLWLTDCRCDIQ
 MTQSPSSLSASVGDRTITCRASEDIYFNLVWYQKPGKAPKLLIYDTNRLADGVPSRFSGSGGTQYTLTISLQPEDFATYCYCQ
 HYKNYPLTFGQGTKEIKRSGGGSGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVS
 SISLNGGSTYYRDSVKGRFTISRDNASTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGGTLVTVSSMPCPAPPVAGPSVFL
 FPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKMALIVLGGVAGLLLFIFGLGIFFMALIVLGGVAGLLLFIFGLGIFFCVRC
 RHRRRSGGGSSHIQIPPGLTELLQGYTVEVLRQQPPDLVEFAVEYFTRLREARA

>DDD1/AD1 ZipCAR control (SEQ ID NO: 37)

ADAPAYQQQNQLYNELNLRREYDVLKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKHDGLYQGL
 STATKDTYDALHMQALPPRAEGRGSLTTCGDVEENPGPMVPTQVLGLLLWLTDCRCDIQMTQSPSSLSASVGDRTITCRASE
 DIYFNLVWYQKPGKAPKLLIYDTNRLADGVPSRFSGSGGTQYTLTISLQPEDFATYCYCQHYKNYPLTFGQGTKEIKRSGGGG
 SGGSGGGSGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEWVSISLNGGSTYYRDSVKGRFTISRD
 NAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGGTLVTVSSMPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV
 SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGCPAPPVAGPSV
 FLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTVLHQDWLNGKEYKCKVSNK
 ALPAPIEKTISKAKMALIVLGGVAGLLLFIFGLGIFFMALIVLGGVAGLLLFIFGLGIFFCVCRHRRRSGGGSSHIQIPPGLTELL
 QGYTVEVLRQQPPDLVEFAVEYFTRLREARA

FIG. 11 (Continued)

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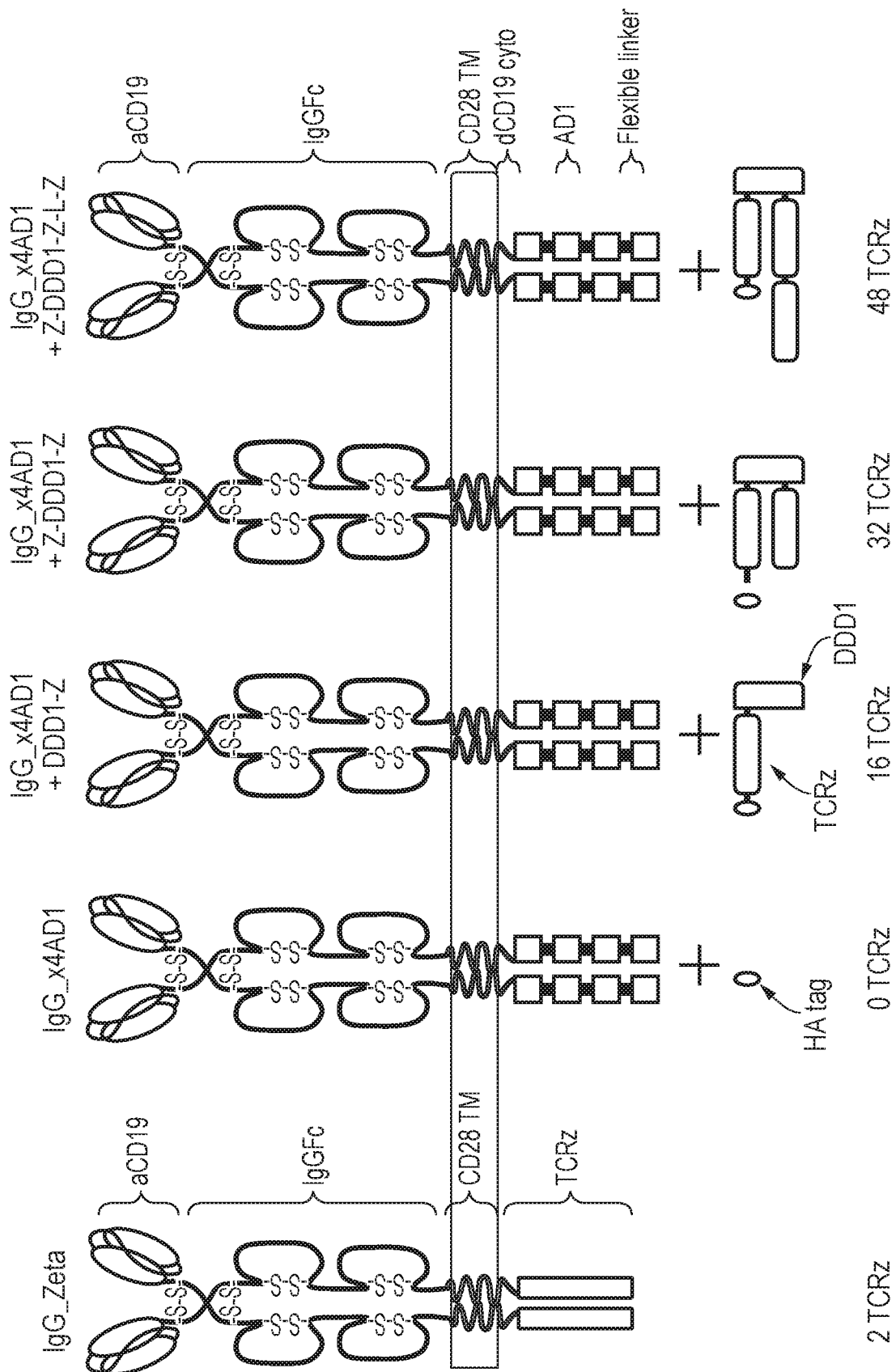


FIG. 12

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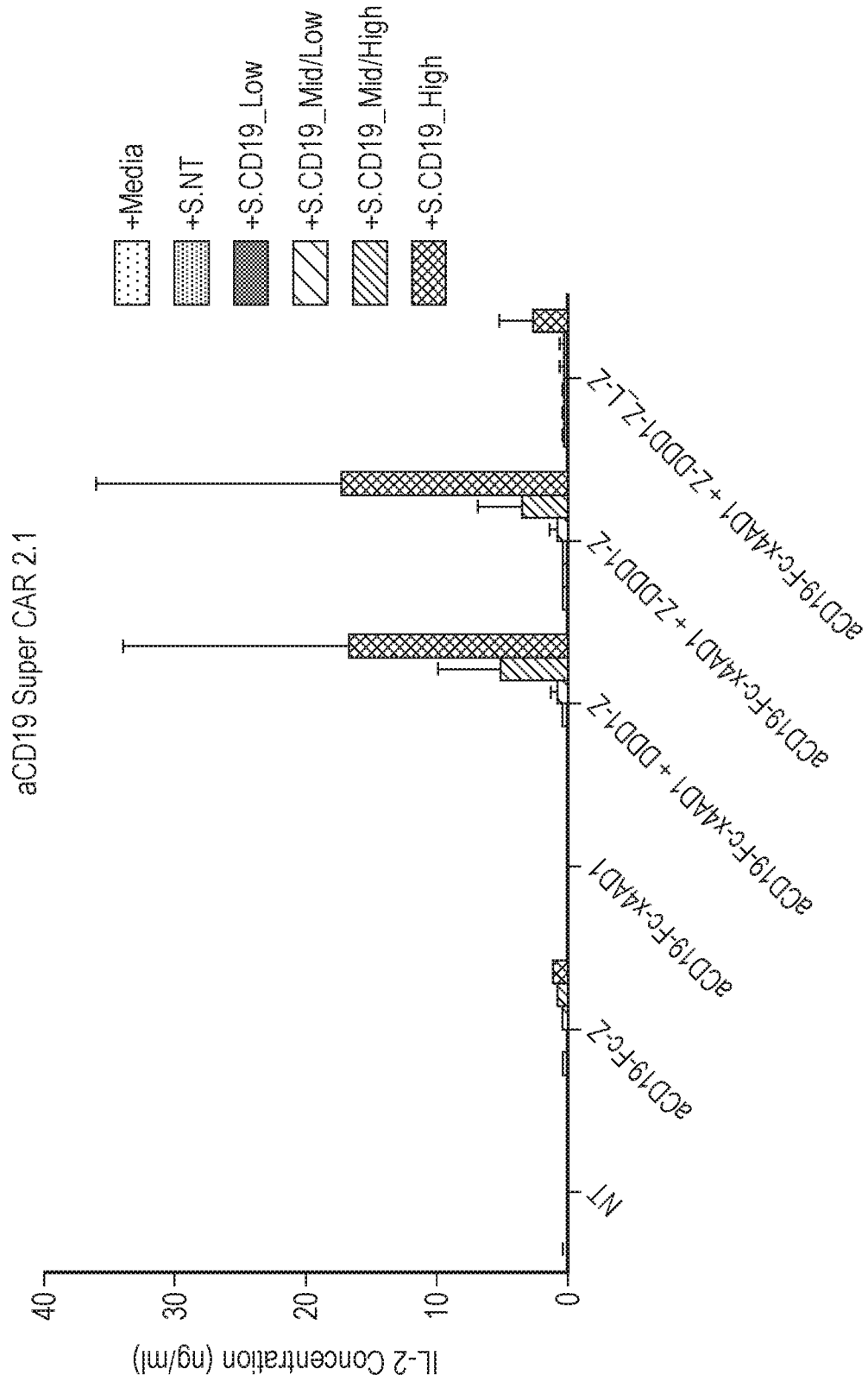


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/050257

A. CLASSIFICATION OF SUBJECT MATTER INV. C07K14/705 C12N5/16 A61K35/17 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 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	WO 2014/127261 A1 (UNIV CALIFORNIA [US]) 21 August 2014 (2014-08-21) abstract paragraphs [0005], [0060] - [0066], [0071], [0101], [0106] - [0113], [0161] - [0183] figures 12-21 ----- -/--	1,6-38
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "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 4 May 2016		Date of mailing of the international search report 13/05/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Chavanne, Franz

INTERNATIONAL SEARCH REPORT

International application No
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E	WO 2016/030691 A1 (UCL BUSINESS PLC [GB]) 3 March 2016 (2016-03-03) abstract page 5, line 22 - page 9, line 20 page 10, line 34 - page 11, line 9 page 19, line 15 - line 26 page 24, line 7 - line 20 figures 1,3,5,6,8-11 -----	1,6-38

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Information on patent family members

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