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Description**FIELD OF THE INVENTION**

5 **[0001]** The present invention relates to a nucleic acid encoding an antigen receptor signalling system.

BACKGROUND TO THE INVENTION

10 **[0002]** 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 bulk population of peripheral blood T-cells.

15 **[0003]** 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 (see Figure 1A).

20 **[0004]** 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 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 treatment of various cancers.

25 **[0005]** 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-target off-tumour" toxicity i.e. recognition of the target antigen on normal tissues.

30 **[0006]** 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.

35 **[0007]** 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.

40 **[0008]** 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.

[0009] 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.

45 **[0010]** 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.

50 **[0011]** There is thus a need for alternative methods for controlling CAR T-cells that are not associated with the disadvantages and problems mentioned above.

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DESCRIPTION OF THE FIGURES

[0012]

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 FcεR1-γ or CD3ζ endodomain, while later designs transmitted additional (c) one or (d) two co-stimulatory signals in the same compound endodomain.

5 **Figure 2** - Structures of Rapamycin analogues.

Figure 3 - Illustration of an example of an inducible CAR signalling system in its simplest incarnation. (a) Receptor component comprising of an extracellular scFv, a spacer, a trans-membrane domain and an intracellular FKBP12. (b) Signalling component: intracellular fusion between FRB fragment of mTOR and the endodomains of CD28, OX40 and CD3 Zeta. (c) A chemical inducer of dimerization (CID), in this case is Rapamycin or an analogue. (d) Receptor component. In the presence of CID, the receptor component and the signalling components dimerize allowing signalling in the presence of cognate antigen.

15 **Figure 4** - Function of an inducible CAR system. T-cells were transduced with a CAR system as illustrated in figure 3 and with SEQ ID No. 19. This CAR recognizes CD19. CAR expressing T-cells were challenged with CD19+ targets at 1:1 and 2:1 effector to target ratios in the presence of increasing concentration of CID. T-cells respond to target cells only in the presence of CID. Stimulation with PMA/Ionomycin is also shown. Response to CD19+ targets of T-cells expressing a standard CD19 CAR (MP15577) to different effector target ratios is also shown.

20 **Figure 5** - Illustration of an inducible CAR system with two receptor components. (a) Two receptor components are co-expressed along with a single signalling component. The two receptor components each have a different single chain which recognizes a different target. The receptor components have endodomains comprising of the FRB fragment of mTOR. A single signalling component comprises of FKBP12 and a fusion of CD28, OX40 and CD3 Zeta endodomains. In (a), the FRB sequences are identical and this CAR will signal equally in response to either target. In (b), the FRB sequences differ such that each has a different affinity for the CID. With this implementation, the CAR will signal with different strength in response to each cognate antigen in the presence of CID.

30 **Figure 6** - Illustration of an example of a multiple transmembrane receptor domain receptor component for use in an inducible CAR signalling system. The receptor component is a chimera of a type I CAR linked to the multi-span protein. A scFv is connected to an Fc spacer domain and a TM domain. This in turn is connected to the CD20 molecule with truncated amino and carboxy termini. Each intracellular entry is attached to an FRB domain. The signalling component comprises of FKBP12 and a fusion of CD28, OX40 and CD3 Zeta endodomains. Addition of the CID allows the stochastic ligation of multiple signalling domains to the multiple endodomains which recognize a single antigen receptor component, resulting in amplified signalling in response to antigen.

35 **Figure 7** - Illustration of an inducible CAR system with multiple signalling component inducible CAR. This CAR system has a single receptor component comprising of a scFv, an Fc spacer, a TM domain and a FKBP12 endodomain. The three different signalling domains comprise of a fusion between the FRB fragment of mTOR and the CD28 endodomain, a fusion between the FRB fragment of mTOR and the 41BB endodomain and a fusion between the FRB fragment of mTOR and the CD3 Zeta endodomain. In (a), the FRB domains are identical so that each signalling component is recruited equally to the receptor component and the CAR transmits an equal CD28, 41BB and CD3 Zeta signal in the presence of CID and upon recognition of the cognate antigen. In (b), the FRB domains are different so that each signalling component can be recruited differently to the receptor component so that the CAR transmits more or less of each of CD28, 41BB and CD3 Zeta signals depending on the optimum needed for the application.

45 **Figure 8** - Illustration of an inducible CAR with signalling component which comprises of two CID binding domains. In this example, there is one receptor component which comprises of a scFv, and Fc spacer, a transmembrane domain and an FKBP12 endodomain. The signalling domain comprises of the FRB domain of mTOR, a fusion of CD28, OX40 and Zeta endodomains and an FKBP12 domain. In the presence of CID, the signalling components multimerize with each other and with the receptor component. Upon antigen recognition, a signal equivalent to the sum of the signalling component multimer is transmitted.

50 **Figure 9** - Chemical structure of RSL1, the synthetic diacylhydrazine (N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine. RSL1 is one of a family of compounds that have been found to act as non-steroidal ecdysone agonists and can function as gene inducers.

Figure 10 - RSL1 inducible CAR (a) an RSL1 inducible CAR was constructed by using retinoic acid receptor domains

and ecodysonic receptor domains to constitute the heterodimerization motifs for the antigen recognition component and the signalling component respectively. The antigen recognition component recognizes CD19. (b) T-cells expressing this CAR along with non-transduced T-cells were stained with anti-Fc which recognizes the CAR spacer. The transduced T-cells expressed the CAR well. (c) Transduced T-cells were next challenged with increasing concentration of RSL1 and challenged with either CD19 negative or CD19 positive target cells. T-cells challenged with CD19 positive targets signal only in the presence of RSL1.

Figure 11 - AP1903 homodimerizer inducible CAR. (a) AP1903 homodimerizer inducible CAR was constructed by using modified FKBP12 domains to constitute the heterodimerization motifs for both the antigen recognition component and the signalling component. The antigen recognition component recognizes CD19. (b) T-cells expressing this CAR along with non-transduced T-cells were stained with anti-Fc which recognizes the CAR spacer. The transduced T-cells expressed the CAR well. (c) Transduced T-cells were next challenged with increasing concentration of CID dimerizer AP1903 and challenged with either CD19 negative or CD19 positive target cells. T-cells challenged with CD19 positive targets signal only in the presence of CID. At high CID concentrations, signalling is reduced likely to saturation of heterodimerization domains with an excess of CID reducing the formation of heterodimers.

SUMMARY OF ASPECTS OF THE INVENTION

[0013] The present inventors have found that it is possible to separate the antigen-recognition and signalling components of a CAR to produce an inducible system, where signalling only takes place in the presence of an agent such as a small molecule which can induce dimerization (henceforth referred to as chemical inducer of dimerization or CID).

[0014] The CID binds two protein domains simultaneously. By incorporating a CID binding domain into each of two non-functional halves of a CAR, the presence of CID will reunite the halves constituting a functional whole.

[0015] Functional signalling by the CAR is hence dependent on the presence of the CID. This provides a mechanism for controlling the CAR T-cell after it has been administered to a patient: The CAR T-cell can be remotely switched on by administering the CID to the patient, and in case of toxicity switched off by not administering further CID. If activity of the CAR is proportional to local concentration of the agent (for instance when CID concentration is not saturating) activity of the CAR T-cells can be "tuned" by altering CID dose administered to the patient.

[0016] Described herein is a chimeric antigen receptor (CAR) signalling system comprising of two separate protein components:

(i) A membrane spanning **receptor component** comprising an extracellular antigen-binding domain, a transmembrane domain and a first intracellular CID binding domain (referred henceforth as CBD1); and

(ii) An intracellular **signalling component** comprising at a minimum a signalling domain and a second CID binding domain (henceforth referred to as CBD2);

wherein CBD1 and CBD2 are capable of simultaneously and specifically binding to the CID.

[0017] The scope of the present invention is as defined in the appended claims.

[0018] Thus, in a first aspect, the present invention provides a nucleic acid encoding a chimeric antigen receptor (CAR) signalling system which comprises;

(i) a receptor component comprising an extracellular antigen-binding domain, a transmembrane domain and a intracellular first chemical inducer of dimerization (CID) binding domain (CBD1); and

(ii) an intracellular signalling component comprising a signalling domain and a second CID binding domain (CBD2);

wherein the nucleic acid encodes both the receptor component and the signalling component of the CAR signalling system;

wherein CBD1 and CBD2 are capable of simultaneously binding to a CID; and wherein, in the absence of CID, binding of the antigen-binding domain to antigen does not result in signalling through the signalling domain; whilst, in the presence of

CID, the receptor component and the signalling component heterodimerize and binding of the antigen-binding domain to antigen results in signalling through the signalling domain.

[0020] CBD1 and CBD2 of the CAR signalling system described herein may comprise different CID binding domains and the CID may comprise two different binding moieties.

[0021] CBD1 may comprise the rapamycin binding domain of FK-binding protein 12 (FKBP12), CBD2 may comprise the FKBP12-Rapamycin Binding (FRB) domain of mTOR, or visa-versa. In this case, the CID may comprise rapamycin or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimer-

ize.

[0022] CBD1 may comprise the FK506 (Tacrolimus) binding domain of FK-binding protein 12 (FKBP12), CBD2 may comprise the cyclosporin binding domain of cyclophilin A (or visa versa). In this case, the CID may comprise an FK506/cyclosporin fusion protein or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize.

[0023] CBD1 may comprise an oestrogen-binding domain (EBD), CBD2 may comprise a streptavidin binding domain, or visa-versa. In this case, the CID may comprise an estrone/biotin fusion protein or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize.

[0024] CBD1 may comprise a glucocorticoid-binding domain (GBD), CBD2 may comprise a dihydrofolate reductase (DHFR) binding domain, or visa-versa. In this case, the CID may comprise a dexamethasone/methotrexate fusion molecule or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize.

[0025] CBD1 may comprise an O⁶-alkylguanine-DNA alkyltransferase (AGT) binding domain, CBD2 may comprise a dihydrofolate reductase (DHFR) binding domain, or vice-versa. In this case, the CID may comprise an O⁶-benzylguanine derivative/methotrexate fusion molecule or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize. CBD1 may comprise a retinoic acid receptor domain, CBD2 may comprise an ecodynone receptor domain, or vice-versa. In this case, the CID may comprise RSL1 or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize.

[0026] CBD1 and CBD2 of the CAR signalling system described herein may comprise the same CID binding domain and the CID may comprise two identical binding moieties.

[0027] CBD1 and CBD2 of the CAR signalling system described herein may comprise FK506 binding protein (FKBP12) binding domains comprising a F36V mutation and the CID may comprise AP1903 or a derivative thereof which is capable of causing the receptor component and the signalling component to heterodimerize. The CAR may comprise a receptor component which spans the membrane multiple times with each intracellular portion constituting a CBD1. Hence a single receptor component may recruit multiple signalling components hence amplifying signal.

[0028] The CAR may further comprise a set of separate receptor components each with an intracellular CBD1 such that in the presence of CID, signalling components connect to each receptor protein component so that the CAR can signal in response to a multiplicity of cognate antigens.

[0029] The CID binding domains may be altered such that affinity to the CID differs. For instance altering amino acids at Positions 2095, 2098, and 2101 of FRB can alter binding to Rapamycin: KTW has high, KHF intermediate and PLW is low (Bayle et al, Chemistry & Biology 13, 99-107, January 2006).

[0030] The CAR may comprise multiple receptor components such that each one has a CBD1 domain of different affinity to the CID. In this way, in the presence of CID, signalling protein components connect to each receptor protein component differentially and hence the CAR signal strengths differentially in response to each cognate antigen.

[0031] The signalling domain of the signalling protein component may comprise of a single endodomain selected from activating or co-stimulating T-cell signalling receptor such as CD3 zeta, CD28, 41BB or OX40.

[0032] The signalling domain of the signalling protein component may comprise of a multiplicity of endodomains selected from activating and co-stimulating T-cell signalling receptor such as CD3 Zeta, CD28, 41BB and OX40.

[0033] If an inhibitory CAR is desired, the signalling domain of the signalling protein may comprise the endodomain of an inhibitory T-cell signalling receptor or a phosphatase.

[0034] The signalling component may comprise a plurality of signalling components, each comprising a different signalling domain and a similar CBD2, wherein the CID agent binding domains each recognise the same CID, but the signalling domains comprise different endodomains. In this way, the CAR can transmit multiple signals but the signalling components being in *trans* rather than *cis* are sterically unencumbered from each other's signalling transmission molecules unlike a single signalling component containing a multiplicity of endodomains.

[0035] The CBD2 of the multiple signalling domains in the aspect described above may bind to the CID molecule with different affinities such that the relative contribution of each signalling component can be tuned. Hence the CAR can transmit a more complex signal rather than the fixed stoichiometry of a single signalling component containing a multiplicity of endodomains.

[0036] The CAR systems described above may comprise signalling domains which contains a CBD1, single or multiple endodomains as well as CBD2. Hence, in the presence of CID, concatenation of multiple signalling domains together to amplify signals.

[0037] Also described is a receptor component suitable for use in the CAR signalling system described above which comprises an antigen-binding domain, a transmembrane domain and a CID binding domain.

[0038] Described herein is a signalling component suitable for use in the CAR signalling system described above which comprises a signalling domain and a CID binding domain.

[0039] The present invention provides a nucleic acid encoding the receptor component described herein.

[0040] The present invention provides a nucleic acid encoding the signalling component described herein.

[0041] The present invention provides a nucleic acid sequence encoding the receptor component described herein and the signalling component described herein, wherein the expressed molecule is a self-cleaving peptide which is cleaved between the receptor component and the signalling component.

[0042] In a second aspect, the present invention provides a vector comprising a nucleic acid sequence according to the first aspect of the invention.

[0043] In a third aspect, the present invention provides a retroviral vector or a lentiviral vector or a transposon comprising a vector according to the second aspect of the invention.

[0044] Described herein is a T cell or NK cell which expresses a receptor component described above and at least one signalling component described above.

[0045] In a fourth aspect, the present invention provides a T cell or NK cell which comprises the nucleic acid according to the first aspect of the invention or the vector according to the second or third aspects of the invention.

[0046] In a fifth aspect, the present invention provides a pharmaceutical composition comprising a plurality of T cells or NK cells according to the fourth aspect of the invention.

[0047] In a sixth aspect, the present invention provides a pharmaceutical composition according to the fifth aspect of the invention for use in treating and/or preventing a disease.

[0048] The disclosure provides a method for treating and/or preventing a disease, which comprises the step of administering a pharmaceutical composition according to the fifth aspect of the invention to a subject.

[0049] The pharmaceutical composition for use according to the sixth aspect of the invention may comprise the following steps:

- (i) isolation of a T or NK cell-containing sample from a subject;
- (ii) transduction or transfection of the T or NK cells with a nucleic acid sequence according to the first aspect or a vector according to the second or third aspects; and
- (iii) administering the cells from (ii) to the subject.

[0050] The pharmaceutical composition for use according to the sixth aspect of the invention may further comprise the step of administering a CID suitable for use in the CAR signalling system described herein.

[0051] The present disclosure provides a method for treating and/or preventing a disease in a subject which subject comprises T cells according to the fourth aspect of the invention, which method comprises the step of administering a CID suitable for use in the CAR signalling system described above to the subject.

[0052] The pharmaceutical composition for use according to the sixth aspect of the invention may involve monitoring the progression of disease and/or monitoring toxic activity in the subject and adjusting the dose of the CID to provide acceptable levels of disease progression and/or toxic activity.

[0053] The disease to be prevented and/or treated according to the sixth aspect of the invention may be cancer.

[0054] The present disclosure provides the use of a pharmaceutical composition according to the fifth aspect of the invention in the manufacture of a medicament for the treatment and/or prevention of a disease.

[0055] The present disclosure provides a kit which comprises a nucleic acid according to the first aspect of the invention or a vector according to the second or third aspects of the invention.

[0056] The kit may also comprise a CID suitable for use in the CAR signalling system described herein.

[0057] The present disclosure provides a kit which comprises a T cell or NK cell according to the fourth aspect of the invention and a CID suitable for use in the CAR signalling system described herein.

[0058] In a seventh aspect, the present invention provides a method for making a T cell or NK cell according to the fourth aspect of the invention, which comprises the step of introducing: a nucleic acid sequence according to the first aspect of the invention or a vector according to the second or third aspects of the invention, into a T or NK cell.

[0059] The T or NK cell may be from a sample isolated from a subject.

[0060] The present disclosure provides a method for activating the CAR signalling system described herein in a subject which comprises a T-cell according to the fourth aspect of the invention, which method comprises the step of administering the CID to the subject.

[0061] The present disclosure provides a method for reducing the activity of the CAR signalling system in a subject which comprises a T-cell or NK cell according to the fourth aspect of the invention, which method comprises reducing or stopping administration of the CID to the subject.

[0062] The present disclosure provides a method for activating signalling in the CAR signalling system described herein in a T cell or NK cell according to the fourth aspect of the invention in culture, which comprises the step of introducing the CID agent to the T or NK cell culture.

[0063] The present disclosure provides a method for reducing or stopping signalling via the CAR signalling system described herein in a T or NK cell according to the fourth aspect of the invention in culture, which comprises the step of removing the CID from the T or NK cell culture.

[0064] Described herein is a method for inducing dimerization *in vivo* between the receptor component described

herein and a signalling component described herein, which comprises the step of administering a chemical inducer of dimerization (CID) to a subject comprising a cell according to the fourth aspect of the invention.

[0065] Also described is a means of tuning the CAR activity to the presence of an agent, such as a small molecule. This allows the potency of CAR cells to be controlled pharmacologically and tuned to an acceptable balance between achieving the desired therapeutic effect, while avoiding CAR-associated toxicities.

DETAILED DESCRIPTION

CHIMERIC ANTIGEN RECEPTORS (CARs)

[0066] 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 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 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.

[0067] Early CAR designs had endodomains derived from the intracellular parts of either the γ chain of the Fc ϵ R1 or CD3 ζ . Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3 ζ results in second generation receptors which can transmit an activating and co-stimulatory signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most potent co-stimulatory signal - namely immunological signal 2, which triggers T-cell proliferation. Some receptors have also been described which include TNF receptor family endodomains, such as the closely related OX40 and 41BB which transmit survival signals. Even more potent third generation CARs have now been described which have endodomains capable of transmitting activation, proliferation and survival signals.

[0068] 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.

[0069] The present disclosure relates to a CAR signalling system in which the antigen-recognizing/antigen binding domain and transmembrane domain are provided on a first molecule (termed herein 'receptor component'), which localizes to the cell membrane. The intracellular signalling domain is provided on a second, intracellular molecule (termed herein 'signaling component'). Certain small molecules have been described which bind simultaneously to two protein domains. Such small molecules can hence act to dimerize proteins (termed herein "chemical inducer of dimerization" or "CID"). Both the receptor component and signaling components comprise a CID binding domain (termed herein 'CID Binding Domain 1', or 'CBD1' and 'CID Binding Domain 2' or 'CBD2' respectively) which allows each receptor component and signalling component to bind simultaneously to the same CID molecule (figure 3).

[0070] When antigen binds to the antigen-binding domain of the receptor component in the absence of the CID, there is no signalling through the signaling component. When the CID is present, receptor binding of antigen to the antigen-binding domain of the receptor component results in signaling through the signalling component.

[0071] Specifically, in the absence of CID, the receptor component and signalling component are located in a stochastically dispersed manner and binding of antigen by the antigen-binding domain of the receptor component does not result in signalling through the signaling component. In the presence of the CID, both the receptor and signalling component of the CAR bind the CID via their CID binding domains, resulting in co-localization of the receptor component and signaling components (heterodimerization). This co-localisation allows signalling through the signalling domain of the signalling component when antigen is bound by the antigen-binding domain of the receptor component.

[0072] Herein 'co-localization' or 'heterodimerization' of the receptor and signalling components is analogous to ligation/recruitment of the signalling component to the receptor component via the CID agent.

[0073] Antigen binding by the receptor component in the absence of the CID may be termed as resulting in 'non-productive' signaling through the signaling component. Such signaling does not result in cell activation, for example T cell activation. Antigen binding by the receptor component in the presence of CID may be termed as resulting in 'productive' signaling through the signaling component. This signaling results in T-cell activation, triggering for example target cell killing and T cell activation.

[0074] Antigen binding by the receptor component in the presence of CID may result in signalling through the signalling component which is 2, 5, 10, 50, 100, 1,000 or 10,000-fold higher than the signalling which occurs when antigen is bound by the receptor domain in the absence of CID.

[0075] Signaling through the signaling 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-biphosphate (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 receptor component and signalling component of the CAR signalling system according to the present disclosure upon binding of antigen to the receptor component in the presence of varying concentrations of CID.

CHEMICAL INDUCER OF DIMERIZATION & BINDING DOMAINS

[0076] The CID and CID binding domains of the CAR signalling system described herein may be any combination of molecules/peptides/domains which enables the selective co-localisation and dimerization of the receptor component and signalling component in the presence of the CID.

[0077] As such, the CID agent is a molecule which is able to simultaneously bind to the receptor component and the signalling component. The CID agent therefore comprises at least two binding moieties.

[0078] The CID may be any pharmaceutically acceptable molecule which can simultaneously be bound by at least two binding domains.

[0079] The CID is capable being delivered to the cytoplasm of a target cell and being available for intracellular binding.

[0080] Small molecule dimerization systems for facilitating the co-localization of peptides are known in the art (Corson et al.; 2008; ACS Chemical Biology; 3(11); 667).

[0081] The binding moieties of the CID may interact with identical binding domains present on the receptor component and the signalling component. That is, the CID may comprise two identical binding moieties such that it can simultaneously interact with a binding domain on the receptor component and an identical binding domain on the signalling component.

[0082] The CID and CID binding domains may be the FK506 binding protein (FKBP) ligand dimerization system described by Clackson et al. (PNAS; 1998; 95; 10437-10442). This dimerization system comprises two FKBP-like binding domains with a F36V mutation in the FKBP binding domain and a dimerization agent (AP1903) with complementary amino acid substitutions. Exposing cells engineered to express FKBP-like binding domain fusion proteins to AP103 results in the dimerization of the proteins comprising the FKBP-like binding domains but no interactions involving endogenous FKBP.

[0083] The dimerization system described by Farrar *et al.*, which utilises bacterial DNA gyrase B (GyrB) binding domains and the antibiotic coumermycin as CID may also be used in the signalling system described herein (Methods Enzymol; 2000; 327; 421-419 and Nature; 1996; 383; 178-181).

[0084] The binding moieties of the CID may interact with different binding domains on the receptor component and the signalling component. That is, the CID may comprise two different binding moieties which can simultaneously interact with a binding domain on the receptor component and a different binding domain on the signalling component.

[0085] The CID and CID binding domains may comprise the dimerization system described by Belshaw et al. (Nature; 1996; 93; 4604-4607), which utilises a FK506 (Tacrolimus)/cyclosporin fusion molecule as the CID agent with FK-binding protein 12 (FKBP12) and cyclophilin A as the binding domains.

[0086] The CID / CID binding domain may also be the rapamycin and FKBP12/FKBP12-Rapamycin Binding (FRB) domain of mTOR system described by Rivera et al. (Nature Med; 1996; 2; 1028-1032) or the non-immunosuppressive rapamycin analogs (rapalogs) and FKBP12/FRB system described by Bayle et al. (Chem Bio; 2006; 13; 99-107). For example the CID may be C-20-methyllylrapamycin (MaRap) or C16(S)-Butylsulfonamidrapamycin (C16-BS-Rap), as described by Bayle *et al.* and illustrated in Figure 2 in combination with the corresponding binding domains. The CID may be C16-(S)-3-methylindolerapamycin (C16-iRap) or C16-(S)-7-methylindolerapamycin (AP21976/C16-AiRap) as described by Bayle *et al.*, in combination with the respective complementary binding domains for each.

[0087] Other dimerization systems suitable for use in the signalling system described herein include an estrone/biotin CID in combination with an oestrogen-binding domain (EBD) and a streptavidin binding domain (Muddana & Peterson; Org. Lett; 2004; 6; 1409-1412; Hussey et al.; J. Am. Chem. Soc.; 125; 3692-3693); a dexamethasone/methotrexate CID in combination with a glucocorticoid-binding domain (GBD) and a dihydrofolate reductase (DHFR) binding domain (Lin et al.; J. Am. Chem. Soc.; 2000; 122; 4247-4248); a similar system in which the methotrexate portion of the CID is replaced with the bacterial specific DHFR inhibitor trimethoprim (Gallagher et al.; Anal. Biochem.; 2007; 363; 160-162) and an O⁶-benzylguanine derivative/methotrexate CID in combination with an O⁶-alkylguanine-DNA alkyltransferase (AGT) binding domain and a DHFR binding domain (Gendreizig et al.; J. Am. Chem. Soc.; 125; 14970-14971).

[0088] RSL1 is a synthetic non-steroidal analogue of 20-hydroxyecdysone (see Figure 9). It is a member of a class of insecticides known as diacylhydrazines and can function to act as a non-steroidal ecdysone agonist. These molecules induce premature moulting and larvae death but are well tolerated in vertebrates. RSL1 has been used in artificial transcription switches in which a two-protein transcription switch is used consisting of a fusion between the ecdysone

receptor (EcR) and GAL4, and the retinoid X receptor (RXR) and VP16. In such systems, EcR is modified to interact specifically with RSL13, and RXR is chimeric comprising of helices 1-8 replaced with helices 1-8 of human PXP β , and helices 9-12 from Locusta migratoria RXR. The signalling system described herein may use EcR and RXR domains for heterodimerisation in the presence of RSL1 or a derivative thereof.

5 **[0089]** For example the CID binding domains may be or comprise the sequences shown as SEQ ID NO: 1 to SEQ ID NO: 5, SEQ ID No. 25 or 26.

SEQ ID No 1 - FKBP12 domain

10 MG VQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRDRNKPFK FMLGKQE
VIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE

SEQ ID No 2 - wild-type FRB segment of mTOR

15 MASRILWHEMWHEGLEEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
QAYGRDLMEAEQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISKLES

20 SEQ ID No 3 - FRB with T to L substitution at 2098 which allows binding to AP21967

MASRILWHEMWHEGLEEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
25 QAYGRDLMEAEQEWCRKYMKSGNVKDLLQAWDLYYHVFRRISKLES

SEQ ID No 4 - FRB segment of mTOR with T to H substitution at 2098 and to W at F at residue 2101 of the full mTOR which binds Rapamycin with reduced affinity to wild type

30 MASRILWHEMWHEGLEEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
QAYGRDLMEAEQEWCRKYMKSGNVKDLHQAFDLYYHVFRRISKLES

SEQ ID No 5 - FRB segment of mTOR with K to P substitution at residue 2095 of the full mTOR which binds Rapamycin with reduced affinity

35 MASRILWHEMWHEGLEEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFN
QAYGRDLMEAEQEWCRKYMKSGNVPDLTQAWDLYYHVFRRISKLES

40 SEQ ID No. 25-EcR

MPVDRIEAELAVEQKSDQGVEGPGGTGGSGSSPNDPVTNICQAADKQLFTLVEW
45 AKRIPHFSSLPLDDQVILLRAGWNELLIASFSHRSIDVRDGILLATGLHVHRNSAHSAG
VGAIFDRVLTELVSKMRDMRMDKTELGCLRAILFNPEVRGLKSAQEVELLREKVYAA
LEEYTRTTHPDEPGRFAKLLLRLPSLRSIGLKCLEHLFFFRLIGDVPIDTFLMEMLESP
SDS

50 SEQ ID No. 26-RXR

55

RPECVVPETQCAMKRKEKKAQKEKDKLPVSTTTVDDHMPPIMQCEPPPPEAARIHE
 VVPRFLSDKLLETNRQKNIPQLTANQQFLIARLIWYQDGYEQPSDEDLKRITQTWQQ
 5 ADDENEESDTPFRQITEMTILTVQLIVEFAKGLPGFAKISQPDQITLLKACSSEVMMLR
 VARRYDAASDSILFANNQAYTRDNYRKAGMAEVIEDLLHFCRCMYSMALDNIHYALL
 TAVVIFSDRPGLEQPQLVEEIQRYYLNTLRIYILNQLSGSARSSVIYGKILSELRTLG
 10 MQNSNMCISLKLKNRKLPPFLEEIWDVADMSHTQPPPILESPTNL

[0090] Variant sequences may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID No. 1 to 5, 25 or 26 provided that the sequences provide an effective dimerization system. That is, provided that the sequences facilitate sufficient co-localisation of the antigen-binding and intracellular signalling components, in the presence of the CID, for successful signaling to occur upon binding of the antigen-binding domain to antigen.

[0091] The signalling system described herein is not limited by the arrangement of a specific dimerization system. The receptor component may comprise either binding domain of a given dimerization system so long as the signalling component comprises the corresponding, complementary binding domain which enables the receptor component and signalling component to co-localize in the presence of the CID.

[0092] The present disclosure also relates to a method for activating the CAR signalling system of the first aspect which comprises the step of administering the CID. As described above, administration of the CID results in co-localization of the receptor component and signalling component, such that signalling through the signalling domain occurs upon binding of the antigen-binding domain to antigen.

[0093] The CID and CID binding domains may facilitate signalling through the CAR signalling system which is proportional to the concentration of CID present.

[0094] The present disclosure further provides a method for reducing the activity of the CAR system described herein which comprises reducing or stopping the administration of the CID. Reducing the level of the CID results in fewer receptor : signalling component dimers and thus the total level of signalling through the CAR signaling system is decreased in the presence of the antigen. Stopping the administration of the CID results in the absence of receptor : signalling component dimers and termination of signalling despite the presence of antigen binding to the antigen-binding domain.

[0095] Another possibility of this system is to "tune" the signal strength the receptor transmits. If the affinity between one or both of the CID binding domains is lessened, less CAR systems will be active and hence signal propagation upon antigen binding is lessened. This may avoid toxicity.

RECEPTOR COMPONENT

[0096] The present disclosure provides a receptor component comprising an antigen-binding domain, an optional spacer domain, a transmembrane domain and a CID binding domain (CBD1). When expressed in a cell, the receptor 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 CBD is localised to the intracellular side of the membrane.

[0097] The receptor component therefore provides the antigen-binding function of the CAR signalling system described herein.

ANTIGEN BINDING DOMAIN

[0098] The antigen-binding domain is the portion of a classical CAR which recognizes antigen. In the signalling system described herein the antigen-binding is located within the receptor component.

[0099] Numerous antigen-binding domains are known in the art, including those based on the antigen binding site of an antibody, antibody mimetics, and T-cell receptors. For example, the antigen-binding domain may comprise: a single-chain variable fragment (scFv) derived from a monoclonal antibody; a natural ligand of the target antigen; a peptide with sufficient affinity for the target; a single domain binder such as a camelid; an artificial binder single as a Darpin; or a single-chain derived from a T-cell receptor.

[0100] 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.

Table 1

Cancer type	TAA
Diffuse Large B-cell Lymphoma	CD19, CD20
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

[0101] The transmembrane domain is the sequence of a classical CAR that spans the membrane. In the signalling system of the present disclosure the transmembrane domain is located in the receptor component. It may comprise a hydrophobic alpha helix. The transmembrane domain may be derived from CD28, which gives good receptor stability. The transmembrane domain may be from CD148, as shown in SEQ ID No. 23.

SIGNAL PEPTIDE

[0102] The receptor component of the CAR signalling system of the present disclosure may comprise a signal peptide so that when the receptor 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 displayed.

[0103] 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.

[0104] The signal peptide may be at the amino terminus of the molecule.

[0105] The signal peptide may comprise the SEQ ID No. 6, 7 or 8 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. 6: MGTSLLCWMALCLLGADHADG

[0106] The signal peptide of SEQ ID No. 6 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. 7: MSLPVTALLLPLALLLHAARP

[0107] The signal peptide of SEQ ID No. 7 is derived from IgG1.

SEQ ID No. 8: MAVPTQVLGLLLLWLTDARC

[0108] The signal peptide of SEQ ID No. 8 is derived from CD8.

SPACER DOMAIN

[0109] The CAR signalling system described herein may comprise a spacer sequence to connect the antigen-binding domain with the transmembrane domain in the receptor component. A flexible spacer allows the antigen-binding domain to orient in different directions to facilitate binding.

[0110] 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.

[0111] Examples of amino acid sequences for these spacers are given below:

SEQ ID No. 9 (hinge-CH₂CH₃ of human IgG1)

AEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK
SLSLSPGKKD

SEQ ID No. 10 (human CD8 stalk):

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDI

SEQ ID No. 11 (human IgG1 hinge):

AEPKSPDKTHTCPPCPKDPK

SEQ ID No. 12 (CD2 ectodomain)

KEITNALETWGALGQDINLDIPSFQMSDDIDDIKWEKTSKKKIAQFRKEKETFKEKD
TYKLFKNGTLKIKHLKTDDQDIYKVSIDYDTKGKNVLEKIFDLKIQERVSKPKISWTCINT
TLTCEVMNGTDPELNLYQDGKHLKLSQRVITHKWTTSLSAKFKCTAGNKVSKESSV
EPVSCPEKGLD

SEQ ID no. 13 (CD34 ectodomain)

SLDNNGTATPELPTQGTFNSVSTNVSQYQETTTTPSTLGSTSLHPVSQHGNEATTNITE
TTVKFTSTSVITSVYGNTNSSVQSQTSVISTVFTTPANVSTPETTLKPSLSPGNVSDL
STTSTSLATSPTKPYTSSSPILSDIAEIKCSGIREVKLTQGICLEQNKTSSCAEFKKD
RGEGLARVLCGEEQADADAGAQCSSLLLAQSEVRPQCLLLVLANRTEISSKLQLMK
KHQSDLKKLGILDFTEQDVASHQSYSQKT

RECEPTOR COMPONENT COMPRISING MULTIPLE TRANSMEMBRANE DOMAINS

[0112] The receptor component may comprise an appropriate number of transmembrane domains such that each CID binding domain is orientated on the intracellular side of the cell membrane (Figure 6). For example the receptor component may comprise 3, 5, 7, 9, 11, or more transmembrane domains. In this way, a single receptor component may recruit multiple signalling components amplifying signalling in response to antigen. The signalling system encoded by SEQ ID No. 22 is a multi-spanning receptor with 3 FRB domains.

MULTIPLE RECEPTOR COMPONENTS

[0113] In another embodiment of the disclosure, the CAR signalling system may comprise of two or more receptor components each recognizing different antigens but comprising of the same intracellular CID binding domain. Such a CAR system would be capable of recognizing multiple antigens (Figure 5a). This might be useful for instance in avoiding tumour escape. In a further related aspect of the disclosure, the CBD1 of the receptor components differ in residues which dictate their affinity to the CID. In this way, a CAR system can be tuned such that signalling in response to one antigen is greater or lesser than response to another (figure 5b). 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.

[0114] Methods suitable for altering the amino acid residues of the CID binding domain such that its binding affinity for CID is altered are known in the art and include substitution, addition and removal of amino acids using both targeted and random mutagenesis. For example, in the case of CID binding to FRB, a set of mutations have been published with a range of affinity (Bayle et al, Chemistry & Biology 13, 99-107, January 2006). Methods for determining the affinity of a CID for the CID binding 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.

[0115] The signalling system encoded by SEQ ID No. 20 comprises two receptor components and one signalling component.

SIGNALLING COMPONENT

[0116] The present disclosure also provides a signalling component comprising a signalling domain and a CID binding domain (CBD2). 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.

[0117] No signalling occurs through the signalling domain of the signalling component unless it is co-localised with the receptor component provided by the present disclosure. Such co-localisation occurs only in the presence of the CID, as described above.

INTRACELLULAR SIGNALLING DOMAIN

[0118] The intracellular signalling domain is the signal-transmission portion of a classical CAR. In the signalling system described herein the intracellular signalling domain (signalling domain) is located in the signalling component. In the presence of the CID, the membrane-bound, receptor component and the intracellular signalling component are brought into proximity. After antigen recognition, receptors cluster, native CD45 and CD148 are excluded from the synapse and a signal is transmitted to the cell.

[0119] As such the signalling domain of the signalling component is analogous to the endodomain of a classical CAR molecule.

[0120] 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).

[0121] 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 3A).

[0122] The signalling component of a CAR signalling system described herein may comprise the sequence shown as SEQ ID No. 14, 15 or 16 or a variant thereof.

SEQ ID No. 14 - CD3 Z endodomain

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

SEQ ID No. 15 - CD28 and CD3 Zeta endodomains

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQ
 5 GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
 AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No. 16 - CD28, OX40 and CD3 Zeta endodomains.

SKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRDQRLPPDAHKKPPG
 10 GGSFRTPIQEEQADAHSTLAKIRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
 LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
 15 LYQGLSTATKDTYDALHMQALPPR

[0123] A variant sequence may have at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID No. 14, 15 or 16, provided that the sequence provides an effective intracellular signalling domain.

MULTIPLE SIGNALLING COMPONENTS

[0124] The signalling system described herein may comprise a plurality of signalling components, each comprising a signalling domain and a CIDB2, wherein each signalling domain is bound by the same CID but the signalling domains comprise different endodomains (Figure 7a). 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.

[0125] If each signalling component comprises of a CBD2 domains which differ in residues which alter their affinity to CID, the CID enables signalling components comprising different signalling domains to ligate to the CID with differing kinetics (Figure 7b). 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.

[0126] The signalling system encoded by SEQ ID No. 21 has one receptor components and three signalling components.

SIGNALLING COMPONENTS WITH AN ADDITIONAL CID BINDING DOMAIN

[0127] In the case of very low density of target antigen expression, signalling strength may have to be amplified. Take for instance, the native T-cell receptor which is associated with multiple ITAM containing CD3 components. In such circumstances, signalling components can be modified to contain CBD2, a signalling domain and also a CBD1 (Figure 8). In this way, addition of CID would lead to concatenation of multiple signalling components. The average size of the concatenation can be tuned by varying the amount of signalling components which have a CBD2. In the event of antigen recognition and in the presence of CID, signalling through the concatenation of multiple signalling components would be more potent than through individual signalling components.

NUCLEIC ACID

[0128] The first aspect of the invention relates to a nucleic acid encoding the receptor component and a nucleic acid encoding a signalling component.

[0129] The specific encoding nucleic acid sequences for four inducible CARs are given later in description, following the Examples section.

[0130] As used herein, the terms "polynucleotide", "nucleotide", and "nucleic acid" are intended to be synonymous with each other.

[0131] 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.

[0132] Nucleic acids described herein 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.

[0133] 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.

[0134] The nucleic acid described herein may be a nucleic acid which encodes both the receptor component and the signalling component.

[0135] The nucleic acid may produce a polypeptide which comprises the receptor 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.

[0136] Various self-cleaving sites are known, including the Foot-and-Mouth disease virus (FMDV) 2a self-cleaving peptide, which has the sequence shown as SEQ ID No. 17 or SEQ ID No. 18:

SEQ ID No. 17

RAEGRGSLLTCGDVEENPGP. or

SEQ ID No. 18

QCTNYALLKLAGDVESNPGP

[0137] Where the signalling system comprises a plurality of signalling components, each comprising a signalling domain and a CIDB2, the nucleic acid may have the general formula:

$$\text{RC-coexpr1-SC1-coexpr2-SC2}$$

wherein:

RC is a nucleic acid sequence encoding the receptor component;

Coexpr1 and coexpr2, which may be the same or different, are nucleic acid sequences allowing co-expression of the two flanking polypeptides (e.g. a sequence which encodes a cleavage site)

SC1 and SC2 are nucleic acid sequence encoding signalling components.

[0138] The signalling components encoded by SC1 and SC2 may bind the same CID, but have different endodomains. The nucleic acid construct may comprise nucleic acid sequences encoding more than two signalling components e.g. SC3, SC4 ...up to SCx, each separated by a coexpression sequence, coexpr3, coexpr4....up to coexprX.

[0139] The nucleic acid sequences encoding each component may be in any order, for example: RC-coexpr1-SC1-coexpr2-SC2; SC1-coexpr1-SC2-coexpr2-RC; or SC1-coexpr1- RC-coexpr2-SC2.

[0140] Where the signalling system comprises a plurality of receptor components, the nucleic acid may have the general formula:

$$\text{RC1-coexpr1-RC2-coexpr2-SC}$$

wherein:

RC1 and RC2 are nucleic acid sequences encoding receptor components;

Coexpr1 and coexpr2, which may be the same or different, are nucleic acid sequences allowing co-expression of the two flanking polypeptides (e.g. a sequence which encodes a cleavage site)

SC is a nucleic acid sequence encoding the signalling component.

[0141] The receptor components may bind to different antigens but the same CID. They may have the same CIDB1. The nucleic acid construct may comprise nucleic acid sequences encoding more than two receptor components e.g. RC3, RC4 ... up to RCx, each separated by a coexpression sequence, coexpr3, coexpr4.... up to coexprX.

[0142] The nucleic acid sequences encoding each component may be in any order, for example: RC1-coexpr1-RC2-coexpr2-SC; RC1-coexpr1-SC-coexpr2-RC2; or SC-coexpr1-RC1-coexpr2-RC2.

[0143] The co-expressing sequence may be an internal ribosome entry sequence (IRES). The co-expressing sequence may be an internal promoter.

5 **[0144]** Also described is a kit comprising a nucleic acid encoding the receptor component of the second aspect and/or a nucleic acid encoding a signalling component of the third aspect. The kit may also comprise a heterodimerization agent which is suitable for use in the signalling system of the first aspect.

VECTOR

10 **[0145]** The present disclosure also provides a vector, or kit of vectors which comprises one or more nucleic acid sequence(s) encoding a receptor component of the described herein and/or signalling component described herein. Such a vector may be used to introduce the nucleic acid sequence(s) into a host cell so that it expresses the receptor component and signalling component of the CAR signalling system described herein. The kit may also comprise a CID

15 which is suitable for use in the signalling system described herein.

[0146] 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.

[0147] The vector may be capable of transfecting or transducing a T cell.

20 CYTOLYTIC IMMUNE CELL

[0148] The present disclosure also relates to an immune cell comprising the CAR signalling system described herein.

[0149] Cytolytic immune cells can 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 **[0150]** 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 **[0151]** 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 **[0152]** 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.

40 **[0153]** 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.

45 **[0154]** Two major classes of CD4+ Treg cells have been described - naturally occurring Treg cells and adaptive Treg cells.

50 **[0155]** 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.

[0156] Adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response.

55 **[0157]** Natural Killer Cells (or NK cells) are a type of cytolytic cell which form part of the innate immune system. NK cells provide rapid responses to innate signals from virally infected cells in an MHC independent manner

[0158] 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.

[0159] The CAR cells described herein may be any of the cell types mentioned above.

[0160] T or NK cells expressing the molecules of the CAR signalling system described herein 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).

[0161] Alternatively, T or NK cells expressing the molecules of the CAR signalling system described herein may be derived from *ex vivo* differentiation of inducible progenitor cells or embryonic progenitor cells to T cells. Alternatively, an immortalized T-cell line which retains its lytic function and could act as a therapeutic may be used.

[0162] In all these embodiments, CAR cells are generated by introducing DNA or RNA coding for the receptor component and signalling component by one of many means including transduction with a viral vector, transfection with DNA or RNA.

[0163] The CAR cell described herein 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 signalling system described herein, for example by treatment with an anti-CD3 monoclonal antibody.

[0164] The T or NK cell described herein 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 receptor component and/or signalling component of the CAR signalling system described herein.

[0165] 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.

[0166] The present disclosure also provides a kit which comprises a T or NK cell comprising the CAR signalling system described herein and a heterodimerization agent suitable for use in the signalling system.

PHARMACEUTICAL COMPOSITION

[0167] The present invention also relates to a pharmaceutical composition containing a plurality of cytolytic immune cells expressing the molecules of the CAR signalling system described herein. 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.

METHODS

[0168] The present disclosure provides a method for treating and/or preventing a disease which comprises the step of administering the cytolytic immune cells described herein (for example in a pharmaceutical composition as described above) and/or a CID suitable for use in a signalling system described herein.

[0169] A method for treating a disease relates to the therapeutic use of the cytolytic immune cells described herein. 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.

[0170] The method for preventing a disease relates to the prophylactic use of the cytolytic immune cells described herein. 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.

[0171] The method may involve the steps of:

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

[0172] The methods for treating a disease may further comprise the step of administering a CID suitable for use in the signalling system described herein to the subject.

[0173] The present disclosure also provides a method for treating and/or preventing a disease in a subject which subject comprises cells of the invention, which method comprises the step of administering a CID agent suitable for use

in the CAR signalling system described herein. As such, this method involves administering a suitable CID agent to a subject which already comprises CAR cells described herein

[0174] The CID may be administered in the form of a pharmaceutical composition. 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.

[0175] The methods for treating a disease provided by the present disclosure may involve monitoring the progression of the disease and monitoring any toxic activity and adjusting the dose of the CID administered to the subject to provide acceptable levels of disease progression and toxic activity.

[0176] Monitoring the progression of the disease means to assess the symptoms associated with the disease over time to determine if they are reducing/improving or increasing/worsening.

[0177] Toxic activities relate to adverse effects caused by the CAR cells described herein following their administration to a subject. Toxic activities may include, for example, immunological toxicity, biliary toxicity and respiratory distress syndrome.

[0178] The level of signalling through the signalling system described herein and therefore the level of activation CAR cells expressing the signalling system, can be adjusted by altering the amount of CID present. In the present method the level of CAR cell activation may be augmented by increasing the dose of CID administered to the subject. Conversely, the level of CAR cell activation may be reduced by decreasing the dose of CID administered to the subject.

[0179] Higher levels of CAR cell activation are likely to be associated with reduced disease progression but increased toxic activities, whilst lower levels of CAR cell activation are likely to be associated with increased disease progression but reduced toxic activities.

[0180] As such the dose of CID administered to a subject may be altered in order to provide an acceptable level of both disease progression and toxic activity. The specific level of disease progression and toxic activities determined to be 'acceptable' will vary according to the specific circumstances and should be assessed on such a basis. The present disclosure provides a method for altering the activation level of the CAR cells in order to achieve this appropriate level.

[0181] The present disclosure provides a CAR cell described herein for use in treating and/or preventing a disease.

[0182] The disclosure also relates to the use of a CAR cell described herein in the manufacture of a medicament for the treatment and/or prevention of a disease.

[0183] The present disclosure also provides a CID agent suitable for activating a CAR signalling system described herein for use in treating and/or preventing a disease.

[0184] The present disclosure also provides a CID agent for use in activating a CAR signalling system described herein in a CAR cell.

[0185] The disclosure also provides the use of a CID suitable for activating a CAR signalling system described herein in the manufacture of a medicament for the treatment and/or prevention of a disease.

[0186] The disclosure also provides the use of a CID in the manufacture of a medicament for in activating a CAR signalling system described herein in a CAR cell.

[0187] The disease to be treated and/or prevented by the methods described herein may be an infection, such as a viral infection.

[0188] The methods described herein may also be for the control of pathogenic immune responses, for example in autoimmune diseases, allergies and graft-vs-host rejection.

[0189] 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), leukemia, lung cancer, melanoma, non-Hodgkin lymphoma, pancreatic cancer, prostate cancer and thyroid cancer.

[0190] The CAR cells described herein 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 expression of a TAA provided above in Table 1.

[0191] The CAR cells and pharmaceutical compositions described herein may be for use in the treatment and/or prevention of the diseases described above.

[0192] The CAR cells and pharmaceutical compositions described herein may be for use in any of the methods described above.

[0193] The present disclosure also provides a method of administering a cell according to the fourth aspect of the invention to a subject in need of same.

[0194] The present disclosure also provides a method of administering a CID as defined herein to a subject who comprises a cell according to the fourth aspect of the invention.

[0195] 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 - A rapamycin-inducible CAR system

[0196] T-cells were transduced with a retroviral vector coding for an inducible CAR detailed in figure 3 and the sequence of which is shown as SEQ ID 19. This inducible CAR has a single receptor component and a single signalling component. The receptor component comprises of an anti-CD19 scFv, a spacer domain derived from human IgG1, a transmembrane-domain derived from CD28 and FKBP12 as endodomain. The signalling component comprises of the FRB domain from mTOR and a signalling domain derived from endodomains of CD28, OX40 and CD3-Zeta. Expression of the CAR was determined by staining the transduced cells with a conjugated antibody recognizing the spacer domain and analysing by flow cytometry. The T-cells were incubated with either target cells expressing the cognate target CD19 in ratio of 2:1, 1:1 and 0:1 (target:effector) in the absence of rapamycin or at increasing concentrations. Signalling only occurred in the presence of both the cognate antigen and rapamycin. A dose response to rapamycin and target antigen was observed.

Example 2 - An RSL1-inducible CAR system

[0197] RSL1 is a synthetic non-steroidal analogue of 20-hydroxyecdysone (Figure 9). It is a member of a class of insecticides known as diacylhydrazines and can function to act as a non-steroidal ecdysone agonist. These molecules induce premature moulting and larvae death but are well tolerated in vertebrates (Dhadialla et al (1998) Annu. Rev. Entomol. 43, 545-569). RSL1 has been used in artificial transcription switches (Lessard et al (2007) The Prostate 67, 808-819) whereby a two-protein transcription switch consisting of a fusion between the ecdysone receptor (EcR) and GAL4, and the retinoid X receptor (RXR) and VP16. In such systems, EcR is modified to interact specifically with RSL1 (Kumar et al (2004) J. Biol. Chem. 279, 27211-27218), and RXR is chimeric comprising of helices 1-8 replaced with helices 1-8 of human PXP β , and helices 9-12 from Locusta migratoria RXR.

[0198] The present inventors have constructed a split CD19 CAR based on the CD19 system described in Example 1 with the heterodimerization motif on the antigen recognition component and signalling components being EcR and RXR respectively (Figure 10a and SEQ ID No. 23). This CAR was transduced into T-cells and found to express well (Figure 10b). These T-cells were challenged with targets which were CD19 negative and CD19 positive. Only CAR T-cells challenged with SupT1 targets in the presence of RSL1 released IL-2 (Figure 10c).

Example 3 - An AP1903-inducible CAR system

[0199] AP1903 is a synthetic chemical inducer of dimerization which binds to two mutated FKBP12 domains (Clackson et al (1998). Proc. Natl. Acad. Sci. U. S. A. 95, 10437-10442). An inducible CAR was developed based on this system, using the modified FKBP12 domains to constitute the heterodimerization domain of both the antigen recognition component and signalling component in the framework as described in Example 1 with the antigen recognition domain and the signalling domain co-expressed with a FMD-2A like sequence (Figure 11a and SEQ ID No. 24). The FKBP12 domains were codon-wobbled to prevent homologous recombination. T-cells were transduced with this construct and challenged with target cells which were CD19 negative or positive.

[0200] T-cells responded only to CD19 positive targets in the presence of AP1903 (Figure 11b). At very high concentration of dimerizer, signalling was inhibited likely due to saturating conditions where the stoichiometry favours one FKBP12 binding a single dimerizer molecule rather than one dimerizer molecule binding two FKBP12 domains (Figure 11c).

SPECIFIC INDUCIBLE CAR-ENCODING SEQUENCES

[0201] SEQ ID No. 19. Simple inducible CAR comprised of one receptor component and one signalling component. The receptor component comprises of a signal peptide, an anti-CD19 scFv, a spacer from the Fc domain of human IgG1, a CD28 transmembrane domain and FKBP12 as endodomain. A FMD-2A peptide separates this receptor component from the signalling component. The signalling component comprises of FRB and a compound of endodomains from CD28, OX40 and CD3-Zeta.

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SgrAI

1 atggagaccg acaccctgct gctgtgggtg ctgctgctgt gggtgccagg cagcaccggc gacatccaga tgacccagac

>>.....signal peptide.....>>

>>.....aCD19 heavy chain.....>

81 caccagcagc ctgagcgcca gcctggggcg cccgggtgacc atcagctgca gagccagcca ggacatcagc aagtacctga

>.....aCD19 heavy chain.....>

161 actggtacca gcagaagccc gacggcaccg tgaagctgct gatctaccac accagccggc tgcacagcgg cgtgcccagc

>.....aCD19 heavy chain.....>

XcmI

241 cggttcagcg gcagcggcag cggcaccgac tacagcctga ccatcagcaa cctggagcag gaggacatcg ccacctactt

>.....aCD19 heavy chain.....>

321 ctgccagcag ggcaacaccc tgcctacac cttcggaggc ggcaccaagc tggagatcac caaggccgga ggcggaggct

>.....aCD19 heavy chain.....>>

>>..linker...>

401 ctggcggagg cggctctggc ggaggcggct ctggcggagg cggcagcgag gtgaagctgc aggagtctgg cccaggcctg

>.....linker.....>>

>>.....aCD19 light chain.....>

481 gtggcccca gccagagcct gagcgtgacc tgcaccgtga gcggcgtgag cctgcccgac tacggcgtga gctggatcag

>.....aCD19 light chain.....>

561 gcagcccca cggaagggcc tggagtggct gggcgtgatc tggggcagcg agaccaccta ctacaacagc gccctgaaga

>.....aCD19 light chain.....>

641 gccggtgac catcatcaag gacaacagca agagccaggt gtccctgaag atgaacagcc tgcagaccga cgacaccgcc

>.....aCD19 light chain.....>

721 atctactact gcgccaagca ctactactat ggcggcagct acgctatgga ctactggggc cagggcacca gcgtgaccgt

>.....aCD19 light chain.....>

BamHI

FseI

801 gagctcggat cccgccgagc ccaaatctcc tgacaaaact cacacatgcc caccgtgccc agcacctccc gtggccggcc

>.>>

>>.....HCH2CH3pvaa.....>

881 cgtcagtctt cctcttcccc ccaaaacca aggacaccct catgatcgcc cggaccctg aggtcacatg cgtggtggtg

>.....HCH2CH3pvaa.....>

961 gacgtgagcc acgaagaccc tgagggtcaag ttcaactggc acgtggacgg cgtggagggtg cataatgcca agacaaagcc

>.....HCH2CH3pvaa.....>

SacII

1041 gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc gtccctaccg tcctgcacca ggactggctg aatggcaagg

>.....HCH2CH3pvaa.....>

1121 agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga

>.....HCH2CH3pvaa.....>

1201 gaaccacagg tgtacaccct gcccctatcc cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaaagg

>.....HCH2CH3pvaa.....>

1281 cttctatccc agcgacatcg ccgtggagtg ggagagcaat gggcaaccgg agaacaacta caagaccagc cctcccgtgc

>.....HCH2CH3pvaa.....>

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1361 tggactccga cggctccttc ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca
>.....HCH2CH3pvaa.....>

Ppu10I

NsiI

BfrBI

1441 tqctccqtqa tqcatqagc cctgcacaat cactataccc agaaatctct gqgtctgagc ccaqgcaaga agqaccccaa
>.....HCH2CH3pvaa.....>>

1521 gttctgggtc ctgctgggtg tgggagggct gctggcctgt tactctctcc tggtgaccgt ggccttcac cttctctggg
>>.....CD28tm.....>

1601 tggcagtgca ggtcgaaacc atctccccag gagacggggc caccctcccc aagcgcggcc agacctgcgt ggtgcactac
>>
>>.....FKBP12.....>

1681 accgggatgc ttgaagatgg aaagaaatc gattcctccc gggacagaaa caagcccttt aagtttatgc taggcaagca
>.....FKBP12.....>

1761 ggaggtgatc cgaaggctggg aagaagggct tgcccagatg agtggtgggtc agagagccaa actgactata tctccagatt
>.....FKBP12.....>

1841 atgcctatgg tgccactggg caccagggca tcatccacc acatgccact ctgctcttcg atgtggagct tctaaaactg
>.....FKBP12.....>

1921 gaacgcgcag agggccgggg ctcatgtctg acctgtggag atgtcgagga aaatcccgcc ccaatggctt ctagaatcct
>>>
>>.....2A.....>>>
>>..>

2001 ctggcatgag atgtggcatg aaggcctgga agaggcatct cgtttgtact ttggggaaaag gaacgtgaaa ggcattgttg
>.....FRB.....>

SphI

2081 aggtgctgga gcccttgcat gctatgatgg aacggggccc ccagactctg aaggaaacat cctttaatca ggcctatggt
>.....FRB.....>

2161 cgagatttaa tggaggccca agagtggctc aggaagtaca tgaaatcagg gaatgtcaag gacctcctcc aagcctggga
>.....FRB.....>

XhoI

2241 cctctattat catctgttcc gacgaatctc aaagctcgag agtgccggag gaggcagttc aaggcccgcc gacgcgcctg
>.....FRB.....>>
>>.....CD28OXZ.....>

2321 cataccagca ggggcagaat cagctgtaca acgagctcaa cctccgtagg cgcgaggaat acgatgtgct cgataagaga
>.....CD28OXZ.....>

NruI

2401 agaggtcgcg atccccgagat gggaggaaaa cctcagcgcc gcaagaaccc tcaggagggg ctgtataacg aactgcagaa
>.....CD28OXZ.....>

SfiI

2481 ggataaagatg gcaqagccct actccgagat tggcatqaaq ggtgagagga gaagaggtaa aggccatgac ggcctctacc
>.....CD28OXZ.....>

2561 aaggcctctc taccgcaacg aaagacactt atgacgtctt gcatatgcag gctctcccc ctaggcagtg cactaattac
>.....CD28OXZ.....>

2641 gcccttctga aacttgccgg cgatgtggag tctaaccctg gccctatcct gtggcacgag atgtggcatg agggctctgga
>.....CD28OXZ.....>

2721 agagggcagc cggctgtact tcggagagag aaatgttaag ggtatgttcg aggtgctgga gccgcttcac gctatgatgg
>.....CD28OXZ.....>

2801 agagggggccc ccagaccctc aaagaaacca gcttcaatca agcctacggg agggatctta tggaggcaca ggaatggtgt
>.....CD28OXZ.....>

2881 cggaagtaca tgaagagcgg gaacgtcaag gacctgctcc aggcttggga tttgtattat cacgtcttta ggccgatcag
>.....CD28OXZ.....>

5 2961 caagtctgga ggccgaggaa gtctgcacag cgattacatg aacatgaccc cccgaaggcc cggaccacaca cgcaaact
>.....CD28OXZ.....>

3041 atcaacccta tgctcccca cgegacttcg ccgcctaccg gtcacgcgcc gaggggcgcg gctctttgtt gacttgccgg
>.....CD28OXZ.....>

10 3121 gacgttgaag agaatcctgg ccccatcctt tggcacgaga tgtggcacga gggcctggag gaagcctccc ggctgtattt
>.....CD28OXZ.....>

3201 cggagagcgc aacgtcaaag gaatgttga ggtgctggag cctctccatg caatgatgga gaggggcct cagactctta
>.....CD28OXZ.....>

15 3281 aagaaacatc ctttaatcag gcttacggtg gagatttgat ggaggctcaa gaatggtgcc ggaaatacat gaagagtgga
>.....CD28OXZ.....>

3361 aacgttaaag acctgctgca ggcatgggac ctgtactatc acgtattcag acggatctca aagtcagggg gcggtggctc
>.....CD28OXZ.....>

20 3441 cctttatata ttcaagcagc ctttcatgag gccggtgcag accacacaag aagaggatgg gtgctcttgc cggttccccg
>.....CD28OXZ.....>

3521 aggaggagga gggcggatgc gagctctga
>.....CD28OXZ.....>

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SEQ ID No. 20. Inducible CAR comprised of two receptor component and one signalling component. The first receptor component comprises of a signal peptide, an anti-CD19 scFv, a spacer from the stalk region of CD8, a transmembrane domain from CD28, and FKB12 as an endodomain. The second signalling component comprises of an anti-CD29 scFv, spacer derived from the Fc domain of human IgG1, a CD28 transmembrane domain and FKB12 as endodomain. The signalling component comprises of FRB and a compound of endodomains from CD28, OX40 and CD3-Zeta. A foot-and-mouth disease 2A peptide separates the components.

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1 atgagcctgc ccgtgaccgc cctgctgctg cccttgccc tgctgctgca cgccgccaga ccagacatcc agatgacca
>>.....signal peptide 1.....>>
>>.....aCD19_scFv.....>

35 81 gaccaccagc agcctgagcg ccagcctggg cgaccgggtg accatcagct gcagagccag ccaggacatc agcaagtacc
>.....aCD19_scFv.....>

161 tgaactggta ccagcagaag cccgacggca ccgtgaagct gctgatctac cacaccagcc ggctgcacag cggcgtgccc
>.....aCD19_scFv.....>

40 241 agccggttca gcggcagcgg cagcggcacc gactacagcc tgaccatcag caacctggag caggaggaca tcgccaccta
>.....aCD19_scFv.....>

321 cttctgccag cagggcaaca ccctgcccta caccttcgga ggccggcacca agctggagat caccaaggcc ggaggcggag
>.....aCD19_scFv.....>

45 401 gctctggcgg aggcggctct gccggaggcg gctctggcgg aggcggcagc gaggtgaagc tgcaggagtc tggcccaggc
>.....aCD19_scFv.....>

50 481 ctggtggccc caagccagag cctgagcgtg acctgcaccg tgagcggcgt gagcctgccc gactacggcg tgagctggat
>.....aCD19_scFv.....>

561 caggcagccc ccacggaagg gcctggagtg gctgggcgtg atctggggca gcgagaccac ctactacaac agcgcctga

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```
>.....aCD19_scFv.....>

641 agagccggct gaccatcaltc aaggacaaca gcaagagcca gg_gllcctg aagatgaaca gccctgcagac cgaacacacc
>.....aCD19_scFv.....>

721 gccatctact actgcgccaa gcactactac tatgggggca gcaacgctat ggactactgg ggccagggca ccagcgtgac
>.....aCD19_scFv.....>

                                SgrAI
                                -----
801 cgtgagctca gatcccacca cgacgccagc gccgcgacca ccaacaccgg cgcccaccat cgcgtcgcag cccctgtccc
>....>>
                                >>.....CD8STK.....>

881 tgcgcccaga ggctgtccgg ccagcggcgg ggggcgcagt gcacacgagg gggttggaact tcgcctgtga tatcttttgc
>.....CD8STK.....>>
                                >>...>

961 gtgctcgtgg tggttggtgg agtcctgget tgctataget tgctagtaac agtggccttc atcatcttct ggggtgggagt
>.....CD28tmZ.....>>
                                >>...>

1041 gcaggtgga accatctccc caggagacgg gcgcaccttc cccaagcgg gccagacctg cgtggtgcac tacaccggga
>.....FKBP12.....>

1121 tgcttgaaga tggaaagaaa ttcgattcct ccggggacag aaacaagccc ttttaagtta tgctaggcaa gcaggaggtg
>.....FKBP12.....>

1201 atccgaggct ggggaagaagg ggttgcccag atgagtgtgg gtcagagagc caaactgact atatctccag attatgccta
>.....FKBP12.....>

1281 tgggtgccact gggcacccag gcacatccc accacatgcc acctcgtct tcgatgtgga gcttctaaaa ctggaacgag
>.....FKBP12.....>>
                                >>..>

                                NcoI
                                -----
1361 ccgagggcag gggaaagtctt ctaacatgcg gggacgtgga ggaaaatccc gggcccatgg agaccgacac cctgctgctg
>.....2A.....>>
                                >>...signal peptide 2....>

1441 tgggtcctgc tgctgtgggt gcccggcagc accggccagg tgcagctgca gcagcccggc gccgagctgg tgaagcccgg
>.....signal peptide 2.....>>
                                >>.....aCD20_scFv.....>

1521 cgcagcgtg aagatgagct gcaaggccag cggctacacc ttcaccagct acaacatgca ctgggtgaag cagacccccg
>.....aCD20_scFv.....>

1601 gccggggcct ggagtggatc ggogccatct accccggcaa cggcgacacc agctacaacc agaagttcaa gggcaaggcc
>.....aCD20_scFv.....>

1681 accctgaccg ccgacaagag cagcagcacc gcctacatgc agctgagcag cctgaccagc gaggacagcg ccgtgtacta
>.....aCD20_scFv.....>

1761 clgcgcccgg agcaccctact acggcggcga clgglactlc aacglglggg gcgcccggac caccglgacc glgagcggag
>.....aCD20_scFv.....>

1841 ggggcggcag cggaggaggc ggcctctggg gaggcggatc tcagatcgtg ctgagccaga gcccggccat cctgagcgcc
>.....aCD20_scFv.....>

1921 agccccggcg agaaggtgac catgacctgc cgggccagca gcagcgtgag ctacatccac tggttccagc agaagcccgg
>.....aCD20_scFv.....>

                                BstXI
                                -----
2001 cagcagcccc aagccctgga tctacgccac cagcaacctg gccagcggcg tgcccgctcg gttcagcggc agcggcagcg
>.....aCD20_scFv.....>

2081 gcaccagcta cagcctgacc atcagccggg tggaggccga ggacgccgc acctactact gccagcagtg gaccagcaac
>.....aCD20_scFv.....>

                                BamHI
                                -----
2161 ccaccacct tcggcggcgg caccaagctg gagatcaagc gg_cggatcc cggcgagccc aaatctctcg acaaaactca
>.....aCD20_scFv.....>>
```

>>.....HCH2CH3pvaa'.....>

FseI

2241 cacatgccca ccgtgccag cacctccgt ggcggcccg tcagtcttcc tcttcccccc aaaacccaag gacacctca

>.....HCH2CH3pvaa'.....>

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2321 tgatgcccg gacctctgag gtcacatgag tgggtggtgga cgtgagccac gaagacctg aggtcaagtt caactggtag

>.....HCH2CH3pvaa'.....>

SacII

2401 gtggacggcg tggaggtgca taatgccaaag acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggtcagcgt

>.....HCH2CH3pvaa'.....>

10

2481 cctcaccgtc ctccaccagg actggtctgaa tggcaaggag tacaagtcca aggtctccaa caaagccctc ccagccccc

>.....HCH2CH3pvaa'.....>

2561 tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg tacacctgc cccatcccg ggatgagctg

>.....HCH2CH3pvaa'.....>

15

2641 accaagaacc agglcagccl gacctgctg glcaaaggcl tclatcccag cgatcgcgc glggaglggg agagcaalg

>.....HCH2CH3pvaa'.....>

2721 gcaacgggag aacaactaca agaccagcc tcccgctgctg gactccgagc gctccttcll cctclacagc aagclcccg

>.....HCH2CH3pvaa'.....>

20

Epu10I

NsiI

B⁵rBI

2801 tggacaagag caggtggcag caggggaagc tcttctcatg ctccgtgatg catgaggccc tgcacaatca ctatacccag

>.....HCH2CH3pvaa'.....>

2881 aaatctctga gtctgagccc aggcaagaag gaccccaagt tctgggtcct ggtgggtggtg gcaggcgtgc tggcctgtta

>.....HCH2CH3pvaa'.....>>

>>.....CD28tm.....>

30

2961 ctctctctctg gtcaccctgg ccttcacat cttctggctg qgagtgcagq tggaaaccat ctccccagga gacgggcgca

>.....CD28tm.....>>

>>.....FKBP12.....>

3041 ccttccccc

gcgcggccag

acctgcgtgg

tgcactacac

cgggatgctt

gaagatggaa

acaaattcga

ttctcccg

>.....FKBP12.....>

35

3121 gacagaaaca agccctttta gtttatgcta ggcaagcagg aggtgatccg aggtgggaa gaaggggttg ccagatgag

>.....FKBP12.....>

3201 tglgggtcag agagccaaac tgaclatalc tccagattat gctatgggtg ccaclgggca cccaggcatc atcccaccac

>.....FKBP12.....>

40

3281 atgcactct cgtcttogat gtggagcttc taaaactgga acgcgcagag ggcggggct cattgctgac ctgtggagat

>.....FKBP12.....>>

>>.....2A.....>

3361 gtcagggaaa atcccggccc aatggcttct agaatectct ggcagatgat gtggcatgaa gccctggaag aggcactctg

>.....2A.....>>

>>.....FRB.....>

SphI

3441 tttgtacttt ggggaaagga acgtgaaagg catgtttcag gtgctggagc ccttgcagtc tatgatggaa cggggcccc

>.....FRB.....>

50

3521 agactctgaa ggaaacatcc tttaatcagg cctatggctg agatttaatg gaggcccaag agtgggtcag gaagtacatg

>.....FRB.....>

XhoI

3601 aaatcaggga atgtcaagga cctcctccaa gcctgggacc tctattatca tgtgttccga cgaatctcaa agctcgagta

>.....FRB.....>>

>>

3681 tagcggcggc ggcagcagga gtaagaggag caggctcctg cacagtgact acatgaacat gactccccgc cgccccgggc

>....linker....>>

55

>>.....CD28OXZ.....>

5 3761 ccacccgcaa gcattaccag ccctatgcc caccacgcga cttcgcagcc tatcgctcca gggaccagag gctgcccccc
>.....CD28OXZ.....>

3841 gatgcccaca agccccctgg gggaggcagt ttccggaccc ccatccaaga ggagcaggcc gacgcccact ccaccctggc
>.....CD28OXZ.....>

10 3921 caagatcaga gtgaagttca gcaggagcgc agacgcccc gcgtaccagc agggccagaa ccagctctat aacgagctca
>.....CD28OXZ.....>

4001 atctaggacg aagagaggag tacgatgttt tggacaagag acgtggccgg gaccctgaga tggggggaaa gccgagaagg
>.....CD28OXZ.....>

15 4081 aagaaccctc aggaaggcct gtacaatgaa ctgcagaaag ataagatggc ggaggcctac agtgagattg ggatgaaagg
>.....CD28OXZ.....>

4161 cgagcgccgg aggggcaagg ggcacgatgg cttttaccag ggtctcagta cagccacca ggacacctac gacgcccttc
>.....CD28OXZ.....>

20 4241 acatgcaggc cctgcctcct cgctaa
>.....CD28OXZ.....>>

[0202] SEQ ID No. 21. Inducible CAR comprised of one receptor component and three signalling components. The
receptor component comprises of a signal peptide, an anti-CD19 scFv, a spacer derived from the Fc domain of human
IgG1, a CD28 transmembrane domain and FKB12 as endodomain. The first signalling component comprises of FRB
and endodomain from CD3 Zeta, the second comprises FRB and CD28 endodomain, the third FRB and 41BB endodo-
main. A foot-and-mouth disease 2A peptide separates the components.

30 1 atggagaccg acaccctgct gctgtgggtg ctgctgctgt ggggtgccagg cagcaccggc gacatccaga tgaccagac
>>.....signal peptide.....>>
>>.....scFv anti-CD19.....>

35 81 caccagcagc ctgagcgcca gcctggggcga ccgggtgacc atcagctgca gagccagcca ggacatcagc aagtacctga
>.....scFv anti-CD19.....>

161 actggtacca gcaaaagccc gacggcaccg tgaagctgct gatctaccac accagccggc tgcacagcgg cgtgcccagc
>.....scFv anti-CD19.....>

40 241 cggttcagcg gcagcggcag cggcaccgac tacagcctga ccatcagcaa cctggagcag gaggacatcg ccacctactt
>.....scFv anti-CD19.....>

321 ctgccagcag ggcaacaccc tgccctacac cttcggaggc ggcaccaagc tggagatcac caaggccgga ggcggaggct
>.....scFv anti-CD19.....>

45 401 ctggcggagg cggctctggc ggaggcggct ctggcggagg cggcagcag gtgaagctgc aggagtctgg ccagggcctg
>.....scFv anti-CD19.....>

481 gtggccccaa gccagagcct gagcgtgacc tgcaccgtga gcggcgtag cctgcccgac tacggcgtga gctggatcag
>.....scFv anti-CD19.....>

50

55

```

561  gcagcctccca cggaaagggcc tggagtggct gggcgctgac tggggcagcg agaccacctc ctacaacagc gccctgaaga
>.....scFv anti-CD19.....>

641  gccggctgac catcatcaag gacaacagca agagccaagt gttcctgaag atgaacagcc tgcagaccga cgacaccgcc
>.....scFv anti-CD19.....>

721  atctactact ggcccaagca ctactactat gggggcagct acgctatgga ctactggggc cagggcacca gcgtgaccgt
>.....scFv anti-CD19.....>

          BamHI                                     FseI
          -----                                     -----
801  gagctcggat cccgccgagc ccaaattctc tgacaaaact cacacatgcc caccgtgcc agcacctccc gtggccggcc
>.>>
          >>.....HCH2CH3pvaa.....>

881  cgtcagtctt cctcttcccc caaaaccca aggacaccct catgatgcc cggacccttg aggtcacatg cgtcgtggtg
>.....HCH2CH3pvaa.....>

961  gacgtgagcc acgaagacc tgggtcaag ttcaactcgt acgtggacgg cgtggagggt cataatgcc agacaaagcc
>.....HCH2CH3pvaa.....>

          SacII
          -----
1041  gccggaggag cagtacaaca gcacgtaccg tgtggtcagc gtctcaccg tctgcacca ggactggctg aatggcaagg
>.....HCH2CH3pvaa.....>

1121  agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
>.....HCH2CH3pvaa.....>

1201  gaaccacagg tgtacacct gcccccaccc cgggatgagc tgaccaagaa ccagtcagc ctgacctgcc tggtaaaagg
>.....HCH2CH3pvaa.....>

1281  ctcttatccc agccacatcg ccgtggagtg ggagagcaat gggcaaccgg agaacaacta caagaccacg cctcccgctc
>.....HCH2CH3pvaa.....>

1361  tggactccga cggctccttc ttctctaca gcaagtcac cgtggacaag agcagctggc agcaggggaa cgtcttctca
>.....HCH2CH3pvaa.....>

          Ppu10I
          -----
          NsiI
          -----
          BfrBI
          -----
1441  tgctccgtga tgcattgagg cctgcacaat cactataccc agaaatctct gactctgagc ccaggcaaga aggaccccaa
>.....HCH2CH3pvaa.....>>

1521  gttctgggtc ctggtggtgg tgggaggcgt gctggcctgt tactctctcc tggtgaccgt ggccttcac atctctggg
>>.....CD28tm.....>

1601  tgggagtgca ggtcgaaacc atctcccag gagacggcg cacctcccc aagcgggcc agacctgcgt ggtgcactac
>>
>>.....FKBP12.....>

1681  accgggatgc ttgaagatgg aaagaaattc gattcctccc gggacagaaa caagcccttt aagtttatgc taggcaagca
>.....FKBP12.....>

1761  ggaggtgatc cgagcctggg aagaagggtg tgcccagatg agtggtgggc agagaccaa actgactata tctccagatt
>.....FKBP12.....>

1841  atgectatgg tgccactggg ccccaggca tcatccccc acatgccact ctgctcttcg atgtggagct tctaaaactg
>.....FKBP12.....>

1921  gaacgcgcag agggcggggc ctcatctgct acctgtggag atgtcgagga aaatccggc ccaatggctt ctagaatcct
>>>
>>.....FMD-2A.....>>
>>.....>

2001  ctggcatgag atgtggcatg aaggcctgga agagcatct cgtttgtact ttggggaaa gaacgtgaaa ggcattgtt
>.....FRB.....>

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2081 aggtgctgga gcccttgcat gctatgatgg aacggggccc ccagactctg aaggaaacat cctttaatca ggctatggt
>.....FRB.....>

2161 cgagatttaa tggaggccca agagtgggag aggaagtaca tgaaatcagg gaatgtcaag gacctctccc aagcctggga
>.....FRB.....>

                    XhoI
                    -----
2241 cctctattat catgtgttcc gacgaatctc aaagctcgag agtggcggag gaggcagttc aagggtccgc gacgcgctg
>.....FRB.....>>
                                     >>...linker....>>
                                             >>.....CD3-Zeta.....>

10 2321 cataccagca ggggcagaat cagctgtaca acgagctcaa cctcggtagg cgcgaggaat acgatgtgct cgataagaga
>.....CD3-Zeta.....>

                    NruI
                    -----
2401 agagggtcgc atcccagat gggaggaaaa cctcagcgcc gcaagaaccc tcaggagggg ctgtataacg aactgcagaa
>.....CD3-Zeta.....>

                    SfiI
                    -----
2481 ggataagatg gcagaggcct actccgagat tggcatgaag ggtgagagga gaagaggtaa aggccatgac ggctctacc
>.....CD3-Zeta.....>

20 2561 aaggcctctc taccgcaacg aaagacactt atgacgctct gcatatgcag gctctcccc ctaggcagtc cactaattac
>.....CD3-Zeta.....>>
                                             >>..FMD-2A..>

2641 gcccttctga aacttgccgg cgatgtggag tctaaccctg gccctatcct gtggcacgag atgtggcatg agggctctgga
>.....FMD-2A.....>>
                                             >>.....FRB.....>

25 2721 agaggccagc cggtgtact tcggagagag aaatgttaag ggtatgttcg aggtgctgga gccgcttcac gctatgatgg
>.....FRB.....>

30 2801 agaggggccc ccagaccctc aaagaaacca gcttcaatca agcctacggg agggatctta tggaggcaca ggaatgggtg
>.....FRB.....>

2881 cggaagtaca tgaagagcgg gaacgtcaag gacctgctcc aggcttgga tttgtattat cagctcttta gccggatcag
>.....FRB.....>

35 2961 caagtctgca gccggaggaa gtctgcacag cgattacatg aacatgacce cccgaaggcc cggaccaca cgcaaact
>.>>
                >>.....linker....>>
                        >>.....CD28.....>

3041 atcaacccta tgctcccca cgcgacttcg ccgcctaccg gtcacgcgcc gaggggccc gctctttgtt gacttgccgg
>.....CD28.....>>
                                             >>.....FMD-2A.....>

40 3121 gacgttgaag agaatcctgg ccccatcctt tggcacgaga tgtggcacga gggcctggag gaagcctccc ggctgtattt
>.....FMD-2A.....>>
                        >>.....FRB.....>

45 3201 cgagagcgc aacgtcaaag gaatgtttga ggtgctggag cctctccatg caatgatgga gagggggcct cagactctta
>.....FRB.....>

3281 aagaaacatc cttaatcag gcttacggta gagatttgat ggaggctcaa gaatgggtcc ggaaatacat gaagagtgga
>.....FRB.....>

50 3361 aacgttaaag acctgctgca ggcattggac ctgtactatc acgtattcag acggatctca aagtcaggcg gcggtggctc
>.....FRB.....>>
                                             >>...linker....>

3441 cctttatata ttcaagcagc ctttcatgag gccggtgcag accacacaag aagaggatgg gtgctcttgc cggttcccc
>
>>.....41BB.....>

55 3521 aggaggagca gggcggatgc gagctctga
>.....41BB.....>>

```

SEQ ID No. 22. Inducible CAR with multi-spanning receptor component. The receptor component comprises of a signal peptide, an anti-CD19 scFv, a spacer from the Fc domain of human IgG1, a CD28 transmembrane domain, a first FRB, then the first trans-membrane, minor extracellular loop and second transmembrane domain of CD20, then a second FRB, then the third transmembrane domain, the major extracellular loop and the fourth transmembrane domain of CD20, and a third FRB. The signalling component comprises of FRB and a compound of endodomains from CD28, OX40 and CD3-Zeta. A foot-and-mouth disease 2A peptide separates the two components.

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SgrAI

1 atggagaccg acaccctgct gctgtgggtg ctgctgctgt gggtgccagg cagcaccggc gacatccaga tgaccagac

>>.....signal peptide.....>>

>>.....scFv anti-CD19.....>

81 caccagcagc ctgagcgcca gcctggggcga cggggtgacc atcagctgca gagccagcca ggacatcagc aagtacctga

>.....scFv anti-CD19.....>

161 actggtacca gcaaaagccc gacggcaccg tgaagctgct gatctaccac accagccggc tgcacagcgg cgtgcccagc

>.....scFv anti-CD19.....>

XcmI

241 cggttcagcg gcagcggcag cggcaccgac tacagcctga ccatcagcaa cctggagcag gaggacatcg ccacctactt

>.....scFv anti-CD19.....>

321 ctgccagcag ggcaacaccc tgcctacac cttcggaggc ggcaccaagc tggagatcac caaggccgga ggcggaggct

>.....scFv anti-CD19.....>

StuI

401 ctggcggagg cggctctggc ggaggcgget ctggcggagg cggcagcag gtgaagctgc aggagtctgg cccaggcctg

>.....scFv anti-CD19.....>

481 gtggcccca ggcagagcct gagcgtgacc tgcaccgtga gggcggtgag cctgcccgac tacggcgtga gctggatcag

>.....scFv anti-CD19.....>

561 gcagcccca cggaagggcc tggagtggct gggcgtgatc tggggcagcg agaccaccta ctacaacagc gcctgaaga

>.....scFv anti-CD19.....>

641 gccggctgac catcatcaag gacaacagca agagccaggt gttcctgaag atgaacagcc tgcagaccga cgacaccgcc

>.....scFv anti-CD19.....>

721 atctactact gcgccaagca ctactactat ggcggcagct acgctatgga ctactggggc cagggcacca gcgtgaccgt

>.....scFv anti-CD19.....>

BamHI

FseI

801 gagctcggat cccgccgagc ccaaactctc tgacaaaact cacacatgcc caccgtgcc agcacctccc gtggccggcc

>.>>

>>.....HCH2CH3pvaa.....>

881 cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgategcc cggacccctg aggtcacatg cgtggtggtg
>.....HCH2CH3pvaa.....>

5 961 gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg cataatgcc agacaaagcc
>.....HCH2CH3pvaa.....>

SacII

1041 gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc gtctcaccg tcctgcacca ggactggctg aatggcaagg
>.....HCH2CH3pvaa.....>

10 1121 agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga
>.....HCH2CH3pvaa.....>

15 1201 gaaccacagg tgtacacct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc ctgacctgc tggtaaagg
>.....HCH2CH3pvaa.....>

1281 cttctatccc agcgacatcg ccgtggagtg ggagagcaat gggcaaccgg agaacaacta caagaccag cctcccgtag
>.....HCH2CH3pvaa.....>

20 1361 tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca
>.....HCH2CH3pvaa.....>

Ppu10I

NsiI

25 BfrBI

1441 tgctccgtga tgcattgagg cctgcacaat cactataccc agaatctctt gattctgagc ccaggcaaga aggaccccaa
>.....HCH2CH3pvaa.....>>

30 1521 gttctgggtc ctggtggtgg tgggaggcgt gctggcctgt tactctctcc tggtgaccgt ggccttcac atctctctgg
>>.....CD28tm.....>

StuI

1601 tgatcctctg gcatgagatg tggcatgaag gcctggaaga ggcattctgt ttgtactttg gggaaaggaa cgtgaaaggc
>>
35 >>.....FRB.....>

StuI

1681 atgtttgagg tgcaggagcc ctgcatgct atgatggaac ggggccccca gactctgaag gaaacatcct ttaatcaggc
>.....FRB.....>

40 1761 ctatggtcga gatttaattg aggcccaaga gtggtgcagg aagtacatga aatcagggaa tgtcaaggac ctctccaag
>.....FRB.....>

1841 cctgggacct ctattatcat gtgtccgac gaatctcaaa gagcagcctg gtgggaccca ccagagctt cttcatgcgg
>.....FRB.....>>
45 >>.....CD20.....>

StuI

1921 gagagcaaga ccctgggagc cgtgcagatc atgaacggcc tgtccacat cgcctggga ggctgctga tgatccctgc
>.....CD20.....>

50 2001 cggcatctac gcccaatct gcgtgaccgt gtggtacca ctgtggggag gcatcatgta catcatcagc ggcagcctgc
>.....CD20.....>

2081 tggccgccac cgaagaaga agcggagggg gaagcatcct gtggcacgag atgtggcatg aggtcttga agaggccagc
55 >.....CD20.....>>
>>...linker...>>
>>.....FRB.....>

5

2161 cggtgttact tcgagagag aaatgttaag ggtatgttcg aggtgctgga gccgcttcac gctatgatgg agagggggccc
>.....FRB.....>

2241 ccagaccctc aaagaaacca gcttcaatca agcctacggg agggatctta tggaggcaca ggaatgggtg cggaagtaca
>.....FRB.....>

10

2321 tgaagagcgg gaacgtcaag gacctgctcc aggttgggga ttgtattat cacgtcttta gccggatcag caagtctgga
>.....FRB.....>>
>>...>

2401 ggccggaggaa gtcggaagtg cctgggtgaag ggcaagatga tcatgaacag cctgagcctg ttcgccgccca tcagcggcat
>...linker...>>
>>.....CD20.....>

15

2481 gatcctgagc atcatggaca tcctgaacat caagatcagc cacttctga agatggagag cctgaacttc atccggggccc
>.....CD20.....>

20

2561 acaccccata catcaacatc tacaactgcg agcctgccaa cccagcagag aagaacagcc ccagcaccca gtactgctac
>.....CD20.....>

2641 agcatccaga gcctgttcct gggcatcctg agcgtgatgc tgatcttcgc ctctctccag gagctgggtga tcgccggcat
>.....CD20.....>

25

2721 cgtcgagaaac gagtggaagc ggacctgcag ccggcccaag agcatcctgt ggcacgagat gtggcatgag ggtctggaag
>.....CD20.....>>
>>.....FRB.....>

30

2801 aggccagccg gctgtacttc ggagagagaa atgttaaggg tatgttcgag gtgctggagc cgcttcacgc tatgatggag
>.....FRB.....>

2881 agggggcccc agaccctcaa agaaaccagc ttcaatcaag cctacgggag ggatcttatg gaggcacagg aatgggtgtc
>.....FRB.....>

35

2961 gaagtacatg aagagcggga acgtcaagga cctgctccag gcttgggatt tgtattatca cgtctttagg cggatcagca
>.....FRB.....>

3041 agccgcgaga gggccggggc tcattgctga cctgtggaga tgtcgaggaa aatcccggcc caggagtga ggtggaaacc
>>>
>>.....FMD-2A.....>>
>>.....FKBP12.....>

45

3121 atctccccag gagacgggcg cacttcccc aagcggggcc agacctgcgt ggtgcactac accgggatgc ttgaagatgg
>.....FKBP12.....>

3201 aaagaaattc gattcctccc gggacagaaa caagcccttt aagtttatgc taggcaagca ggaggtgatc cgaggctggg
>.....FKBP12.....>

50

3281 aagaaggggt tgcccagatg agtgtgggtc agagagccaa actgactata tctccagatt atgcctatgg tgccactggg
>.....FKBP12.....>

3361 caccagga tcatccacc acatgccact ctgtcttcg atgtggagct tctaaaactg gaaaggagta agaggagcag
>.....FKBP12.....>>
>>.....28OXZ.....>

55

3441 gtcctgcac agtgactaca tgaacatgac tccccgcgc ccggggccca ccgcaagca ttaccagccc tatgccccac
>.....28OXZ.....>

3521 cagcgactt cgcagcctat cgctccaggg accagaggct gccccccgat gccacaagc cccctggggg aggcagtttc
>.....280XZ.....>

5 3601 cggacccccca tccaagagga gcaggccgac gcccaactcca ccctggccaa gatcagagtg aagttcagca ggagcgcaga
>.....280XZ.....>

3681 cgcccccgcg taccagcagg gccagaacca gctctataac gagctcaatc taggacgaag agaggagtac gatgttttgg
>.....280XZ.....>

10 3761 acaagagacg tggccgggac cctgagatgg ggggaaagcc gagaaggaag aaccctcagg aaggcctgta caatgaactg
>.....280XZ.....>

15 3841 cagaaagata agatggcgga ggcctacagt gagattggga tgaaaggcga gcgccggagg ggcaaggggc acgatggcct
>.....280XZ.....>

3921 ttaccagggg ctcagtacag ccaccaagga cacctacgac gcccttcaca tgcaggccct gcctcctcgc tga
>.....280XZ.....>>

20

[0203] SEQ ID No. 23. Simple inducible CAR comprised of one receptor component and one signalling component. The signalling component comprises an ecdysone receptor and the CD3-Zeta endodomain (*italic*). A FMD-2A peptide (shown in **bold**) separates this signalling component from the receptor component. The receptor component comprises an anti-CD19 scFv, a HCH2CH3 spacer (underlined), a CD148 transmembrane domain (highlighted in grey) and the retinoid X receptor as endodomain.

25

SEQ ID No. 23

30 MPVDRILEAEELAVEQKSDQGVGPGGTGGSGSSPNDPVTNICQAADKQLFTLVEWAKRIP
HFSSLPLDDQVILLRAGWNELLIASFHSRSIDVRDGILLATGLHVHRNSAHSAGVGAI
RVLTELVSKMRDMRMDKTELGCLRAIILFNPEVRGLKSAQEVELLREKVYAALEEYTRT
35 HPDEPGRFAKLLRLPSLRSIGLKCLEHLFFFRLIGDVPIDTFLMEMLESPSDSSGGGSA
DAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA
EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR**RAEGRGSLLTCGDVEE**
NPGPMETDTLLLWVLLLWVPGSTGDIQMTQTTSSLSASLGDRVTISCRASQDISKYL
40 NWWYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPY
TFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGV
SLPDYGVSWIRQPPRKGLEWLGVIWGSETTYNSALKSRLTIKDNSKSQVFLKMNSLQT
DDTAIYYCAKHYYYGGSYAMDYWGQGTSTVTVSSDPAEPKSPDKTHTCPPCPAPPVAGPSV
45 FLFPPKPKDTLMIARTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSPGKKDPMALIVLGGVAGLLLFIGLGIFFCVRCRHR
50 RQAERMAQIKRVVSEKKTAQAPHRFQKTCSPISGGGGSRPECVVPETQCAMKRKEKKAQK
EKDKLPVSTTTVDDHMPPIMQCEPPPEAARIHEVVPRFLSDKLLETNRQKNIPQLTANQ
QFLIARLIWYQDGYEQPSDEDLKRITQTWQQADDENEESDTPFRQITEMTILTVQLIVEF
AKGLPGFAKISQPDQITLLKACSEVMMLRVARRYDAASDSILFANNQAYTRDNYRKAGM

55 AEVIEDLLHFCRCMYSMALDNIHYALLTAVVIFSDRPGLEQPQLVEEIQRYYLNTLRIYI
LNQLSGSARSSVIYGKILSILSELRTLGMQNSNMCISLKLKNRKLPPFLEEIWDVADMSH
TQPPPILESPTNL

[0204] SEQ ID No. 24. Simple inducible CAR comprised of one receptor component and one signalling component. The signalling component comprises an FKBP12 domain (underlined) and the CD3-Zeta endodomain (*italic*). A FMD-2A peptide (shown in bold) separates this signalling component from the receptor component. The receptor component comprises an anti-CD19 scFv, a HCH2CH3 spacer (highlighted in grey), a CD148 transmembrane domain and the and FKBP12 domain (underlined).

SEQ ID No. 24

10 MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVIRGW
EEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLESGGGSADAPAYQ
QGQNQLYNE~~LN~~LGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR**RAEGRGSLLTCGDVEENPGP**ME
15 TDTLLLWVLLLWVPGSTGDIQMTQTTSSLSASLGDRVTTISCRASQDISKYLNWYQQKPDG
TVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGT
KLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSGVSLPDYG
VSWIRQPPRKGLEWLGVWVWGSETTYNSALKSRLTI IKDNSKSQVFLKMNSLQTDDTAIY
20 YCAKHYYYGGSYAMDYWGQGTSVTVSSDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPK
PKDTILMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
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CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFFLYSKLTVDKSRWQQGNVFS
25 VMHEALHNHYTQKSLSLSPGKKDPMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQAERM
AQIKRVVSEKKTAQAPHRFQKTCSPISGGGGSGVQVETISPGDGRTFPKRGQTCVVHYTG
MLEDGKKVDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHP
GIIPPHATLVFDVELLKLE

SEQUENCE LISTING

[0205]

35 <110> UCL Business PLC

<120> Signalling system

<130> P104247PCT
40 <150> GB 1405845.7
<151> 2014-04-01

<160> 26
45 <170> PatentIn version 3.5

<210> 1
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<213> Artificial Sequence

<220>
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5	Pro	Lys	Arg	Gly	Gln	Thr	Cys	Val	Val	His	Tyr	Thr	Gly	Met	Leu	Glu
				20					25					30		
10	Asp	Gly	Lys	Lys	Phe	Asp	Ser	Ser	Arg	Asp	Arg	Asn	Lys	Pro	Phe	Lys
			35					40					45			
15	Phe	Met	Leu	Gly	Lys	Gln	Glu	Val	Ile	Arg	Gly	Trp	Glu	Glu	Gly	Val
		50					55					60				
20	Ala	Gln	Met	Ser	Val	Gly	Gln	Arg	Ala	Lys	Leu	Thr	Ile	Ser	Pro	Asp
	65					70					75					80
25	Tyr	Ala	Tyr	Gly	Ala	Thr	Gly	His	Pro	Gly	Ile	Ile	Pro	Pro	His	Ala
					85					90					95	
30	Thr	Leu	Val	Phe	Asp	Val	Glu	Leu	Leu	Lys	Leu	Glu				
				100					105							

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30	<213>	Artificial Sequence
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	<223>	CID binding domain - wild-type FRB segment of mTOR
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5	Glu	Ala	Ser	Arg	Leu	Tyr	Phe	Gly	Glu	Arg	Asn	Val	Lys	Gly	Met	Phe
				20					25					30		
10	Glu	Val	Leu	Glu	Pro	Leu	His	Ala	Met	Met	Glu	Arg	Gly	Pro	Gln	Thr
			35					40					45			
15	Leu	Lys	Glu	Thr	Ser	Phe	Asn	Gln	Ala	Tyr	Gly	Arg	Asp	Leu	Met	Glu
		50					55					60				
20	Ala	Gln	Glu	Trp	Cys	Arg	Lys	Tyr	Met	Lys	Ser	Gly	Asn	Val	Lys	Asp
	65					70					75					80
25	Leu	Thr	Gln	Ala	Trp	Asp	Leu	Tyr	Tyr	His	Val	Phe	Arg	Arg	Ile	Ser
					85					90					95	
30	Lys	Leu	Glu	Ser												
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40	Glu	Ala	Ser	Arg	Leu	Tyr	Phe	Gly	Glu	Arg	Asn	Val	Lys	Gly	Met	Phe
				20					25					30		
45	Glu	Val	Leu	Glu	Pro	Leu	His	Ala	Met	Met	Glu	Arg	Gly	Pro	Gln	Thr
			35					40					45			
50	Leu	Lys	Glu	Thr	Ser	Phe	Asn	Gln	Ala	Tyr	Gly	Arg	Asp	Leu	Met	Glu
		50					55					60				
55	Ala	Gln	Glu	Trp	Cys	Arg	Lys	Tyr	Met	Lys	Ser	Gly	Asn	Val	Lys	Asp
	65					70					75					80
	Leu	Leu	Gln	Ala	Trp	Asp	Leu	Tyr	Tyr	His	Val	Phe	Arg	Arg	Ile	Ser
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Lys Leu Glu Ser
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Glu Ala Ser Arg Leu Tyr Phe Gly Glu Arg Asn Val Lys Gly Met Phe
20 25 30

Glu Val Leu Glu Pro Leu His Ala Met Met Glu Arg Gly Pro Gln Thr
35 40 45

25
Leu Lys Glu Thr Ser Phe Asn Gln Ala Tyr Gly Arg Asp Leu Met Glu
50 55 60

30 Ala Gln Glu Trp Cys Arg Lys Tyr Met Lys Ser Gly Asn Val Lys Asp
65 70 75 80

Leu His Gln Ala Phe Asp Leu Tyr Tyr His Val Phe Arg Arg Ile Ser
85 90 95

Lys Leu Glu Ser
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<400> 5

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				20					25					30		
10	Glu	Val	Leu	Glu	Pro	Leu	His	Ala	Met	Met	Glu	Arg	Gly	Pro	Gln	Thr
			35					40					45			
15	Leu	Lys	Glu	Thr	Ser	Phe	Asn	Gln	Ala	Tyr	Gly	Arg	Asp	Leu	Met	Glu
		50					55					60				
20	Ala	Gln	Glu	Trp	Cys	Arg	Lys	Tyr	Met	Lys	Ser	Gly	Asn	Val	Pro	Asp
	65					70					75					80
25	Leu	Thr	Gln	Ala	Trp	Asp	Leu	Tyr	Tyr	His	Val	Phe	Arg	Arg	Ile	Ser
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30	Lys	Leu	Glu	Ser												
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	1				5					10					15	
45	Asp	His	Ala	Asp	Gly											
				20												
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Met Ser Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
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5 His Ala Ala Arg Pro
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<210> 8

<211> 20

10 <212> PRT

<213> Artificial Sequence

<220>

<223> Signal peptide

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<400> 8

Met Ala Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp Leu Thr
1 5 10 15

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Asp Ala Arg Cys
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25 <210> 9

<211> 234

<212> PRT

<213> Artificial Sequence

30 <220>

<223> Spacer (hinge-CH₂CH₃ of human IgG1)

<400> 9

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5	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro
				20					25					30		
10	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val
			35					40					45			
15	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
		50					55					60				
20	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln
	65					70					75					80
25	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
					85					90					95	
30	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
				100					105					110		
35	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
			115					120					125			
40	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr
		130					135					140				
45	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
	145					150					155					160
50	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
					165					170					175	
55	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr
				180					185					190		
60	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
			195					200					205			
65	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
		210					215					220				
70	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Asp						
	225					230										

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	Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly 20 25 30
15	Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile 35 40 45
20	<210> 11
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25	<220>
	<223> Spacer (human IgG1 hinge)
	<400> 11
30	Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro 1 5 10 15
	Lys Asp Pro Lys 20
35	<210> 12
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	<223> Spacer (CD2 ectodomain)
45	<400> 12
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	Lys	Glu	Ile	Thr	Asn	Ala	Leu	Glu	Thr	Trp	Gly	Ala	Leu	Gly	Gln	Asp
	1				5					10					15	
5	Ile	Asn	Leu	Asp	Ile	Pro	Ser	Phe	Gln	Met	Ser	Asp	Asp	Ile	Asp	Asp
				20					25					30		
10	Ile	Lys	Trp	Glu	Lys	Thr	Ser	Asp	Lys	Lys	Lys	Ile	Ala	Gln	Phe	Arg
			35					40					45			
15	Lys	Glu	Lys	Glu	Thr	Phe	Lys	Glu	Lys	Asp	Thr	Tyr	Lys	Leu	Phe	Lys
		50					55					60				
20	Asn	Gly	Thr	Leu	Lys	Ile	Lys	His	Leu	Lys	Thr	Asp	Asp	Gln	Asp	Ile
	65					70					75					80
25	Tyr	Lys	Val	Ser	Ile	Tyr	Asp	Thr	Lys	Gly	Lys	Asn	Val	Leu	Glu	Lys
					85					90					95	
30	Ile	Phe	Asp	Leu	Lys	Ile	Gln	Glu	Arg	Val	Ser	Lys	Pro	Lys	Ile	Ser
				100					105					110		
35	Trp	Thr	Cys	Ile	Asn	Thr	Thr	Leu	Thr	Cys	Glu	Val	Met	Asn	Gly	Thr
			115					120					125			
40	Asp	Pro	Glu	Leu	Asn	Leu	Tyr	Gln	Asp	Gly	Lys	His	Leu	Lys	Leu	Ser
		130					135					140				
45	Gln	Arg	Val	Ile	Thr	His	Lys	Trp	Thr	Thr	Ser	Leu	Ser	Ala	Lys	Phe
	145					150					155					160
50	Lys	Cys	Thr	Ala	Gly	Asn	Lys	Val	Ser	Lys	Glu	Ser	Ser	Val	Glu	Pro
					165					170					175	
55	Val	Ser	Cys	Pro	Glu	Lys	Gly	Leu	Asp							
				180					185							
55	Ser	Leu	Asp	Asn	Asn	Gly	Thr	Ala	Thr	Pro	Glu	Leu	Pro	Thr	Gln	Gly
	1				5					10					15	
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	<212> PRT															
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	<223> Spacer (CD34 ectodomain)															
	<400> 13															

	Thr	Phe	Ser	Asn	Val	Ser	Thr	Asn	Val	Ser	Tyr	Gln	Glu	Thr	Thr	Thr
				20					25					30		
5	Pro	Ser	Thr	Leu	Gly	Ser	Thr	Ser	Leu	His	Pro	Val	Ser	Gln	His	Gly
			35					40					45			
10	Asn	Glu	Ala	Thr	Thr	Asn	Ile	Thr	Glu	Thr	Thr	Val	Lys	Phe	Thr	Ser
		50					55					60				
15	Thr	Ser	Val	Ile	Thr	Ser	Val	Tyr	Gly	Asn	Thr	Asn	Ser	Ser	Val	Gln
	65					70					75					80
20	Ser	Gln	Thr	Ser	Val	Ile	Ser	Thr	Val	Phe	Thr	Thr	Pro	Ala	Asn	Val
					85					90					95	
25	Ser	Thr	Pro	Glu	Thr	Thr	Leu	Lys	Pro	Ser	Leu	Ser	Pro	Gly	Asn	Val
				100					105					110		
30	Ser	Asp	Leu	Ser	Thr	Thr	Ser	Thr	Ser	Leu	Ala	Thr	Ser	Pro	Thr	Lys
			115					120					125			
35	Pro	Tyr	Thr	Ser	Ser	Ser	Pro	Ile	Leu	Ser	Asp	Ile	Lys	Ala	Glu	Ile
		130					135					140				
40	Lys	Cys	Ser	Gly	Ile	Arg	Glu	Val	Lys	Leu	Thr	Gln	Gly	Ile	Cys	Leu
	145					150					155					160
45	Glu	Gln	Asn	Lys	Thr	Ser	Ser	Cys	Ala	Glu	Phe	Lys	Lys	Asp	Arg	Gly
					165					170					175	
50	Glu	Gly	Leu	Ala	Arg	Val	Leu	Cys	Gly	Glu	Glu	Gln	Ala	Asp	Ala	Asp
				180					185					190		
55	Ala	Gly	Ala	Gln	Val	Cys	Ser	Leu	Leu	Leu	Ala	Gln	Ser	Glu	Val	Arg
			195					200					205			
60	Pro	Gln	Cys	Leu	Leu	Leu	Val	Leu	Ala	Asn	Arg	Thr	Glu	Ile	Ser	Ser
		210					215					220				
65	Lys	Leu	Gln	Leu	Met	Lys	Lys	His	Gln	Ser	Asp	Leu	Lys	Lys	Leu	Gly
	225					230					235					240
70	Ile	Leu	Asp	Phe	Thr	Glu	Gln	Asp	Val	Ala	Ser	His	Gln	Ser	Tyr	Ser
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<223> Signalling component CD3 Z endodomain

<400> 14

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Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly

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Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr

20

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys

25

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys

30

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg

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Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala

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Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg

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<220>

<223> Signalling component CD28 and CD3 Zeta endodomains

<400> 15

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Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro

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Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro

60

Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala

	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu
	50					55						60				
5	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly
	65					70					75					80
10	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys	Asn	Pro	Gln	Glu
					85					90					95	
15	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala	Glu	Ala	Tyr	Ser
				100					105					110		
	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys	Gly	His	Asp	Gly
			115					120					125			
20	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr	Tyr	Asp	Ala	Leu
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25	His	Met	Gln	Ala	Leu	Pro	Pro	Arg								
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	<223> Signalling component CD28, OX40 and CD3 Zeta endodomains
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5	Arg	Arg	Pro	Gly	Pro	Thr	Arg	Lys	His	Tyr	Gln	Pro	Tyr	Ala	Pro	Pro
				20					25					30		
10	Arg	Asp	Phe	Ala	Ala	Tyr	Arg	Ser	Arg	Asp	Gln	Arg	Leu	Pro	Pro	Asp
			35					40					45			
15	Ala	His	Lys	Pro	Pro	Gly	Gly	Gly	Ser	Phe	Arg	Thr	Pro	Ile	Gln	Glu
		50					55					60				
20	Glu	Gln	Ala	Asp	Ala	His	Ser	Thr	Leu	Ala	Lys	Ile	Arg	Val	Lys	Phe
	65					70					75					80
25	Ser	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu
					85					90					95	
30	Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp
					100				105					110		
35	Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly	Gly	Lys	Pro	Arg	Arg	Lys
			115					120					125			
40	Asn	Pro	Gln	Glu	Gly	Leu	Tyr	Asn	Glu	Leu	Gln	Lys	Asp	Lys	Met	Ala
		130					135					140				
45	Glu	Ala	Tyr	Ser	Glu	Ile	Gly	Met	Lys	Gly	Glu	Arg	Arg	Arg	Gly	Lys
	145					150					155					160
50	Gly	His	Asp	Gly	Leu	Tyr	Gln	Gly	Leu	Ser	Thr	Ala	Thr	Lys	Asp	Thr
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55	Tyr	Asp	Ala	Leu	His	Met	Gln	Ala	Leu	Pro	Pro	Arg				
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<212> PRT
<213> Foot-and-mouth disease virus

<400> 17

Arg Ala Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu Glu
1 5 10 15

5 Asn Pro Gly Pro
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<210> 18
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<213> Foot-and-mouth disease virus

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20 Asn Pro Gly Pro
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<210> 19
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- <210> 23

<211> 1273

<212> PRT

<213> Artificial Sequence
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- <220>

<223> Simple inducible CAR
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- <400> 23

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20																
25																
30																
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40																
45																
50																
55																

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40	Glu	Val	Glu	Leu	Leu	Arg	Glu	Lys	Val	Tyr	Ala	Ala	Leu	Glu	Glu	Tyr
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65	Asp	Ala	Pro	Ala	Tyr	Gln	Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu
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70	Asn	Leu	Gly	Arg	Arg	Glu	Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly
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15	Ala	Lys	His	Tyr	Tyr	Tyr	Gly 615	Gly	Ser	Tyr	Ala	Met 620	Asp	Tyr	Trp	Gly
20	Gln 625	Gly	Thr	Ser	Val	Thr 630	Val	Ser	Ser	Asp	Pro 635	Ala	Glu	Pro	Lys	Ser 640
25	Pro	Asp	Lys	Thr 645	His	Thr	Cys	Pro	Pro	Cys 650	Pro	Ala	Pro	Pro	Val 655	Ala
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30	Ile	Ala	Arg 675	Thr	Pro	Glu	Val	Thr 680	Cys	Val	Val	Val	Asp 685	Val	Ser	His
35	Glu 690	Asp	Pro	Glu	Val	Lys 695	Phe	Asn	Trp	Tyr	Val	Asp 700	Gly	Val	Glu	Val
40	His 705	Asn	Ala	Lys	Thr	Lys 710	Pro	Arg	Glu	Glu	Gln 715	Tyr	Asn	Ser	Thr	Tyr 720
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45	Lys	Glu	Tyr	Lys 740	Cys	Lys	Val	Ser	Asn 745	Lys	Ala	Leu	Pro	Ala 750	Pro	Ile
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70	Ile	Ala	Arg	Leu	Ile	Trp	Tyr	Gln	Asp	Gly	Tyr	Glu	Gln	Pro	Ser	
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15	Leu	Pro	Gly	Phe	Ala	Lys	Ile	Ser	Gln	Pro	Asp	Gln	Ile	Thr	Leu
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25	Ala	Tyr	Thr	Arg	Asp	Asn	Tyr	Arg	Lys	Ala	Gly	Met	Ala	Glu	Val
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40	Ser	Asp	Arg	Pro	Gly	Leu	Glu	Gln	Pro	Gln	Leu	Val	Glu	Glu	Ile
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 <211> 919
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> Simple inducible CAR

10 <400> 24

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REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Nukleinsyre, der koder et kimærisk antigen receptor-signalsystem (CAR), der omfatter:

- 5 (i) en receptorkomponent, der omfatter et ekstracellulært antigen-bindende domæne, et transmembran-domæne og en intracellulær første CID-bindende (kemisk inducerede dimerisering) domæne (CBD1), og
- (ii) en intracellulær signalkomponent, der omfatter et
10 signaldomæne og et andet CID-bindende domæne (CBD2),
hvor nukleinsyren koder både receptorkomponenten og signalkomponenten af CAR-signalsystemet,
hvor CBD1 og CBD2 kan bindes samtidig til en CID, og
hvor, i fravær af CID, binding af det antigenbindende domæne til
15 antigen ikke resulterer i signalering gennem signaldomænet,
mens, ved tilstedeværelse af CID, receptorkomponenten og signalkomponenten heterodimeriserer, og binding af det antigenbindende domæne til antigen resulterer i signalering gennem signaldomænet.

20

2. Nukleinsyre ifølge krav 1, der producerer en polypeptid, der omfatter receptorkomponenten og signalkomponenten, der er forbundet af et spaltningssted.

- 25 3. Nukleinsyre ifølge krav 2, hvor spaltningsstedet er selvspaltende.

4. Nukleinsyre ifølge krav 3, hvor spaltningsstedet omfatter et 2A-selvspaltende peptid fra mund- og klovsygevirus (FMDV).

30

5. Nukleinsyre ifølge et hvilket som helst af de foregående krav, hvor:

- (i) CBD1 omfatter det rapamycin-bindende domæne af FK-bindende protein 12 (FKBP12), og CBD2 omfatter det FKBP12-rapamycin-
35 bindende (FRB) domæne af mTOR, eller
- (ii) CBD2 omfatter det rapamycin-bindende domæne af FK-bindende protein 12 (FKBP12), og CBD1 omfatter det FKBP12-rapamycin-bindende (FRB) domæne af mTOR, og

hvor CID'et er rapamycin eller et derivativ af denne, som er i stand til at forårsage, at receptorkomponenten og signalkomponenten heterodimeriserer.

5 6. Nukleinsyre ifølge et hvilket som helst af kravene 1 til 4, hvor CBD1 og CBD2 omfatter Fk506-bindende protein (FKBP) bindende domæner, der omfatter en F36V-mutation, hvor CID'et er AP1903 eller et derivativ af denne, som er i stand til at forårsage, at receptorkomponenten og signalkomponenten
10 heterodimeriserer.

7. Vektor, der omfatter en nukleinsyre ifølge et hvilket som helst af de foregående krav.

15 8. Vektor ifølge krav 7, der er en en retroviral vektor eller en lentiviral vektor eller en transposon.

9. T-celle eller NK-celle, der omfatter nukleinsyren ifølge et hvilket som helst af kravene 1 til 6 eller en vektor ifølge
20 krav 7 eller 8.

10. Farmaceutisk sammensætning, der omfatter en flerhed af T-celler eller NK-celler ifølge krav 9.

25 11. Farmaceutisk sammensætning ifølge krav 10 til brug ved behandling af en sygdom.

12. Farmaceutisk sammensætning til brug ifølge krav 11, hvor behandlingen omfatter følgende trin:

30 (i) isolation af en T-celle eller NK-celle, der indeholder prøve fra en person,
(ii) transduktion eller transfektion af T-cellen eller NK-cellen med en nukleinsyresekvens ifølge et hvilket som helst af kravene 1 til 6 eller en vektor ifølge krav 7 eller 8, og
35 (iii) indgivelse af T-cellerne eller NK-cellerne fra (ii) til personen.

13. Farmaceutisk sammensætning til brug ifølge krav 11 eller

12, hvor behandlingen yderligere omfatter trinnet med indgivelse af et CID til brig i CAR-signalsystemet til personen.

5 14. Farmaceutisk sammensætning til brug ifølge krav 13, der involverer overvågning af progressionen af sygdommen og/eller overvågning af toksisk aktivitet i personen og justering af dosis af CID'et for at tilvejebringe acceptable niveauer af sygdomsudvikling og/eller toksisk aktivitet.

10 15. Farmaceutisk sammensætning til brug ifølge et hvilket som helst af kravene 11 til 14, hvor sygdommen er en cancertype.

15 16. Fremgangsmåde til at fremstille en T- eller NK-celle ifølge krav 9, der omfatter trinnet med at introducere: en nukleinsyre ifølge et hvilket som helst af kravene 1 til 6 eller vektoren ifølge krav 7 eller 8, i en T-celle ex vivo.

17. Fremgangsmåde ifølge krav 16, hvor T- eller NK-cellen er fra en prøve, der er isoleret fra en person.

DRAWINGS

FIGURE 1

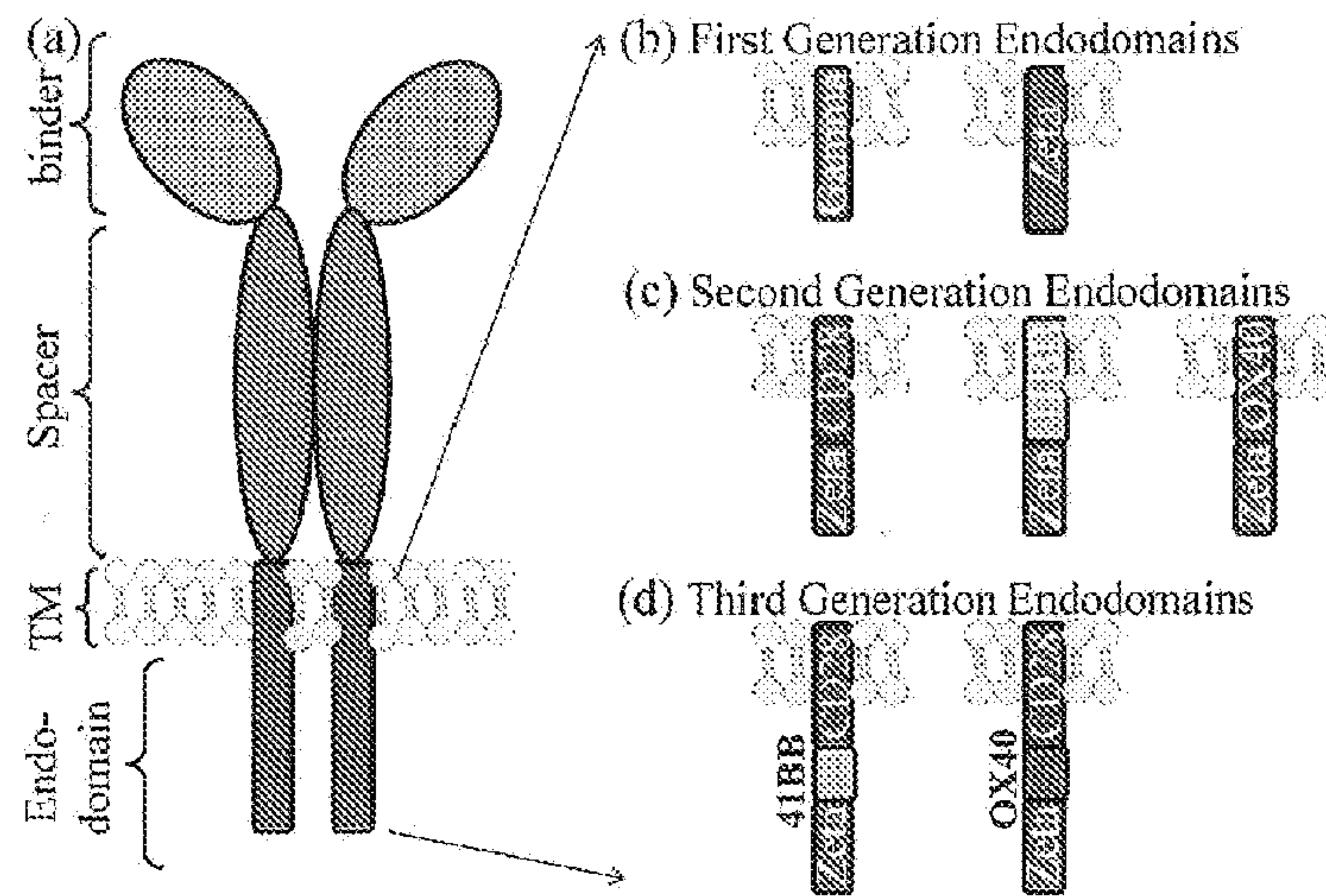


FIGURE 2

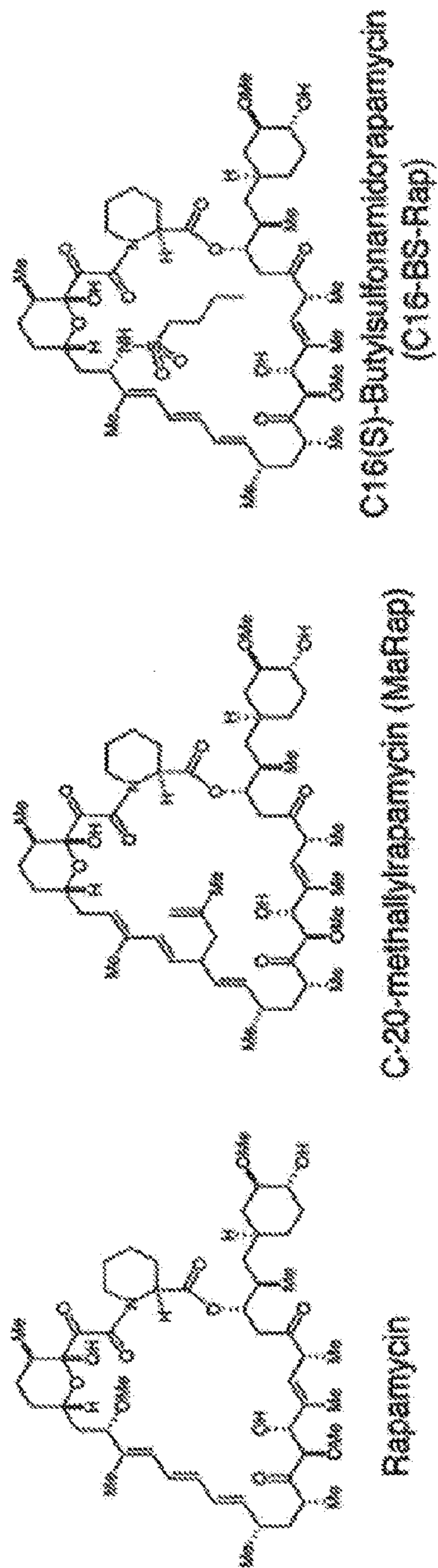


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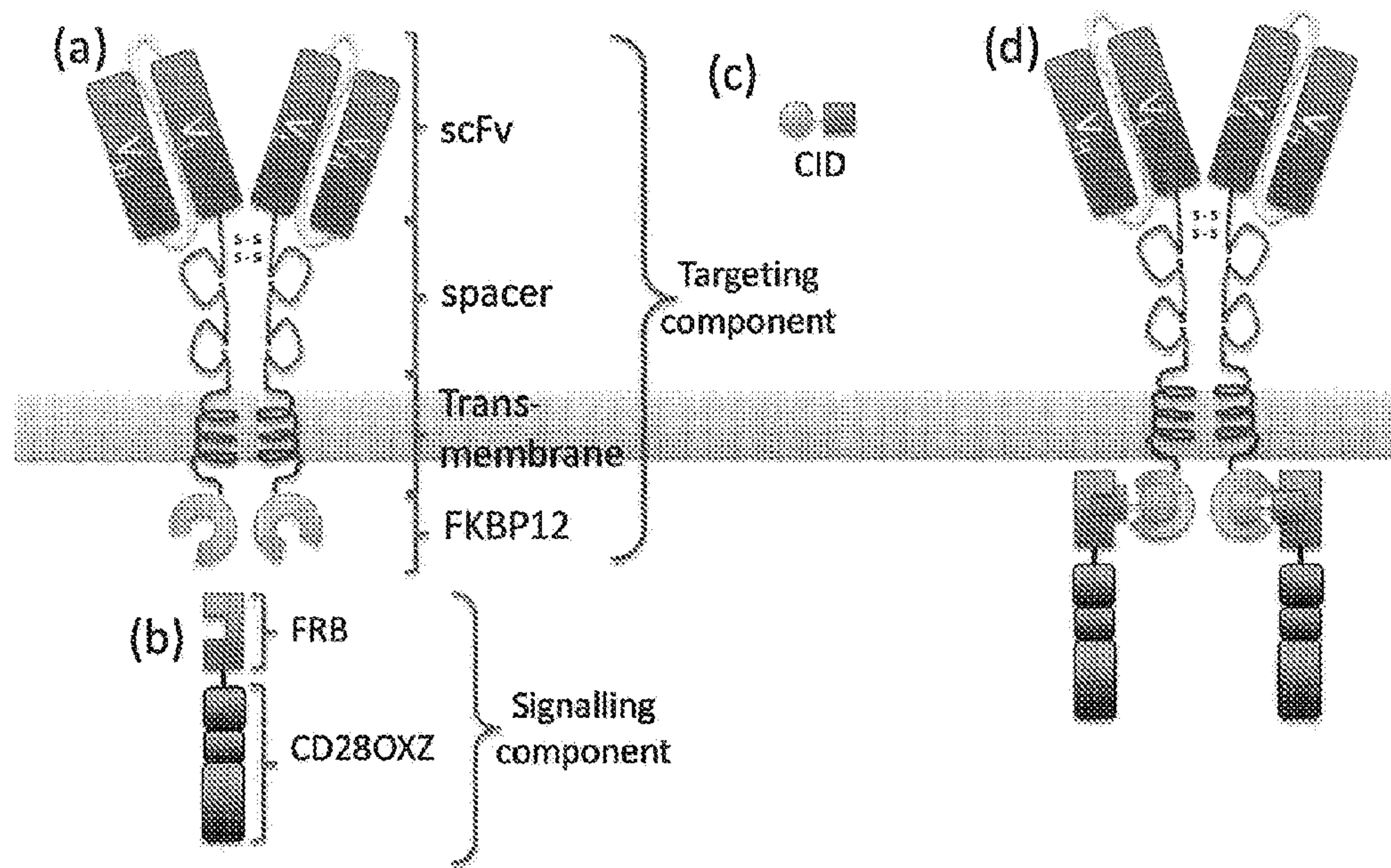


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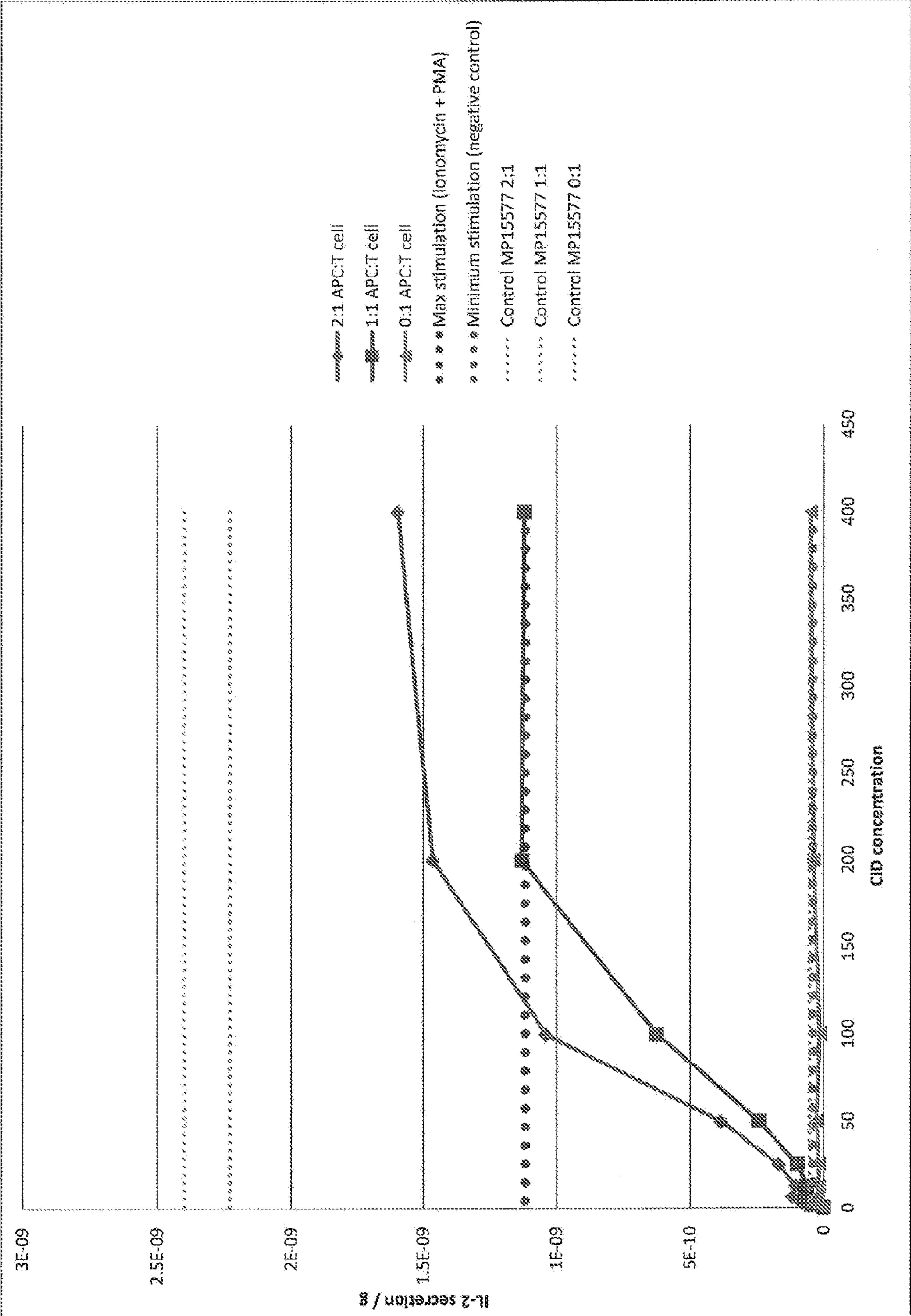
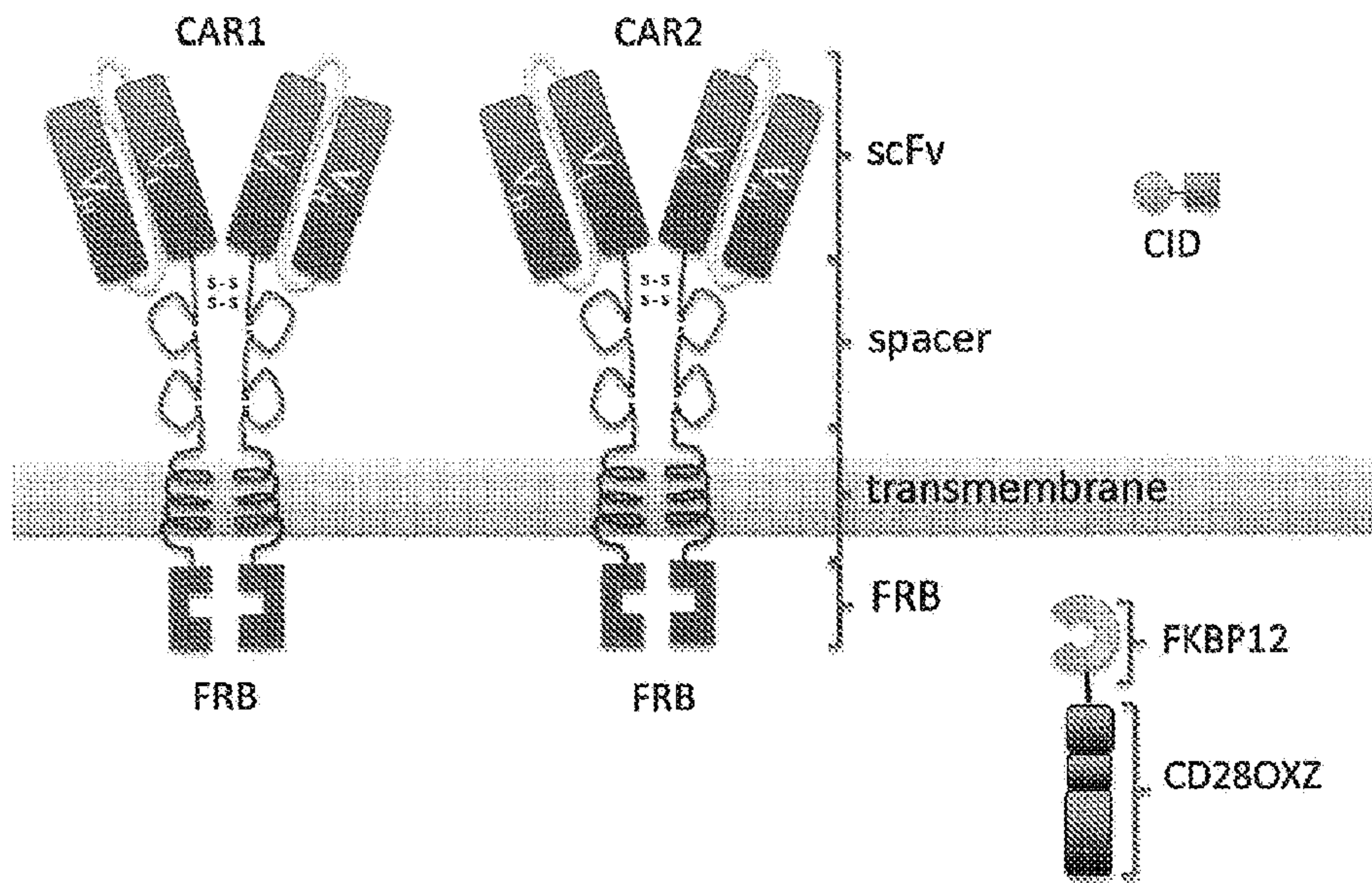


FIGURE 5

(a)



(b)

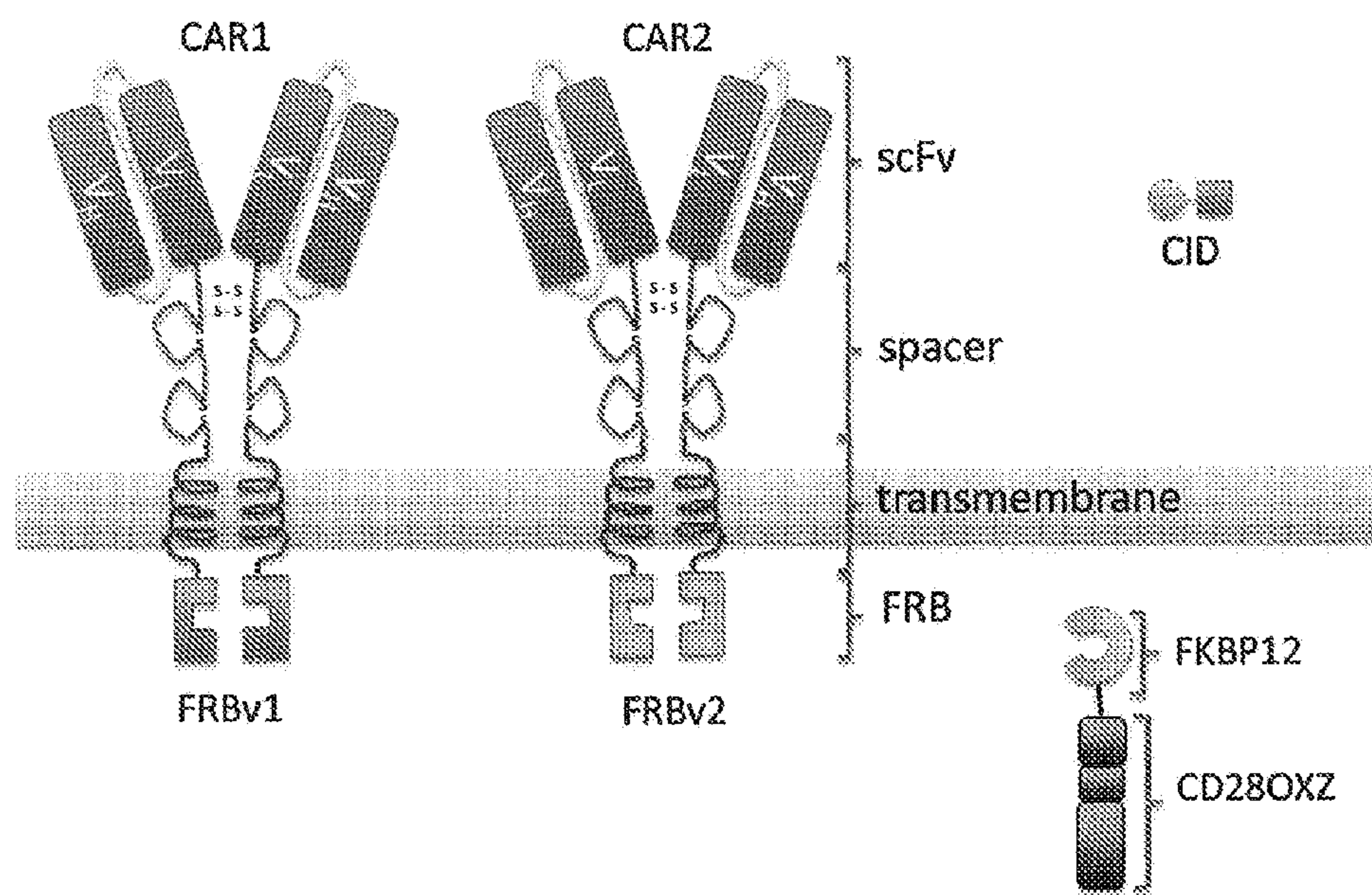


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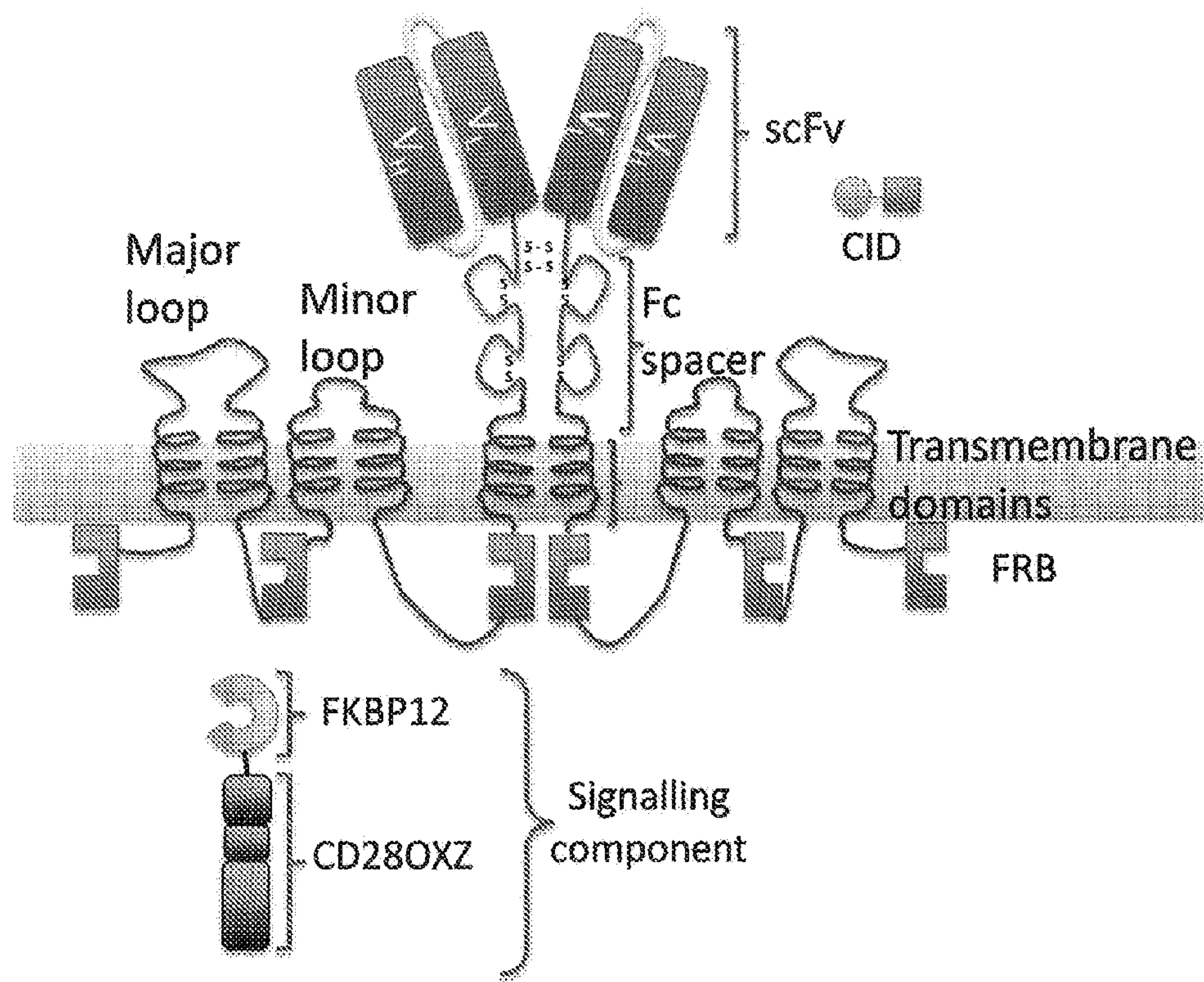
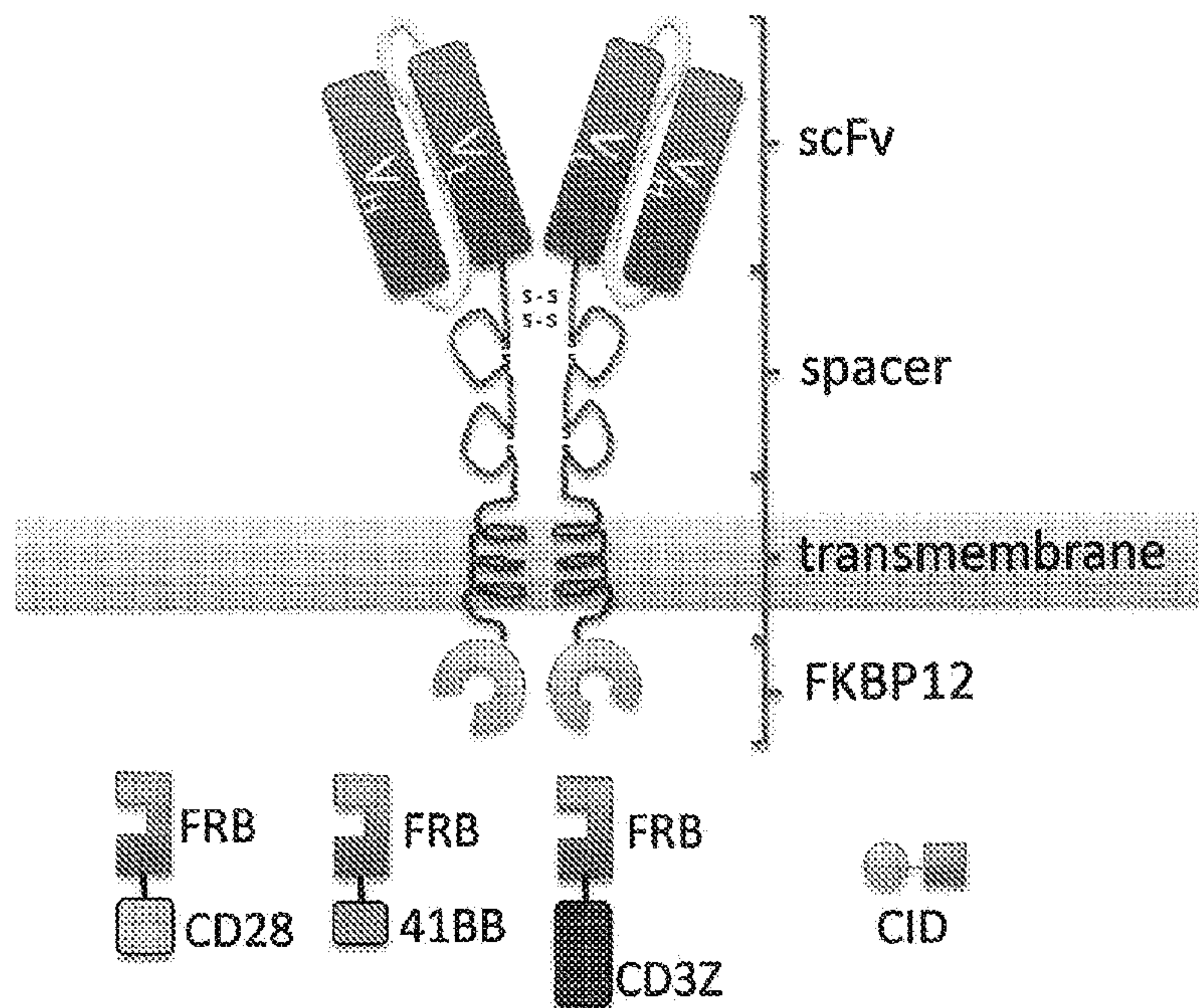


FIGURE 7

(a)



(b)

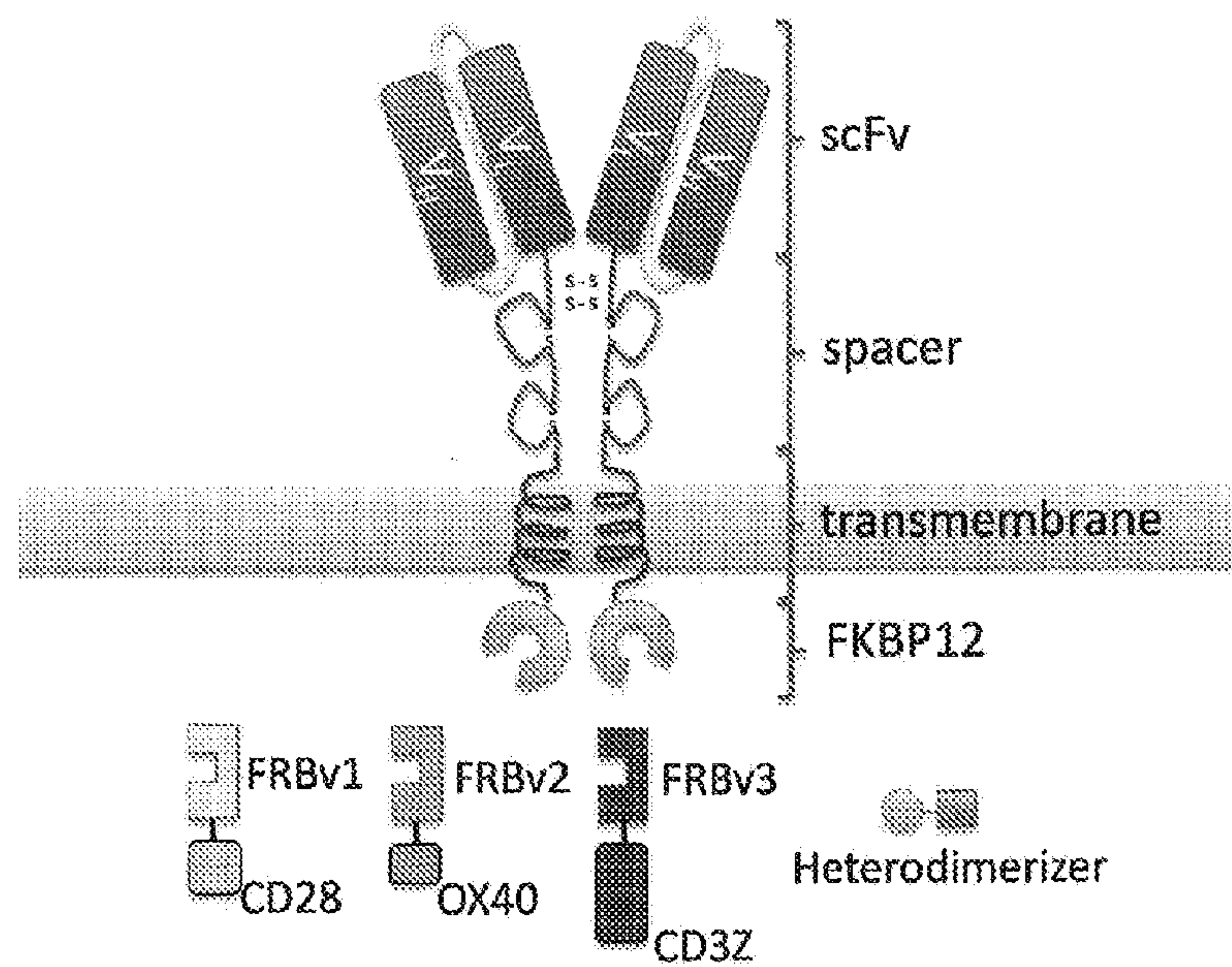


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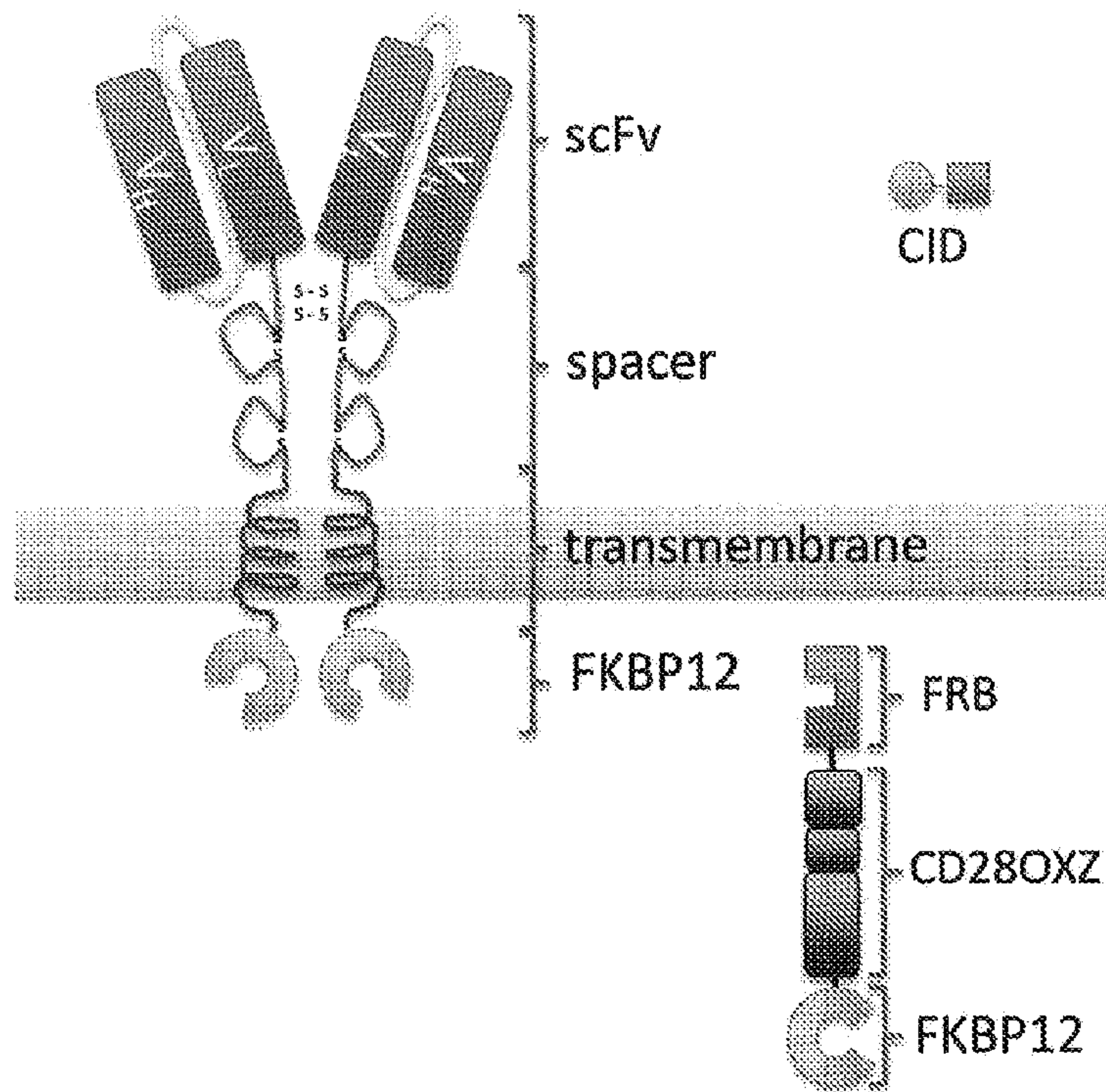


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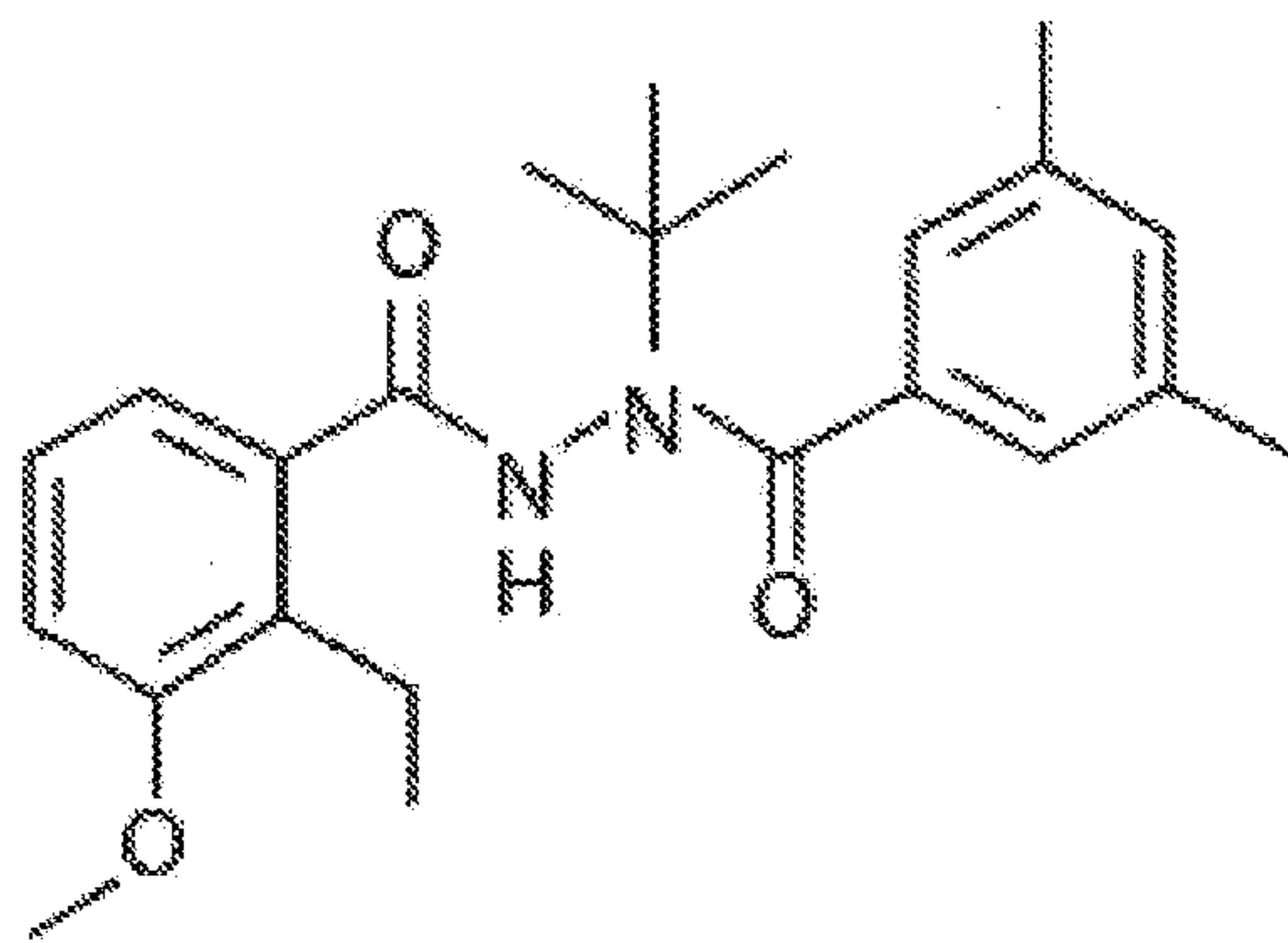
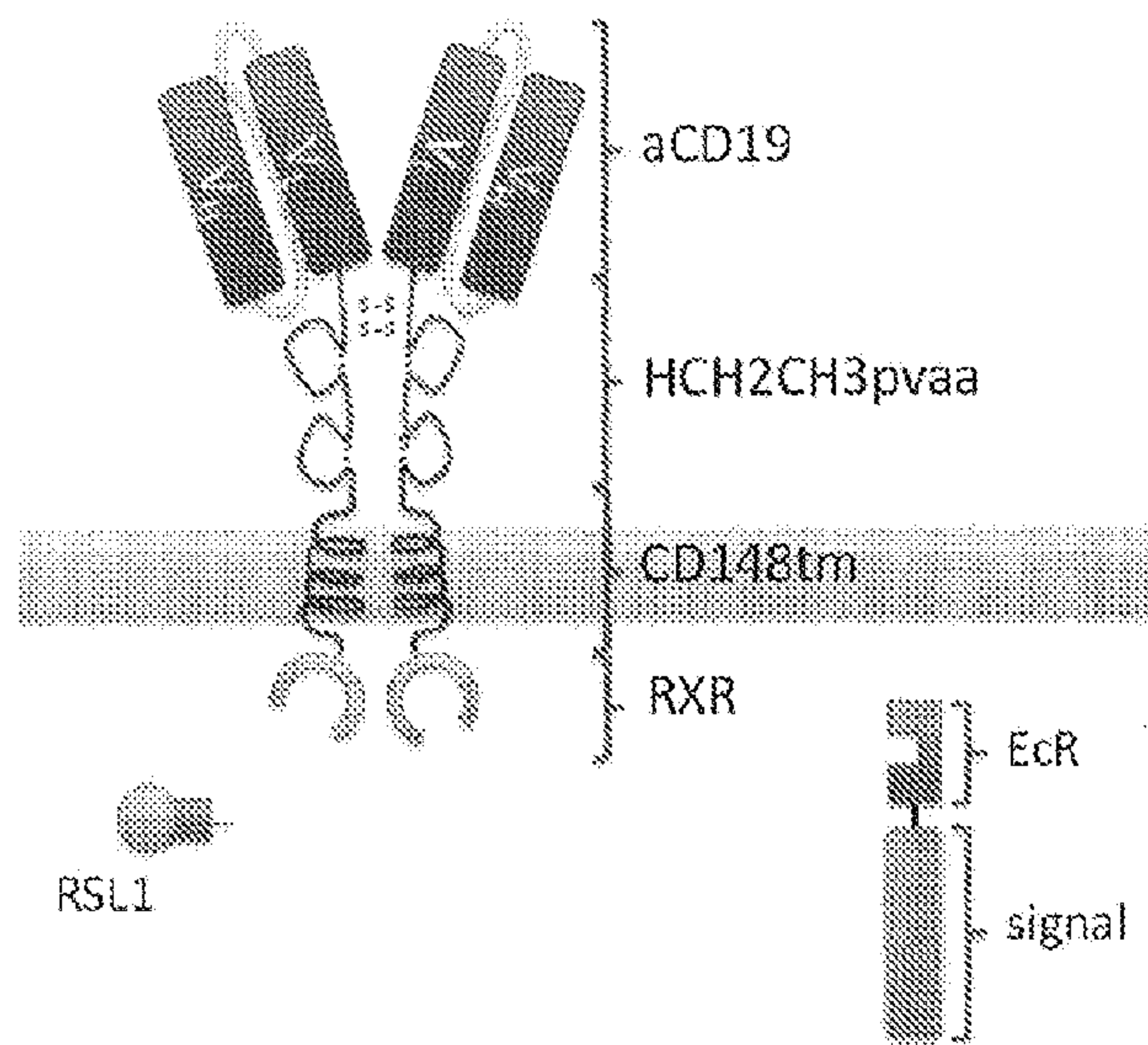
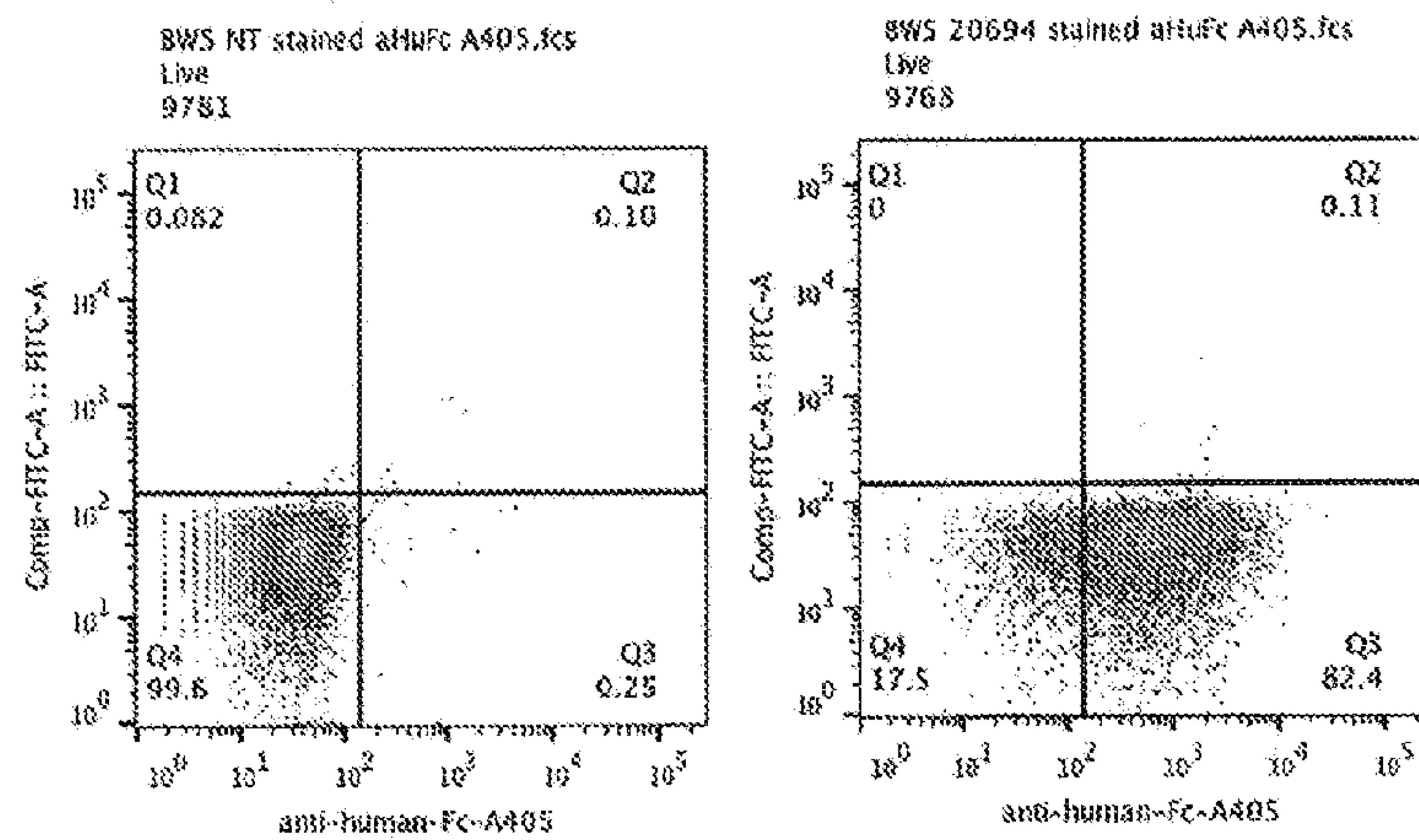


FIGURE 10

(a)



(b)



(c)

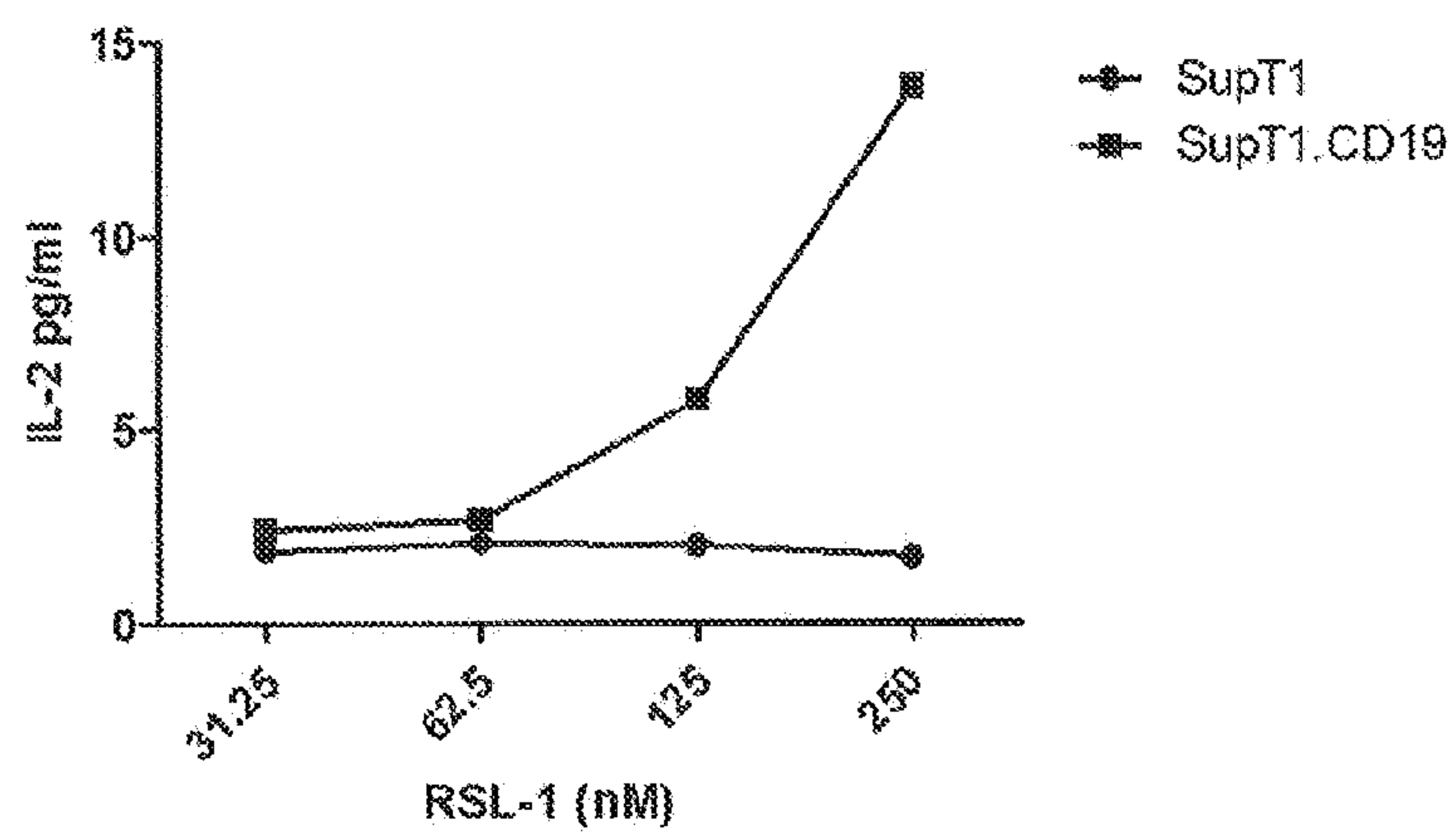


FIGURE 11

