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(54) Titre : RECEPTEURS D'ANTIGENES CHIMERIQUES SPECIFIQUES DE LA GLYCOPROTEINE
TROPHOBLASTIQUE (5T4, TPBG) UTILISES POUR L'IMMUNOTHERAPIE DU CANCER
(54) Title: TROPHOBLAST GLYCOPROTEIN (5T4, TPBG) SPECIFIC CHIMERIC ANTIGEN RECEPTORS FOR
CANCER IMMUNOTHERAPY

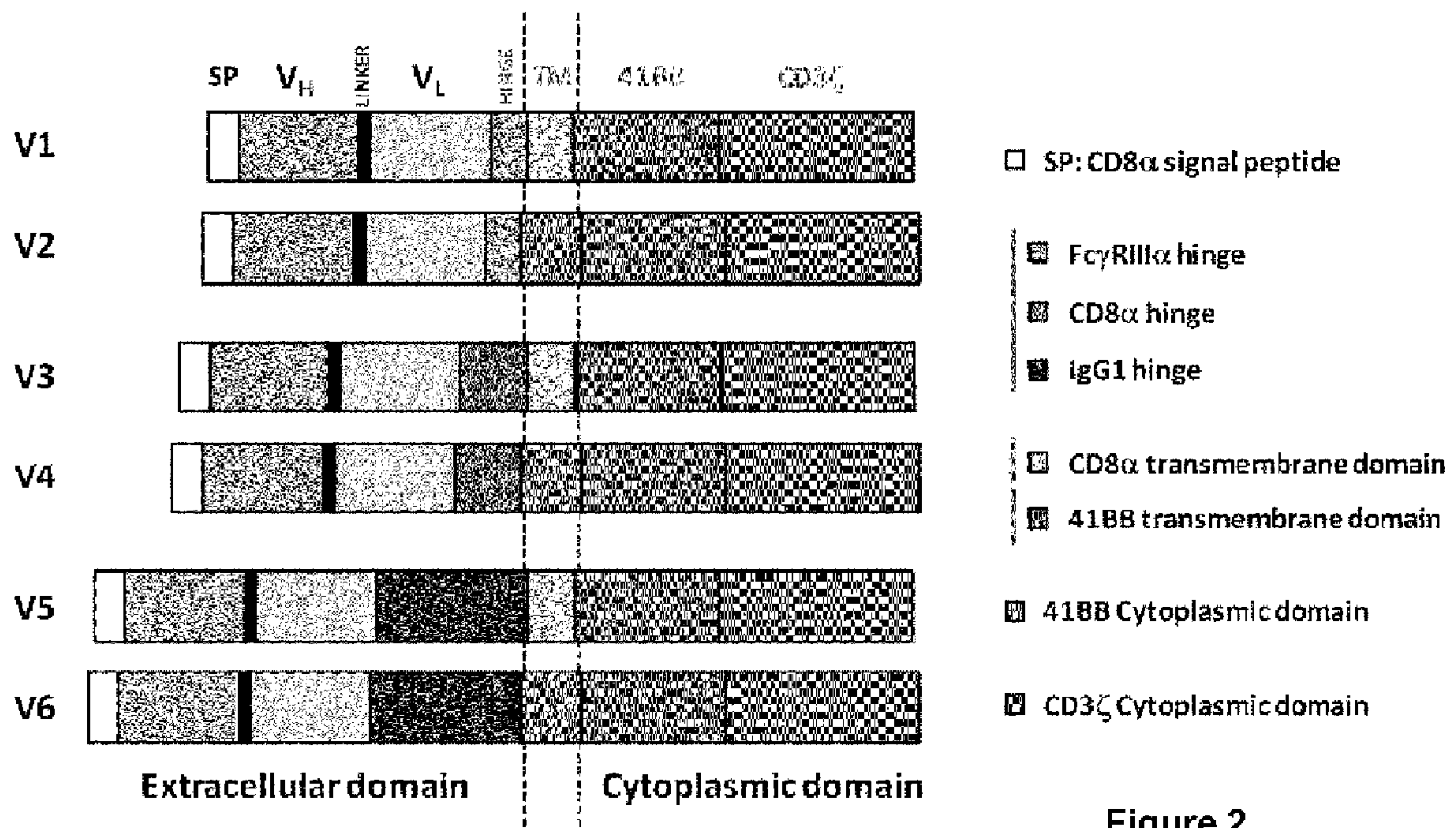


Figure 2

(57) Abrégé/Abstract:

The present invention relates to Chimeric Antigen Receptors (CAR) that are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward selected membrane antigens, and more particularly in which extracellular ligand binding is a



(57) **Abrégé(suite)/Abstract(continued):**

scFV derived from a 5T4 monoclonal antibody, conferring specific immunity against 5T4 positive cells. The engineered immune cells endowed with such CARs are particularly suited for treating lymphomas and leukemia, and for solid tumors such as colon, stomach, and ovarian tumors.

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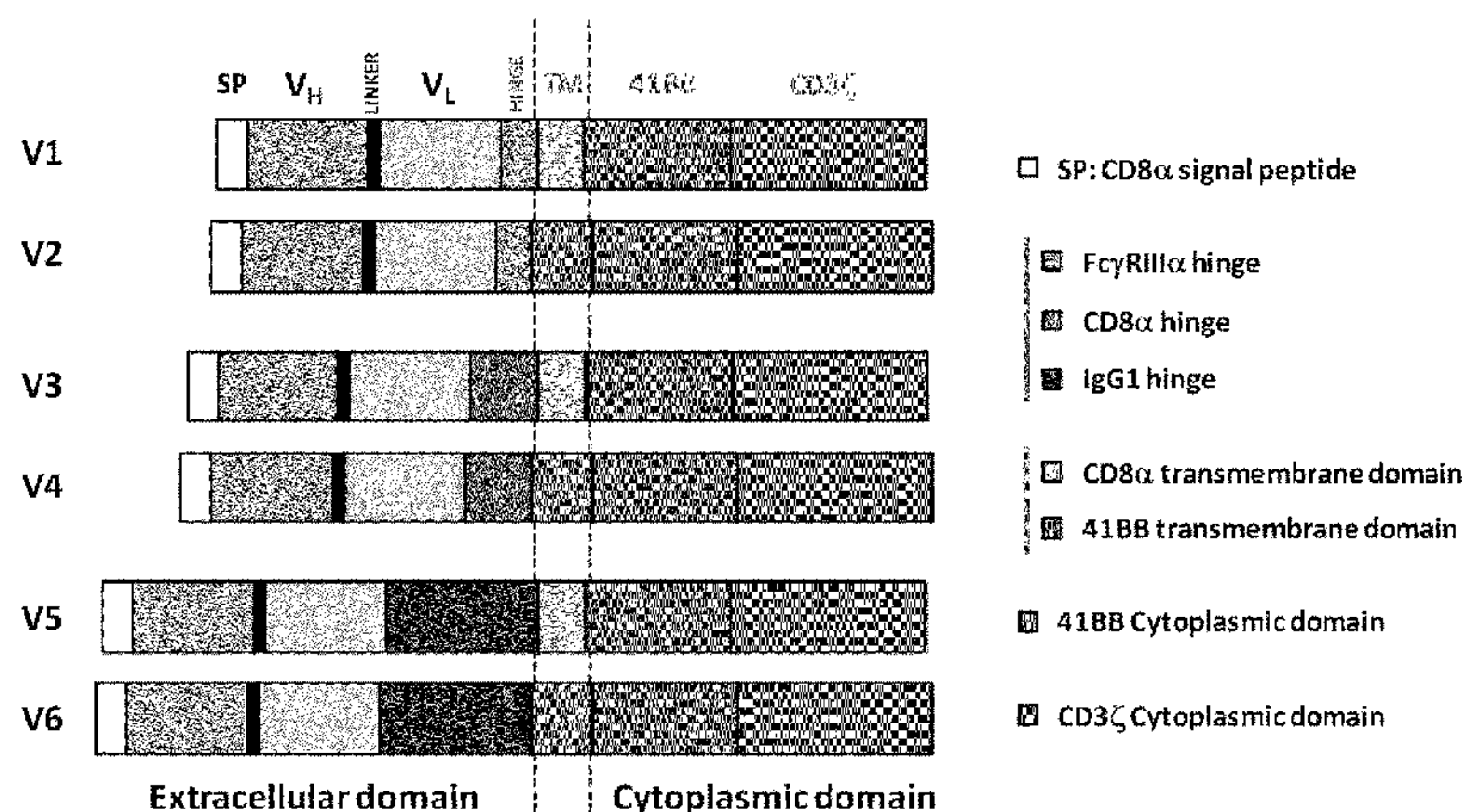


Figure 2

(57) Abstract: The present invention relates to Chimeric Antigen Receptors (CAR) that are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward selected membrane antigens, and more particularly in which extracellular ligand binding is a scFV derived from a 5T4 monoclonal antibody, conferring specific immunity against 5T4 positive cells. The engineered immune cells endowed with such CARs are particularly suited for treating lymphomas and leukemia, and for solid tumors such as colon, stomach, and ovarian tumors.

TROPHOBLAST GLYCOPROTEIN (5T4, TPBG) SPECIFIC CHIMERIC ANTIGEN RECEPTORS FOR
CANCER IMMUNOTHERAPY

Field of the invention

5 The present invention relates to Chimeric Antigen Receptors (CAR) that are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward 5T4, a cell surface glycoprotein found on most myeloid cells and used to diagnose solid tumors such as stomach, colon and ovarian tumors, and pre-B acute lymphocytic leukemia (ALL) in patients. The CARs according to the invention are particularly useful to treat malignant cells bearing 5T4 antigen, when expressed in T-cells or NK
10 cells. The resulting engineered immune cells display high level of specificity toward malignant cells, conferring safety and efficiency for immunotherapy.

Background of the invention

Adoptive immunotherapy, which involves the transfer of autologous antigen-specific T cells
15 generated *ex vivo*, is a promising strategy to treat viral infections and cancer. The T cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T cells or redirection of T cells through genetic engineering (Park, Rosenberg et al. 2011). Transfer of viral antigen specific T cells is a well-established procedure used for the treatment of transplant associated viral infections and rare viral-related malignancies. Similarly, isolation and transfer of
20 tumor specific T cells has been shown to be successful in treating melanoma.

Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs) (Jena, Dotti et al. 2010). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. In general, the binding moiety of a CAR consists of an antigen-
25 binding domain of a single-chain antibody (scFv), comprising the light and variable fragments of a monoclonal antibody joined by a flexible linker. Binding moieties based on receptor or ligand domains have also been used successfully. The signaling domains for first generation CARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. First generation CARs have been shown to successfully redirect T-cell cytotoxicity. However, they failed to
30 provide prolonged expansion and anti-tumor activity *in vivo*. Signaling domains from co-stimulatory molecules, as well as transmembrane and hinge domains have been added to form CARs of second

and third generations, leading to some successful therapeutic trials in humans, where T-cells could be redirected against malignant cells expressing CD19 (June et al., 2011). However, the particular combination of signaling domains, transmembrane and co-stimulatory domains used with respect to CD19 ScFv, was rather antigen-specific and cannot be expanded to any antigen markers.

5 According to the data from the Centers for Disease Control and Prevention. [<http://www.cdc.gov/cancer/colorectal/statistics/race.htm>], incidence of colorectal cancer in the US population over the year 2011 was about 50 per 100 000 people for women and up to 60 for males in the black people population, leading to 50 % mortality. This incidence has only decreased by 10 % over the last decade.

10 One candidate antigen of immunotherapies for solid tumors, including the colorectal, ovarian and gastric and also for non-solid tumors such as childhood acute lymphoblastic leukemia (ALL) is the trophoblast glycoprotein, also known as TPBG or 5T4 (UniProt: Q13641). 5T4 is often referred to as an oncofetal antigen due to its expression in foetal trophoblast (where it was first discovered) or trophoblast glycoprotein (TPBG). 5T4 protein is an N-glycosylated transmembrane 72 kDa
15 glycoprotein containing seven leucine-rich repeat regions (Hole et al, 1988). The 5T4 antigen was found to be expressed in number of carcinoma including gastric (Starzynska et al. 1995), ovarian and carcinoma (Wrigley et al. 1995). Also, 5T4 oncofetal antigen is expressed in high risk of relapse childhood pre-B acute lymphoblastic leukemia (Castro et al. 2012). It has very limited expression in normal tissue but is widespread in malignant tumors throughout their development (Carsberg et al.
20 1995).

The present inventors have thus considered that 5T4 could be a valuable target antigen for treating solid tumors such as colorectal, ovarian and gastric tumors, by using CAR-expressing T cells.

As an alternative to the previous strategies, the present invention provides with 5T4 specific
25 CARs, which can be expressed in immune cells to target 5T4 malignant cells with significant clinical advantage.

There is still the need for the improvement of CAR functionality by designing CAR architecture and using suitable components since these parameters play a role important and a fine tuning may be necessary.

The inventors have found that, by combining CAR architecture to the choice of suitable
30 components, they could obtain specific 5T4 single chain CARs with high cytotoxicity towards cancerous target cells.

Summary of the invention

The inventors have generated 5T4 specific CAR having different structure and comprising different scFV derived from different 5T4 specific antibodies.

5 In the framework of the present invention, they have designed and implemented at 5T4 specific CAR having one of the polypeptide structure selected from V1 to V6 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-5T4 antibody, a hinge, a transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB. Preferred CAR
10 polypeptides of the invention comprise an amino acid sequence selected from SEQ ID NO.19 to 42. Following non-specific activation in vitro (e.g. with anti CD3/CD28 coated beads and recombinant IL2), T-cells from donors have been transformed with polynucleotides expressing these CARs using viral transduction. More preferred CAR polypeptides having a polypeptide structure selected from V3, V5, V1 (i.e. having the CD8 α transmembrane domain) have shown the best and unexpected
15 results.

In particular, the 5T4 specific CARs containing the scFvs from A1, A2, A3 and H8 antibodies represent suitable candidates for immunotherapy as shown by their activity and specificity tested against selected tumor cell lines expressing the 5T4 antigen.

In certain instances, the T-cells were further engineered to create non-alloreactive T-cells,
20 more especially by disruption of a component of TCR ($\alpha\beta$ – T-Cell receptors) to prevent Graft versus host reaction.

The resulting engineered T-cells displayed reactivity in-vitro against 5T4 positive cells to various extend, showing that the CARs of the present invention contribute to antigen dependent activation, and also proliferation, of the T-cells, making them useful for immunotherapy.

25 The polypeptides and polynucleotide sequences encoding the CARs of the present invention are detailed in the present specification.

The engineered immune cells of the present invention are particularly useful for therapeutic applications, such as for treating chronic lymphocytic leukemia or on solid tumors such as breast, colon, lung, and kidney tumors.

Brief description of the figures

Figure 1: Schematic representation of an engineered immune cell according to the invention. The engineered immune cell presented in this figure is a T-cell transduced with a retroviral polypeptide encoding CAR. This T-cell is further engineered to allow a better and safer engraftment into the patient, which is optional within the frame of the present invention. X gene may be for instance a gene expressing a component of TCR (TCRalpha or TCRbeta), Y may be a gene involved into the sensitivity of T-cells to immune-suppressive drugs like CD52 (with respect to Campath) or HPRT (with respect to 6-Thioguanine).

Figure 2: schematic representation of the different CAR Architecture (V1 to V6).

Figures 3 to 6: schematic representation of the v1 to v6 T cell CARs accordingly to figure 2 with the VH and VL chains from A1, A2, A3 and H8 antibodies.

Figure 7: T cell degranulation test for eight 5T4-CAR-engineered T cells lines according to the invention to assess their activity.

Figure 8: T cell specific lysis for seven 5T4-CAR-engineered T cells lines according to the invention to assess their specificity.

Table 1: Sequence of the different CAR components

Functional domains	SEQ ID #	Raw amino acid sequence
CD8 α signal peptide	SEQ ID NO.1	MALPVTALLPLALLHAARP
Alternative signal peptide	SEQ ID NO.2	METDTLLLVLLLWVPGSTG
Fc ϵ R11y hinge	SEQ ID NO.3	GLAVSTISSFFPPGYQ
CD8 α hinge	SEQ ID NO.4	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL DFACD
IgG1 hinge	SEQ ID NO.5	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVD KSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
CD8 α transmembrane domain	SEQ ID NO.6	IYIWAPLAGTCGVLLLSLVITLYC
41BB transmembrane domain	SEQ ID NO.7	IISFFLALTSTALLFLLFLLTLRFSVV
41BB intracellular domain	SEQ ID NO.8	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGG CEL
CD3 ζ intracellular domain	SEQ ID NO.9	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
G4Sx3 linker	SEQ ID NO.10	GGGGSGGGGSGGGGS

Table 2: Sequence of the VH and VL chains of different scFvs and their respective CDRs

ScFv sequences	SEQ ID #	Raw amino acid sequence
H8 heavy chain variable region	SEQ ID NO.11	EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVVKQSHGKSLEWIGRINP NNGVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVM DYWGQVTSVTVSS
	SEQ ID NO.48	CDR1 GYSFTGY
	SEQ ID NO.49	CDR2 INPNNGVT
	SEQ ID NO.50	CDR3 ARSTMITNYVMDY
H8 light chain variable region	SEQ ID NO.12	SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRY AGVPDRFIGSGYGTDFTFTISTLQAEDLAVYFCQQDYNSPPTFGGGTKLEIKR
	SEQ ID NO.51	CDR1 QSVSND
	SEQ ID NO.52	CDR2 YTS
	SEQ ID NO.53	CDR3 QQDYNSPPT
A1 heavy chain variable region	SEQ ID NO.13	QIQLVQSGPELKKPGETVKISCKASGYFTNFGMNWVKQGPGEGLKWMGWIN TNTGEPRYAEFEKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFD YWGQGTTLTVSS
	SEQ ID NO.54	CDR1 GYTFTNFG
	SEQ ID NO.55	CDR2 INTNTGEP
	SEQ ID NO.56	CDR3 ARDWDGAYFFDY
A1 light chain variable region	SEQ ID NO.14	SIVMTQTPKFLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLINFATNR YTGVPNRFTGSGYGTDFTFTISTVQAEDLALYFCQQDYSSPWTFGGGTKLEIK
	SEQ ID NO.57	CDR1 QSVSND
	SEQ ID NO.58	CDR2 FAT
	SEQ ID NO.59	CDR3 QQDYSSPWT
A2 heavy chain variable region	SEQ ID NO.15	QVQLQQSRPELVKPGASVKMSCKASGYFTDYVISWVKQRTGQGLEWIGEIYP GSNSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYW GQGTTLTVSS
	SEQ ID NO.60	CDR1 GYTFTDYV
	SEQ ID NO.61	CDR2 IYPGSNSI
	SEQ ID NO.62	CDR3 AMGGNYGFDY

A2 light chain variable region	SEQ ID NO.16	QIVLTQSPAIMASASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNL ASGVPARFSGSGSGTSYSLTISSMEDAATYYCHQYHRSPLTFGAGTKLELK
	SEQ ID NO.63	CDR1 SSVNSNY
	SEQ ID NO.64	CDR2 STS
	SEQ ID NO.65	CDR3 HQYHRSPLT
A3 heavy chain variable region	SEQ ID NO.17	EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIR SKSNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMYYCVRQWDY DVRAMNYWGQGTSVTVSS
	SEQ ID NO.66	CDR1 GFTFNTYA
	SEQ ID NO.67	CDR2 IRSKSNNYAT
	SEQ ID NO.68	CDR3 VRQWDYDVRAMNY
A3 light chain variable region	SEQ ID NO.18	DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIYWAST RLTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGGTKLEIK
	SEQ ID NO.69	CDR1 QDVDTA
	SEQ ID NO.70	CDR2 WAS
	SEQ ID NO.71	CDR3 QQYSSYPYT

Table 3: CAR of structure V-1

CAR Designation	CAR Structure						
V-1	signal peptide (optional)	VH	VL	FcεRIIIy hinge	CD8α TM	41BB -IC	CD3ζζCD
H8 scCAR-v1 (SEQ ID NO.19)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A1 scCAR-v1 (SEQ ID NO.25)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v1 (SEQ ID NO.31)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v1 (SEQ ID NO.37)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 4: CAR of structure V-2

CAR Designation	CAR Structure						
V-2	signal peptide (optional)	VH	VL	FcεRIIIy hinge	41BB-TM	41BB -IC	CD3ζζCD
H8 scCAR-v2 (SEQ ID NO.20)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A1-scCAR-v2 (SEQ ID NO.26)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v2 (SEQ ID NO.32)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v2 (SEQ ID NO.38)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

5

Table 5: CAR of structure V-3

CAR Designation	CAR Structure						
V-3	signal peptide (optional)	VH	VL	CD8α hinge	CD8α TM	41BB -IC	CD3ζζCD
H8 scCAR-v3 (SEQ ID NO.21)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A1-scCAR-v3 (SEQ ID NO.27)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v3 (SEQ ID NO.33)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v3 (SEQ ID NO.39)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 6: CAR of structure V-4

CAR Designation	CAR Structure						
V-4	signal peptide (optional)	VH	VL	CD8α hinge	41BB-TM	41BB -IC	CD3ζζCD
H8 scCAR-v4 (SEQ ID NO.22)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A1 scCAR-v4 (SEQ ID NO.28)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v4 (SEQ ID NO.34)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v4 (SEQ ID NO.40)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

5

Table 7: CAR of structure V-5

CAR Designation	CAR Structure						
V-5	signal peptide (optional)	VH	VL	IgG1 hinge	CD8α TM	41BB -IC	CD3ζζCD
H8 scCAR-v5 (SEQ ID NO.23)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A1 scCAR-v5 (SEQ ID NO.29)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v5 (SEQ ID NO.35)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v5 (SEQ ID NO.41)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 8: CAR of structure V-6

CAR Designation	CAR Structure						
V-6	signal peptide (optional)	VH	VL	IgG1 hinge	41BB-TM	41BB -IC	CD3 ζ CD
H8 scCAR-v6 (SEQ ID NO.24)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A1 scCAR-v6 (SEQ ID NO.30)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A2 scCAR-v6 (SEQ ID NO.36)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
A3 scCAR-v6 (SEQ ID NO.42)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

5

Detailed description of the invention

Unless specifically defined herein, all technical and scientific terms used have the same meaning as commonly understood by a skilled artisan in the fields of gene therapy, biochemistry, genetics, and molecular biology.

All methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, with suitable methods and materials being described herein. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will prevail. Further, the materials, methods, and examples are illustrative only and are not intended to be limiting, unless otherwise specified.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are

explained fully in the literature. See, for example, Current Protocols in Molecular Biology (Frederick M. AUSUBEL, 2000, Wiley and son Inc, Library of Congress, USA); Molecular Cloning: A Laboratory Manual, Third Edition, (Sambrook et al, 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Harries & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the series, Methods In ENZYMOLOGY (J. Abelson and M. Simon, eds.-in-chief, Academic Press, Inc., New York), specifically, Vols.154 and 155 (Wu et al. eds.) and Vol. 185, "Gene Expression Technology" (D. Goeddel, ed.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); and Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

5T4 specific Chimeric Antigen Receptors

The present invention relates to new designs of anti-5T4 chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain, a transmembrane domain and a signaling transducing domain.

The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising the light (V_L) and the heavy (V_H) variable fragment of a target antigen specific monoclonal anti 5T4 antibody joined by a flexible linker.

The antigen binding domain of the 5T4 CARs of the invention can be any domain that binds to the off-tissue antigen including but not limited to a monoclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof.

By the term "recombinant antibody" as used herein, is meant an antibody or antibody fragment which is generated using recombinant DNA technology, such as, for example, an antibody or antibody fragment expressed by a bacteriophage, a yeast expression system or a mammalian cell expression system, and more especially by a T cell transduced with a viral vector comprising a nucleic acid sequence encoding CDR regions of an antibody. The term should also be construed to mean an antibody or antibody fragment which has been generated by the synthesis of a DNA molecule encoding the antibody or antibody fragment and which DNA molecule expresses an antibody or antibody fragment protein, or an amino acid sequence specifying the antibody or antibody fragment, wherein the DNA or amino acid sequence has been obtained using recombinant or synthetic DNA or amino acid sequence technology which is available and well known in the art.

By the term "monoclonal antibody" as used herein, is meant antibody produced by a laboratory-grown cell clone, either of a hybridoma or a virus-transformed lymphocyte, that is more abundant and uniform than natural antibody and is able to bind specifically to a single site on ROR1 antigen. They are monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope.

In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising the light (V_L) and the heavy (V_H) variable fragment of a target antigen specific monoclonal 5T4 antibody joined by a flexible linker. Said V_L and V_H are preferably selected from the antibodies referred to as H8, A1, A2 and A3 as indicated in Table 2. They are preferably linked together by a flexible linker comprising for instance the sequence SEQ ID NO.10. In other words, said CARs preferentially comprise an extracellular ligand-binding domain comprising a polypeptide sequence displaying at least 90 %, 95 % 97 % or 99 % identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 11 to SEQ ID NO: 18.

According to a preferred embodiment, the 5T4 specific CAR according to the present invention contains an extracellular ligand-binding domain, wherein said V_H and V_L have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:13 (A1- V_H) and SEQ ID NO:14 (A1- V_L).

According to another preferred embodiment, the 5T4 specific CAR according to the present invention contains an extracellular ligand-binding domain, wherein said V_H and V_L have at least 80%,

preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:15 (A2-VH) and SEQ ID NO:16 (A2-VL).

According to another preferred embodiment, the 5T4 specific CAR according to the present invention contains an extracellular ligand-binding domain, wherein said VH and VL have at least 80%,
5 preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:17 (A3-VH) and SEQ ID NO:18 (A3-VL).

According to another preferred embodiment, the 5T4 specific CAR according to the present invention contains an extracellular ligand-binding domain, wherein said VH and VL have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99%
10 sequence identity respectively with SEQ ID NO:11 (H18-VH) and SEQ ID NO:12 (H18-VL).

The present invention discloses a 5T4 specific chimeric antigen receptor (5T4 CAR) as above, wherein said extra cellular ligand binding-domain comprises VH and VL chains which are humanized.

By the term "humanized antibody" as used herein, is meant the polypeptides include a humanized heavy chain variable region and a humanized light chain variable region. For example, the
15 polypeptides may include the framework (FR) regions of the light and heavy chain variable regions of a human antibody, while retaining substantially the antigen-binding specificity of a parental monoclonal antibody. The humanized heavy chain variable region and/or the humanized light chain variable region are at least about 87% humanized, at least about 90% humanized, at least about 95% humanized, at least about 98% humanized, or at least about 100% humanized, excluding the
20 complementary-determining regions (CDRs). The antigen-binding polypeptides molecules may be derived from monoclonal antibody donors (*e.g.*, mouse monoclonal antibody donors) and may include CDRs from the monoclonal antibodies (*e.g.*, mouse monoclonal CDRs).

A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, *e.g.*, European Patent No. EP 239,400; International
25 Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, *e.g.*, European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; each of which is incorporated herein by its entirety by reference), chain shuffling (see, *e.g.*, U.S. Pat. No. 5,565,332, which is incorporated herein
30 in its entirety by reference), and techniques disclosed in, *e.g.*, U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213,

U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and which is incorporated herein by reference in their entireties).

According to a preferred embodiment, the A 5T4 specific CAR of the present invention comprises VH and VL chains which have at least 80 %, preferably 90%, more preferably wherein said extra cellular ligand binding-domain comprising:

- a VH chain comprising the CDRs from the mouse monoclonal antibody H8 of SEQ ID NO. 48 (CDR1), SEQ ID NO.49 (CDR2) and SEQ ID NO.50 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody H18 of NO. 51 (CDR1), SEQ ID NO.52 (CDR2) and SEQ ID NO:53 (CDR3), or;

- a VH chain comprising the CDRs from the mouse monoclonal antibody A1 of SEQ ID NO. 54 (CDR1), SEQ ID NO.55 (CDR2) and SEQ ID NO.56 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody A1 of NO. 57 (CDR1), SEQ ID NO.58 (CDR2) and SEQ ID NO:59 (CDR3), or;

- a VH chain comprising the CDRs from the mouse monoclonal antibody A2 of SEQ ID NO. 61 (CDR1), SEQ ID NO.61 (CDR2) and SEQ ID NO.63 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody A2 of NO. 64 (CD1), SEQ ID NO.65 (CD2) and SEQ ID NO:65 (CDR3), or;

- a VH chain comprising the CDRs from the mouse monoclonal antibody A3 of SEQ ID NO. 66 (CDR1), SEQ ID NO.67 (CDR2) and SEQ ID NO.68 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody A3 of NO. 69 (CDR1), SEQ ID NO.70 (CDR2) and SEQ ID NO:71 (CDR3).

Table 2 shows the sequences VH and VL chains corresponding to the H8, A1, A2 and A3 anti-5T4 antibodies and of their respective CDRs.

The signal transducing domain or intracellular signaling domain of a CAR according to the present invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In

other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines. Thus, the term “signal transducing domain” refers to the portion of a protein which
5 transduces the effector signal function signal and directs the cell to perform a specialized function.

Preferred examples of signal transducing domain for use in a CAR can be the cytoplasmic sequences of the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivate or variant of these sequences and any synthetic sequence that has the same functional capability. Signal transduction domain
10 comprises two distinct classes of cytoplasmic signaling sequence, those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequence can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as
15 binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non-limiting examples those derived from TCRzeta, FcRgamma, FcRbeta, FcRepsilon, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d. In a preferred embodiment, the signaling transducing domain of the CAR can comprise the CD3zeta signaling domain which has amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90 %, 95 %
20 97 % or 99 % sequence identity with amino acid sequence selected from the group consisting of (SEQ ID NO: 9).

In particular embodiment the signal transduction domain of the CAR of the present invention comprises a co-stimulatory signal molecule. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient immune response.
25 “Co-stimulatory ligand” refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1
30 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia,

an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

A “co-stimulatory molecule” refers to the cognate binding partner on a T-cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to, an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

In a preferred embodiment, the signal transduction domain of the CAR of the present invention comprises a part of co-stimulatory signal molecule selected from the group consisting of fragment of 4-1BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In particular the signal transduction domain of the CAR of the present invention comprises amino acid sequence which comprises at least 70%, preferably at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8.

A CAR according to the present invention is expressed on the surface membrane of the cell. Thus, such CAR further comprises a transmembrane domain. The distinguishing features of appropriate transmembrane domains comprise the ability to be expressed at the surface of a cell, preferably in the present invention an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and to interact together for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T-cell receptor such as α , β , γ or ζ , polypeptide constituting CD3 complex, IL2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fc γ receptor III or CD proteins. Alternatively the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In a preferred embodiment said transmembrane domain is derived from the human CD8 alpha chain (e.g. NP_001139345.1) The transmembrane domain can further comprise a hinge region between said extracellular ligand-binding domain and said transmembrane domain. The term “hinge region” used herein generally means any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, hinge region are used to provide more flexibility and accessibility for the

extracellular ligand-binding domain. A hinge region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Hinge region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively the hinge region may
5 be a synthetic sequence that corresponds to a naturally occurring hinge sequence, or may be an entirely synthetic hinge sequence. In a preferred embodiment said hinge domain comprises a part of human CD8 alpha chain, FcγRIIIα receptor or IgG1 respectively referred to in this specification as SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO.5, or hinge polypeptides which display preferably at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with these polypeptides.

10 A car according to the invention generally further comprises a transmembrane domain (TM) more particularly selected from CD8α and 4-1BB, showing identity with the polypeptides of SEQ ID NO. 6 or 7.

Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell more
15 specific to target, the 5T4 specific CAR according to the invention can comprise another extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In one embodiment, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In another embodiment, said different extracellular ligand-binding domains can be
20 placed on different transmembrane polypeptides composing the CAR. In another embodiment, the present invention relates to a population of CARs comprising each one different extracellular ligand binding domains. In a particular, the present invention relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of said cell a population of CAR each one comprising different extracellular ligand binding domains. In another particular
25 embodiment, the present invention relates to a method of engineering an immune cell comprising providing an immune cell and introducing into said cell polynucleotides encoding polypeptides composing a population of CAR each one comprising different extracellular ligand binding domains. By population of CARs, it is meant at least two, three, four, five, six or more CARs each one comprising different extracellular ligand binding domains. The different extracellular ligand binding
30 domains according to the present invention can preferably simultaneously bind different elements in target thereby augmenting immune cell activation and function. The present invention also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand binding domains.

According to a preferred embodiment, the 5T4 specific CAR according to the invention has a structure V3 as displayed in Figure 2, thus comprising a CD8 α hinge and a CD8 α transmembrane domain.

According to another preferred embodiment, the 5T4 specific CAR according to the invention has a structure V5 as displayed in Figure 2, thus comprising an IgG1 hinge and a CD8 α transmembrane domain.

According to another preferred embodiment, the 5T4 specific CAR according to the invention has a structure V1 as displayed in Figure 2, thus comprising a Fc γ RIII α hinge and CD8 α transmembrane domain.

Polynucleotides, vectors:

The present invention also relates to polynucleotides, vectors encoding the above described CAR according to the invention.

The polynucleotide may consist in an expression cassette or expression vector (e.g. a plasmid for introduction into a bacterial host cell, or a viral vector such as a baculovirus vector for transfection of an insect host cell, or a plasmid or viral vector such as a lentivirus for transfection of a mammalian host cell).

In a particular embodiment, the different nucleic acid sequences can be included in one polynucleotide or vector which comprises a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By "codon" is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

To direct transmembrane polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading
5 frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleic acid sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleic acid sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). In a preferred embodiment the signal peptide comprises
10 the amino acid sequence SEQ ID NO: 1 and 2.

Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. Preferably, the nucleic acid sequences of the present invention are codon-optimized for expression in mammalian cells, preferably for expression in human cells. Codon-optimization refers to the exchange in a
15 sequence of interest of codons that are generally rare in highly expressed genes of a given species by codons that are generally frequent in highly expressed genes of such species, such codons encoding the amino acids as the codons that are being exchanged.

Methods of engineering immune cells endowed with CARs:

20 The present invention encompasses the method of preparing immune cells for immunotherapy comprising introducing *ex-vivo* into said immune cells the polynucleotides or vectors encoding one of the 5T4 CAR as previously described.

In a preferred embodiment, said polynucleotides are included in lentiviral vectors in view of being stably expressed in the immune cells.

25 According to further embodiments, said method further comprises the step of genetically modifying said cell to make them more suitable for allogeneic transplantation.

According to a first aspect, the immune cell can be made allogeneic, for instance, by inactivating at least one gene expressing one or more component of T-cell receptor (TCR) as described in WO 2013/176915, which can be combined with the inactivation of a gene encoding or
30 regulating HLA or $\beta 2m$ protein expression. Accordingly the risk of graft versus host syndrome and graft rejection is significantly reduced.

According to another aspect, the immune cells can be further genetically engineered to improve their resistance to immunosuppressive drugs or chemotherapy treatments, which are used as standard care for treating 5T4 positive malignant cells. For instance, CD52 and glucocorticoid receptors (GR), which are drug targets of Campath (alemtuzumab) and glucocorticoids treatments, 5 can be inactivated to make the cells resistant to these treatments and give them a competitive advantage over patient's own T-cells not endowed with specific 5T4 CARs. Expression of CD3 gene can also be suppressed or reduced to confer resistance to Teplizumab, which is another immune suppressive drug. Expression of HPRT can also be suppressed or reduced according to the invention to confer resistance to 6- thioguanine, a cytostatic agent commonly used in chemotherapy especially 10 for the treatment of acute lymphoblastic leukemia.

According to further aspect of the invention, the immune cells can be further manipulated to make them more active or limit exhaustion, by inactivating genes encoding proteins that act as "immune checkpoints" that act as regulators of T-cells activation, such as PDCD1 or CTLA-4. Examples of genes, which expression could be reduced or suppressed are indicated in Table 9.

Table 9: List of genes encoding immune checkpoint proteins.

Pathway		Genes that can be inactivated In the pathway
Co-inhibitory receptors	CTLA4 (CD152)	CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22
	PDCD1 (PD-1, CD279)	PDCD1
	CD223 (lag3)	LAG3
	HAVCR2 (tim3)	HAVCR2
	BTLA(cd272)	BTLA
	CD160(by55)	CD160
	IgSF family	TIGIT
		CD96
		CRTAM
	LAIR1(cd305)	LAIR1
	SIGLECs	SIGLEC7
		SIGLEC9
	CD244(2b4)	CD244
Death receptors	TRAIL	TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7
	FAS	FADD, FAS
Cytokine signalling	TGF-beta signaling	TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1
	IL10 signalling	IL10RA, IL10RB, HMOX2
	IL6 signalling	IL6R, IL6ST
Prevention of TCR signalling		CSK, PAG1
		SIT1
Induced Treg	induced Treg	FOXP3
Transcription factors controlling exhaustion	transcription factors controlling exhaustion	PRDM1 (=blimp1, heterozygotes mice control chronic viral infection better than wt or conditional KO)
		BATF
Hypoxia mediated tolerance	iNOS induced guanylated cyclase	GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3

In a preferred embodiment said method of further engineering the immune cells involves
5 introducing into said T cells polynucleotides, in particular mRNAs, encoding specific rare-cutting
endonuclease to selectively inactivate the genes, as those mentioned above, by DNA cleavage. In a
more preferred embodiment said rare-cutting endonucleases are TALE-nucleases or Cas9
endonuclease. TAL-nucleases have so far proven higher specificity and cleavage efficiency over the

other types of rare-cutting endonucleases, making them the endonucleases of choice for producing of the engineered immune cells on a large scale with a constant turn-over.

Delivery methods

The different methods described above involve introducing CAR into a cell. As non-limiting
5 example, said CAR can be introduced as transgenes encoded by one plasmid vector. Said plasmid vector can also contain a selection marker which provides for identification and/or selection of cells which received said vector.

Polypeptides may be synthesized *in situ* in the cell as a result of the introduction of polynucleotides encoding said polypeptides into the cell. Alternatively, said polypeptides could be
10 produced outside the cell and then introduced thereto. Methods for introducing a polynucleotide construct into cells are known in the art and including as non-limiting examples stable transformation methods wherein the polynucleotide construct is integrated into the genome of the cell, transient transformation methods wherein the polynucleotide construct is not integrated into the genome of the cell and virus mediated methods. Said polynucleotides may be introduced into a cell by for
15 example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, transient transformation methods include for example microinjection, electroporation or particle bombardment. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells.

Engineered immune cells

20 The present invention also relates to isolated cells or cell lines susceptible to be obtained by said method to engineer cells. In particular said isolated cell comprises at least one CAR as described above. In another embodiment, said isolated cell comprises a population of CARs each one comprising different extracellular ligand binding domains. In particular, said isolated cell comprises exogenous polynucleotide sequence encoding CAR. Genetically modified immune cells of the present
25 invention are activated and proliferate independently of antigen binding mechanisms.

In the scope of the present invention is also encompassed an isolated immune cell, preferably a T-cell obtained according to any one of the methods previously described. Said immune cell refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response. Said immune cell according to the present invention can
30 be derived from a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells.

Representative human cells are CD34+ cells. Said isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T-cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In another embodiment, said cell can be derived from the group consisting of CD4+ T-lymphocytes and CD8+ T-lymphocytes. Prior to expansion and genetic modification of the cells of the invention, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available and known to those skilled in the art, may be used. In another embodiment, said cell can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In another embodiment, said cell is part of a mixed population of cells which present different phenotypic characteristics. In the scope of the present invention is also encompassed a cell line obtained from a transformed T- cell according to the method previously described. Modified cells resistant to an immunosuppressive treatment and susceptible to be obtained by the previous method are encompassed in the scope of the present invention.

As a preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a 5T4 CAR as described above, that do not express functional TCR and that are reactive towards 5T4 positive cells, for their allogeneic transplantation into patients.

Activation and expansion of T cells

Whether prior to or after genetic modification of the T cells, even if the genetically modified immune cells of the present invention are activated and proliferate independently of antigen binding mechanisms, the immune cells, particularly T-cells of the present invention can be further activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded *in vitro* or *in vivo*.

Generally, the T cells of the invention are expanded by contact with an agent that stimulates a CD3 TCR complex and a co-stimulatory molecule on the surface of the T cells to create an activation signal for the T-cell. For example, chemicals such as calcium ionophore A23187, phorbol 12-myristate

13-acetate (PMA), or mitogenic lectins like phytohemagglutinin (PHA) can be used to create an activation signal for the T-cell.

As non-limiting examples, T cell populations may be stimulated *in vitro* such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized
5 on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include an appropriate media
10 (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-g, 1L-4, 1L-7, GM-CSF, -10, - 2, 1L-15, TGFp, and TNF- or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-
15 cysteine and 2-mercaptoethanoi. Media can include RPMI 1640, A1M-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in
20 cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO₂). T cells that have been exposed to varied stimulation times may exhibit different characteristics

In another particular embodiment, said cells can be expanded by co-culturing with tissue or
25 cells. Said cells can also be expanded *in vivo*, for example in the subject's blood after administrating said cell into the subject.

Therapeutic applications

In another embodiment, isolated cell obtained by the different methods or cell line derived from said isolated cell as previously described can be used as a medicament. In another embodiment,
30 said medicament can be used for treating cancer, particularly for the treatment of carcinoma and leukemia in a patient in need thereof. In another embodiment, said isolated cell according to the

invention or cell line derived from said isolated cell can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

In another aspect, the present invention relies on methods for treating patients in need thereof, said method comprising at least one of the following steps:

- 5 (a) providing an immune-cell obtainable by any one of the methods previously described;
- (b) Administering said transformed immune cells to said patient,

On one embodiment, said T cells of the invention can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time.

10 Said treatment can be ameliorating, curative or prophylactic. It may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment. By autologous, it is meant that cells, cell line or population of cells used for treating patients are originating from said patient or from a Human Leucocyte Antigen (HLA) compatible donor. By allogeneic is meant that the cells or population of cells used for treating patients are not originating from said patient but from a donor.

15 Cells that can be used with the disclosed methods are described in the previous section. Said treatment can be used to treat patients diagnosed wherein a pre-malignant or malignant cancer condition characterized by 5T4-expressing cells, especially by an overabundance of 5T4-expressing cells. Such conditions are found in solid cancers or in hematologic cancers, such as childhood pre-B acute lymphoblastic leukemia.

20 Solid tumors can be gastric tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, renal tumors or ovarian tumors.

More specifically, such treatment may be useful for progressive hormone refractory prostate cancer in combination or not of drug(s) such as docetaxel or granulocyte macrophage-colony stimulating factor (GM-CSF).

25 Also, the engineered T cell of the invention may be used for treating advanced solid tumors such as non-small cell lung cancer, renal clear cell carcinoma or pancreatic cancer, in conjunction with other drug(s) such as interleukin-2 (IL-2), docetaxel or pemetrexed/cisplatin.

Moreover, the engineered T cell of the invention may be used for treating prostate cancer with or without cyclophosphamide.

Lymphoproliferative disorder can be leukemia, in particular childhood pre-B acute lymphoblastic leukemia.

Cancers that may be treated may comprise nonsolid tumors (such as hematological tumors, including but not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma and the like. Types of cancers to be treated with the CARs of the invention include, but are not limited leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included.

Also, solid tumors such as stomach, colon, and ovarian tumors can be treated by the CARs of the invention

The treatment with the engineered immune cells according to the invention may be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

According to a preferred embodiment of the invention, said treatment can be administrated into patients undergoing an immunosuppressive treatment. Indeed, the present invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T-cells according to the invention within the patient.

The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

The administration of the cells or population of cells can consist of the administration of 10^4 - 10^9 cells per kg body weight, preferably 10^5 to 10^6 cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as more than one

dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

In another embodiment, said effective amount of cells or composition comprising those cells are administered parenterally. Said administration can be an intravenous administration. Said administration can be directly done by injection within a tumor.

In certain embodiments of the present invention, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizimab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH, In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present

invention. In an additional embodiment, expanded cells are administered before or following surgery.

Other definitions

- Amino acid residues in a polypeptide sequence are designated herein according to the one-letter code, in which, for example, Q means Gln or Glutamine residue, R means Arg or Arginine residue and D means Asp or Aspartic acid residue.

- Amino acid substitution means the replacement of one amino acid residue with another, for instance the replacement of an Arginine residue with a Glutamine residue in a peptide sequence is an amino acid substitution.

- Nucleotides are designated as follows: one-letter code is used for designating the base of a nucleoside: a is adenine, t is thymine, c is cytosine, and g is guanine. For the degenerated nucleotides, r represents g or a (purine nucleotides), k represents g or t, s represents g or c, w represents a or t, m represents a or c, y represents t or c (pyrimidine nucleotides), d represents g, a or t, v represents g, a or c, b represents g, t or c, h represents a, t or c, and n represents g, a, t or c.

- "As used herein, "nucleic acid" or "polynucleotides" refers to nucleotides and/or polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Nucleic acids can be either single stranded or double stranded.

- By chimeric antigen receptor (CAR) is intended molecules that combine a binding domain against a component present on the target cell, for example an antibody-based specificity for a desired antigen (e.g., tumor antigen) with a T cell receptor-activating intracellular domain to

generate a chimeric protein that exhibits a specific anti-target cellular immune activity. Generally, CAR consists of an extracellular single chain antibody (scFvFc), fused to the intracellular signaling domain of the T cell antigen receptor complex zeta chain (scFvFc:ζ) and have the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity.

5 CAR may sometimes comprise multiple transmembrane polypeptides (multi-chain CARs) as described in WO2014039523. One example of CAR used in the present invention is a CAR directing against 5T4 antigen and can comprise as non-limiting example the amino acid sequences : SEQ ID NO: 19 to 42.

- The term “endonuclease” refers to any wild-type or variant enzyme capable of catalyzing the hydrolysis (cleavage) of bonds between nucleic acids within a DNA or RNA molecule, preferably a

10 DNA molecule. Endonucleases do not cleave the DNA or RNA molecule irrespective of its sequence, but recognize and cleave the DNA or RNA molecule at specific polynucleotide sequences, further referred to as “target sequences” or “target sites”. Endonucleases can be classified as rare-cutting endonucleases when having typically a polynucleotide recognition site greater than 12 base pairs (bp) in length, more preferably of 14-55 bp. Rare-cutting endonucleases significantly increase HR by

15 inducing DNA double-strand breaks (DSBs) at a defined locus (Perrin, Buckle et al. 1993; Rouet, Smih et al. 1994; Chouluka, Perrin et al. 1995; Pingoud and Silva 2007). Rare-cutting endonucleases can for example be a homing endonuclease (Paques and Duchateau 2007), a chimeric Zinc-Finger nuclease (ZFN) resulting from the fusion of engineered zinc-finger domains with the catalytic domain of a restriction enzyme such as FokI (Porteus and Carroll 2005), a Cas9 endonuclease from CRISPR system

20 (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) or a chemical endonuclease (Eisenschmidt, Lanio et al. 2005; Arimondo, Thomas et al. 2006). In chemical endonucleases, a chemical or peptidic cleaver is conjugated either to a polymer of nucleic acids or to another DNA recognizing a specific target sequence, thereby targeting the cleavage activity to a specific sequence. Chemical endonucleases also encompass synthetic nucleases like

25 conjugates of orthophenanthroline, a DNA cleaving molecule, and triplex-forming oligonucleotides (TFOs), known to bind specific DNA sequences (Kalish and Glazer 2005). Such chemical endonucleases are comprised in the term “endonuclease” according to the present invention.

- By a “TALE-nuclease” (TALEN) is intended a fusion protein consisting of a nucleic acid-binding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease

30 catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, like for instance I-TevI, ColE7, NucA and Fok-I. In a particular embodiment, the TALE domain can be fused to a meganuclease like for instance I-CreI and I-OnuI or functional variant thereof. In a more preferred embodiment, said

nuclease is a monomeric TALE-Nuclease. A monomeric TALE-Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927. Transcription Activator like Effector (TALE) are proteins from the bacterial species *Xanthomonas* comprise a plurality of repeated sequences, each repeat comprising di-residues in position 12 and 13 (RVD) that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered by the applicant in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALE-nuclease have been already described and used to stimulate gene targeting and gene modifications (Boch, Scholze et al. 2009; Moscou and Bogdanove 2009; Christian, Cermak et al. 2010; Li, Huang et al. 2011). Custom-made TAL-nucleases are commercially available under the trade name TALENTM (Cellestis, 8 rue de la Croix Jarry, 75013 Paris, France).

The rare-cutting endonuclease according to the present invention can also be a Cas9 endonuclease. Recently, a new genome engineering tool has been developed based on the RNA-guided Cas9 nuclease (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) from the type II prokaryotic CRISPR (Clustered Regularly Interspaced Short palindromic Repeats) adaptive immune system (see for review (Sorek, Lawrence et al. 2013)). The CRISPR Associated (Cas) system was first discovered in bacteria and functions as a defense against foreign DNA, either viral or plasmid. CRISPR-mediated genome engineering first proceeds by the selection of target sequence often flanked by a short sequence motif, referred as the proto-spacer adjacent motif (PAM). Following target sequence selection, a specific crRNA, complementary to this target sequence is engineered. Trans-activating crRNA (tracrRNA) required in the CRISPR type II systems paired to the crRNA and bound to the provided Cas9 protein. Cas9 acts as a molecular anchor facilitating the base pairing of tracrRNA with crRNA (Deltcheva, Chylinski et al. 2011). In this ternary complex, the dual tracrRNA:crRNA structure acts as guide RNA that directs the endonuclease

Cas9 to the cognate target sequence. Target recognition by the Cas9-tracrRNA:crRNA complex is initiated by scanning the target sequence for homology between the target sequence and the crRNA. In addition to the target sequence-crRNA complementarity, DNA targeting requires the presence of a short motif adjacent to the protospacer (protospacer adjacent motif - PAM). Following pairing
5 between the dual-RNA and the target sequence, Cas9 subsequently introduces a blunt double strand break 3 bases upstream of the PAM motif (Garneau, Dupuis et al. 2010).

Rare-cutting endonuclease can be a homing endonuclease, also known under the name of meganuclease. Such homing endonucleases are well-known to the art (Stoddard 2005). Homing endonucleases recognize a DNA target sequence and generate a single- or double-strand break.
10 Homing endonucleases are highly specific, recognizing DNA target sites ranging from 12 to 45 base pairs (bp) in length, usually ranging from 14 to 40 bp in length. The homing endonuclease according to the invention may for example correspond to a LAGLIDADG endonuclease, to a HNH endonuclease, or to a GIY-YIG endonuclease. Preferred homing endonuclease according to the present invention can be an *I-CreI* variant.

15 - By “delivery vector” or “delivery vectors” is intended any delivery vector which can be used in the present invention to put into cell contact (i.e “contacting”) or deliver inside cells or subcellular compartments (i.e “introducing”) agents/chemicals and molecules (proteins or nucleic acids) needed in the present invention. It includes, but is not limited to liposomal delivery vectors, viral delivery vectors, drug delivery vectors, chemical carriers, polymeric carriers, lipoplexes,
20 polyplexes, dendrimers, microbubbles (ultrasound contrast agents), nanoparticles, emulsions or other appropriate transfer vectors. These delivery vectors allow delivery of molecules, chemicals, macromolecules (genes, proteins), or other vectors such as plasmids, peptides developed by Diatos. In these cases, delivery vectors are molecule carriers. By “delivery vector” or “delivery vectors” is also intended delivery methods to perform transfection.

25 - The terms “vector” or “vectors” refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A “vector” in the present invention includes, but is not limited to, a viral vector, a plasmid, a RNA vector or a linear or circular DNA or RNA molecule which may consists of a chromosomal, non-chromosomal, semi-synthetic or synthetic nucleic acids. Preferred vectors are those capable of autonomous replication (episomal vector) and/or expression
30 of nucleic acids to which they are linked (expression vectors). Large numbers of suitable vectors are known to those of skill in the art and commercially available.

Viral vectors include retrovirus, adenovirus, parvovirus (e. g. adenoassociated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e. g., influenza virus), rhabdovirus (e. g., rabies and vesicular stomatitis virus), paramyxovirus (e. g. measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including
5 adenovirus, herpesvirus (e. g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e. g., vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their
10 replication, In Fundamental Virology, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

- By “lentiviral vector” is meant HIV-Based lentiviral vectors that are very promising for gene delivery because of their relatively large packaging capacity, reduced immunogenicity and their ability to stably transduce with high efficiency a large range of different cell types. Lentiviral vectors
15 are usually generated following transient transfection of three (packaging, envelope and transfer) or more plasmids into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface glycoproteins with receptors on the cell surface. On entry, the viral RNA undergoes reverse transcription, which is mediated by the viral reverse transcriptase complex. The product of reverse transcription is a double-stranded linear viral DNA, which is the substrate for viral
20 integration in the DNA of infected cells. By “integrative lentiviral vectors (or LV)”, is meant such vectors as nonlimiting example, that are able to integrate the genome of a target cell. At the opposite by “non-integrative lentiviral vectors (or NILV)” is meant efficient gene delivery vectors that do not integrate the genome of a target cell through the action of the virus integrase.

- Delivery vectors and vectors can be associated or combined with any cellular
25 permeabilization techniques such as sonoporation or electroporation or derivatives of these techniques.

- By cell or cells is intended any eukaryotic living cells, primary cells and cell lines derived from these organisms for *in vitro* cultures.

- By “primary cell” or “primary cells” are intended cells taken directly from living tissue (i.e. biopsy material) and established for growth *in vitro*, that have undergone very few population
30 doublings and are therefore more representative of the main functional components and

characteristics of tissues from which they are derived from, in comparison to continuous tumorigenic or artificially immortalized cell lines.

As non-limiting examples cell lines can be selected from the group consisting of CHO-K1 cells; HEK293 cells; Caco2 cells; U2-OS cells; NIH 3T3 cells; NSO cells; SP2 cells; CHO-S cells; DG44 cells; K-562 cells, U-937 cells; MRC5 cells; IMR90 cells; Jurkat cells; HepG2 cells; HeLa cells; HT-1080 cells; HCT-116 cells; Hu-h7 cells; Huvec cells; Molt 4 cells.

All these cell lines can be modified by the method of the present invention to provide cell line models to produce, express, quantify, detect, study a gene or a protein of interest; these models can also be used to screen biologically active molecules of interest in research and production and various fields such as chemical, biofuels, therapeutics and agronomy as non-limiting examples.

- by "mutation" is intended the substitution, deletion, insertion of up to one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty five, thirty, forty, fifty, or more nucleotides/amino acids in a polynucleotide (cDNA, gene) or a polypeptide sequence. The mutation can affect the coding sequence of a gene or its regulatory sequence. It may also affect the structure of the genomic sequence or the structure/stability of the encoded mRNA.

- by "variant(s)", it is intended a repeat variant, a variant, a DNA binding variant, a TALE-nuclease variant, a polypeptide variant obtained by mutation or replacement of at least one residue in the amino acid sequence of the parent molecule.

- by "functional variant" is intended a catalytically active mutant of a protein or a protein domain; such mutant may have the same activity compared to its parent protein or protein domain or additional properties, or higher or lower activity.

-"identity" refers to sequence identity between two nucleic acid molecules or polypeptides. Identity can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base, then the molecules are identical at that position. A degree of similarity or identity between nucleic acid or amino acid sequences is a function of the number of identical or matching nucleotides at positions shared by the nucleic acid sequences. Various alignment algorithms and/or programs may be used to calculate the identity between two sequences, including FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default setting. For example, polypeptides having at least 70%, 85%, 90%, 95%, 98% or 99% identity to specific polypeptides described herein and preferably exhibiting

substantially the same functions, as well as polynucleotide encoding such polypeptides, are contemplated. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP “Identities” shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP “Positives” shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity of similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. The polynucleotide sequences of similar polypeptides are deduced using the genetic code and may be obtained by conventional means, in particular by reverse translating its amino acid sequence using the genetic code.

- “signal-transducing domain” or “co-stimulatory ligand” refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

A “co-stimulatory molecule” refers to the cognate binding partner on a Tcell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor.

A “co-stimulatory signal” as used herein refers to a signal, which in combination with primary signal, such as TCR/CD3 ligation, leads to T cell proliferation and/or upregulation or downregulation of key molecules.

The term “extracellular ligand-binding domain” as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus examples of cell surface markers that may act as ligands include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

The term “subject” or “patient” as used herein includes all members of the animal kingdom including non-human primates and humans.

The above written description of the invention provides a manner and process of making and using it such that any person skilled in this art is enabled to make and use the same, this enablement being provided in particular for the subject matter of the appended claims, which make up a part of the original description.

Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and subranges within a numerical limit or range are specifically included as if explicitly written out.

The above description is presented to enable a person skilled in the art to make and use the invention, and is provided in the context of a particular application and its requirements. Various modifications to the preferred embodiments will be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the invention. Thus, this invention is not intended to be limited to the embodiments shown, but is to be accorded the widest scope consistent with the principles and features disclosed herein.

Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

Examples

Materials and Methods

Primary cells

Peripheral blood mononuclear cells were isolated by density gradient centrifugation from buffy coats from healthy volunteer donors (Etablissement Français du Sang). T lymphocytes were

then purified using the EasySep human T cell enrichment kit (Stemcell Technologies), and activated with Dynabeads Human T-Activator CD3/CD28 (Life Technologies) in X-vivo 15 medium (Lonza) supplemented with 20 ng/ml IL-2 (Miltenyi) and 5% human AB serum (Seralab).

5 *Cell lines*

The HCT116, MCF-7, SK-MEL-28 and Daudi cell lines were obtained from the American Type Culture Collection. HCT116 cells were cultured in McCoy supplemented with 10% heat-inactivated FCS, 2mmol/L L-glutamine and 100 units/ml penicillin, and 100µg/mL streptomycin. MCF-7 cells were cultured in DMEM supplemented with 10% heat-inactivated FCS, 2mmol/L L-glutamine and 100
10 units/ml penicillin, and 100µg/mL streptomycin and 0.01mg/ml human insulin. SK-MEL-28 cells were cultured in MEM supplemented with 10% heat-inactivated FCS, 2mmol/L L-glutamine and 100 units/ml penicillin, and 100µg/mL streptomycin. Daudi cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2mmol/L L-glutamine and 100 units/ml penicillin, and 100µg/mL streptomycin.

15

Synthesis and cloning of scCARs coding sequences

The DNA sequences encoding the scCARs were synthesized by GenScript and cloned in a plasmid containing the T7 promoter for the in vitro synthesis of CAR mRNA.

20 *In vitro synthesis of CAR mRNA*

mRNA encoding the scCARs were synthesized using as templates linearized plasmids in which the sequence encoding the CARs is under the control of the T7 promoter. In vitro transcription and polyadenylation were done using the mMessage mMachine T7 Ultra kit (Life technologies) according to the manufacturer's instructions. RNAs were purified with RNeasy columns (Qiagen), eluted in
25 cytoporation medium T (Harvard Apparatus), and quantified by measuring absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer. Quality of the RNA was verified on a denaturing formaldehyde/MOPS agarose gel.

RNA electroporation of T cells

30 After a period of 11-12 days of activation, T lymphocytes were transfected by electrotransfer of messenger RNA using an AgilePulse MAX system (Harvard Apparatus). Following removal of activation beads, cells were pelleted, resuspended in cytoporation medium T at 25×10^6 cells/ml.

5×10^6 cells were mixed with 15 μ g of the mRNA encoding the scCAR into a 0.4 cm cuvette. The electroporation consisted of two 0.1 ms pulses at 1200 V followed by four 0.2ms pulses at 130V. Following electroporation, cells were diluted into culture medium and incubated at 37°C/ 5% CO₂.

5 *Degranulation assay*

A batch of 5×10^4 T cells were co-cultured with 5×10^4 5T4-positive (MCF7 or HCT116) or -negative cells (Daudi) in 0.1 ml per well in a 96-well plate. APC-labeled anti-CD107a (BD Biosciences) was added at the beginning of the co-culture in addition to 1 μ g/ml of anti-CD49d (BD Biosciences), 1 μ g/ml of anti-CD28 (Miltenyi), and 1x Monensin solution (eBioscience). After a 6h incubation, the
 10 cells were stained with a fixable viability dye (eBioscience) and vioblue-labeled anti-CD8 (Miltenyi) and analyzed using the MACSQuant flow cytometer (Miltenyi). Degranulating cytotoxic T cells correspond to CD8+CD107a+ cells.

Cytotoxicity assay

15 5T4-positive and -negative cells were respectively labeled with CellTrace CFSE and CellTrace Violet. Un batch of 2×10^4 5T4-positive cells (MCF7 or HCT116) were co-cultured with 2×10^4 5T4-negative cells (SKMEL28) with 4×10^5 T cells in 0.1ml per well in a 96-well plate. After a 4 hours incubation, the cells were harvested and stained with a fixable viability dye (eBioscience) and analyzed using the MACSQuant flow cytometer (Miltenyi).

20 The percentage of specific lysis was calculated using the following formula:

$$\% \text{ cell lysis} = 100\% - \frac{\frac{\% \text{ viable target cells upon coculture with CAR modified T cells}}{\% \text{ viable control cells upon coculture with CAR modified T cells}}}{\frac{\% \text{ viable target cells upon coculture with non modified T cells}}{\% \text{ viable control cells upon coculture with non modified T cells}}}$$

Example 1: Proliferation of TCRalpha inactivated cells expressing a 5T4-CAR.

25 Heterodimeric TALE-nuclease targeting two 17-bp long sequences (called half targets) separated by an 15-bp spacer within T-cell receptor alpha constant chain region (TRAC) gene were designed and produced. Each half target is recognized by repeats of the half TALE-nucleases listed in Table 10

Table 10: TAL-nucleases targeting TCRalpha gene

Target	Target sequence	Polynucleotid encode TALEN	Half TALE-nuclease
TRAC_T01	TTGTCCCACAGATATCC Agaaccctgaccctg CCGTGTACCAGCTGAGA (SEQ ID NO: 43)	T01-L (SEQ ID NO: 44)	TRAC_T01-L TALEN (SEQ ID NO: 46)
		T01-R (SEQ ID NO: 45)	TRAC_T01-R TALEN (SEQ ID NO: 47)

5 Each TALE-nuclease construct was subcloned using restriction enzyme digestion in a mammalian expression vector under the control of the T7 promoter. mRNA encoding TALE-nuclease cleaving TRAC genomic sequence were synthesized from plasmid carrying the coding sequence downstream from the T7 promoter.

10 Purified T cells preactivated during 72 hours with anti-CD3/CD28 coated beads were transfected with each of the 2 mRNAs encoding both half TRAC_T01 TALE-nucleases. 48 hours post-transfection, different groups of T cells from the same donor were respectively transduced with a lentiviral vector encoding one of the 5T4 CAR previously described (SEQ ID NO: 19 to 42). 2 days post-transduction, CD3_{NEG} cells were purified using anti-CD3 magnetic beads and 5 days post-transduction cells were reactivated with soluble anti-CD28 (5 µg/ml).

15 Cell proliferation was followed for up to 30 days after reactivation by counting cell 2 times per week. Increased proliferation in TCR alpha inactivated cells expressing the 5T4 CARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

To investigate whether the human T cells expressing the 5T4 CAR display activated state, the expression of the activation marker CD25 are analyzed by FACS 7 days post transduction. The purified
20 cells transduced with the lentiviral vector encoding 5T4 CAR assayed for CD25 expression at their surface in order to assess their activation in comparison with the non-transduced cells. Increased CD25 expression is expected both in CD28 reactivation or no reactivation conditions.

25 **Example 2: Selection of 5T4 -positive and -negative cell line**

Eight human cell lines were screened for 5T4 expression by western blot and flow cytometry (see Table 11 below).

Table 11: Expression of 5T4 antigen in 8 human cell lines

Cell line	Description	Cell type
MCF7	adherent	adenocarcinoma
HCT116	adherent	colorectal carcinoma
MKN45	adherent	gastric carcinoma
LS174T	adherent	colorectal adenocarcinoma
SK-MEL-28	adherent	malignant melanoma
SupT1	suspension	T-cell lymphoblastic lymphoma
Daudi	suspension	Burkitt's lymphoma

5T4 was not detected in extracts from Daudi (ATCC CCL-213), SupT1 (ATT CRL-1942) and SK-MEL-28 (ATCC HTB-72) cells but was detected in extracts from MCF7 (ATCC HTB-22), HCT116 (ATCC CCL-247), MKN45 (JCRB0254) and LS174T (ATCC CL-188) cells. Among the cells that were positive for 5T4 antigen following western blot analysis, only two were found to express 5T4 at the cell surface: MCF7 and HCT116 cells, MCF7 expressing highest levels of 5T4 antigen than HCT116 cells.

Example 3: Generation of anti-5T4 scCARs

Second generation singlechain CARs specific for 5T4 (shown schematically in Figures 3 to 6 and in Table 3 to Table 6) were created by combining the sequences of 4 different scFv with the sequences of 3 different spacers, 2 different transmembrane domains, 1 costimulatory domain and 1 stimulatory domain as represented in Figure 2.

The sequences used in the CARs (presented in Table 1 and Table 2) derive from:

- the H8, A1, A2 or A3 antibodies for the scFv;
- the IgG1, FcεRIIIy or CD8α molecules for the spacer domain;
- the CD8α or 4-1BB molecules for the transmembrane domain;
- the 4-1BB molecule for the costimulatory domain;
- the CD3ζ molecule for the stimulatory domain.

Example 4: *In vitro* testing of anti-5T4 scCARs

To evaluate the activity of 5T4-specific singlechain CARs, human T cells from healthy volunteers were activated with CD3/CD28 beads and, eleven days post activation, were electroporated with mRNA encoding the CARs. CAR's activity and specificity were analysed 1-2 days

post transfection by measuring T cells degranulation and T cell cytotoxicity against 5T4-positive and – negative target cells.

The results are presented below for the testing on one case (N=1), however experiments were performed on two other cases showing similar results (not shown).

5 Figure 7 shows that all the CARs tested induced significant level ($\geq 20\%$) of T cells degranulation upon coculture with MCF7 but not upon coculture with Daudi cells. Among the eight CARs tested seven were also able to mediate T cells degranulation following coculture with HCT116 cells, a cell line expressing lower level of 5T4 than MCF7.

10 Figure 8 shows that all the T cells modified with the A1-v3, A1-v5, A2-v3, A2-v5, A3-v3, H8-v2 and H8-v3 CARs lysed significantly and specifically MCF7 cells. T cells modified with the A1-v3, A1-v5, A2-v3, A2-v5, A3-v3 and H8-v3 CARs were also able to lyse HCT116 cells, a cell line expressing lower level of 5T4 than MCF7 cells.

Examples of CAR polypeptide sequences:

Framed sequences correspond to preferred VH and VL sequences. VH and VL may be swapped to improve CAR efficiency.

H8 v1

MALPVTALLPLALLLHAARP EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS GGGGSGGGGS
 GGGGS SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR GLAVSTISSFFPPGYQIYWAPLAGTCGVLLSLVITLYCKRGR
 KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
 RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP
 R

H8 v2

MALPVTALLPLALLLHAARP EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS GGGGSGGGGS
 GGGGS SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFLLTLRFSVVKRG
 RKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
 RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
 PR

H8 v3

MALPVTALLPLALLLHAARP EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS GGGGSGGGGS
 GGGGS SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR TTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDF
 ACDIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA
 PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
 KGHDGLYQGLSTATKDTYDALHMQALPPR

H8 v4

MALPVTALLPLALLHAARP[EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS]GGGGSGGGGS
 GGGGS[SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDF
 ACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD
 APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
 GKGHDGLYQGLSTATKDTYDALHMQALPPR

H8 v5

MALPVTALLPLALLHAARP[EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS]GGGGSGGGGS
 GGGGS[SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPE
 VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMR
 PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

H8 v6

MALPVTALLPLALLHAARP[EVQLQQSGPDLVKPGASVKISCKASGYSFTGYMHVWKQSHGKSLEWIGRINPNN
 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDYWGQVTSVTVSS]GGGGSGGGGS
 GGGGS[SIVMTQTPTFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYAGVPDRFIGSGYGTDF
 FTISTLQAEDLAVYFCQQDYNPPTFGGGTKLEIKR]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPE
 VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLT
 VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMR
 RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A1 v1

MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT
NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSSGGGSGGGGS
GGGGSIVMTQTPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD
FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLSLVITLYCKRG
RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
RRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

A1 v2

MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT
NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSSGGGSGGGGS
GGGGSIVMTQTPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD
FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFLLTLRFSVVKR
GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
PPR

A1 v3

MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT
NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSSGGGSGGGGS
GGGGSIVMTQTPKFLLVSAGDRVITITCKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD
FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD
FACDIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD
APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGHDGLYQGLSTATKDTYDALHMQALPPR

A1 v4

MALPVTALLLPLALLLHAARP[QIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT]
[NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSS]GGGSGGGGS
GGGGS[SIVMTQTPKFLLVSAGDRVITICKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD]
[FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLD
FACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR

A1 v5

MALPVTALLLPLALLLHAARP[QIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT]
[NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSS]GGGSGGGGS
GGGGS[SIVMTQTPKFLLVSAGDRVITICKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD]
[FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP
EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM
RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A1 v6

MALPVTALLLPLALLLHAARP[QIQLVQSGPELKKPGETVKISCKASGYTFTNFGMNWVKQGPGEGLKWMGWINT]
[NTGEPRYAEEFKGRFAFSLETTASTAYLQINNLIKNETATYFCARDWDGAYFFDYWGQGTTTLTVSS]GGGSGGGGS
GGGGS[SIVMTQTPKFLLVSAGDRVITICKASQSVSNDVAWYQQKPGQSPKLLINFATNRYTGVPNRFTGSGYGTD]
[FTFTISTVQAEDLALYFCQQDYSSPWTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP
EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKL
TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A2 v1

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
GGSQIVLTQSPAISASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL
TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKGLAVSTISSFFPPGYQIYWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A2 v2

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
GGSQIVLTQSPAISASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL
TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTRFSVVKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
GRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A2 v3

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
GGSQIVLTQSPAISASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL
TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
HDGLYQGLSTATKDTYDALHMQALPPR

A2 v4

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
GGSQIVLTQSPAISASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL

TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
 DIISFFLALTSTALLFLLFFLT LRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAP
 AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
 GHDGLYQGLSTATKDTYDALHMQALPPR

A2 v5

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
 NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
 GGSQIVLTQSPAIMSASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL
 TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPV
 QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A2 v6

MALPVTALLPLALLHAARPQVQLQQSRPELVKPGASVKMSCKASGYTFTDYVISWVKQRTGQGLEWIGEIYPGS
 NSIYYNEKFKGRATLTADKSSSTAYMQLSSLTSEDSAVYFCAMGGNYGFDYWGGQGTTLTVSSGGGGSGGGGSGG
 GGSQIVLTQSPAIMSASLGERVTLTCTASSSVNSNYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSL
 TISSMEAEDAATYYCHQYHRSPLTFGAGTKLELKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLT LRFSVVKRGRKKLLYIFKQPFMRP
 VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A3 v1

MALPVTALLPLALLHAARP EVQLVESGGGLVQPGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK
 SNNYATYYADSVKDRFTISRDDSQSMYLYQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSTVTVSSGGG
 GSGGGGSGGGGS DIVMTQSHIFMSTSVGDRVSITCKASQDQDVAWAQYQQKPGQSPKLLIYWASTRLTGVPDRF
 TGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGKLEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRRE
EYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
HMQALPPR

A3 v2

MALPVTALLPLALLHAARP[EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK]
[SNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSVTVSS]GGG
GSGGGGSGGGGS[DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIWASTRLTGVPDRF]
[TGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGKLEIK]GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLT
RFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRR
EEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

A3 v3

MALPVTALLPLALLHAARP[EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK]
[SNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSVTVSS]GGG
GSGGGGSGGGGS[DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIWASTRLTGVPDRF]
[TGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA
VHTRGLDFACDIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRV
KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A3 v4

MALPVTALLPLALLHAARP[EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK]
[SNNYATYYADSVKDRFTISRDDSQSMLYLQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSVTVSS]GGG
GSGGGGSGGGGS[DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIWASTRLTGVPDRF]
[TGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA
VHTRGLDFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A3 v5

MALPVTALLPLALLHAARP[EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK]
[SNNYATYYADSVKDRFTISRDDSQSMYLYQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSVTVSS]GGG
GSGGGGSGGGGS[DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIWASTRLTGVPDRF]
[TGSGSGTDFTLTISNVQSEDLADYFCQQYSSYPYTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKD
TLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSG
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EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

A3 v6

MALPVTALLPLALLHAARP[EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSK]
[SNNYATYYADSVKDRFTISRDDSQSMYLYQMNNLKTEDTAMYYCVRQWDYDVRAMNYWGQGTSVTVSS]GGG
GSGGGGSGGGGS[DIVMTQSHIFMSTSVGDRVSITCKASQDVDTAVAWYQQKPGQSPKLLIWASTRLTGVPDRF]
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TLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSG
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YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

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CLAIMS

1. A 5T4 (NTRKR1) specific chimeric antigen receptor (CAR) having one of the polypeptide structure selected from V1 to V6 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-5T4 antibody, a hinge, a transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

2. A 5T4 specific CAR according to claim 1, wherein said structure V3 comprises a CD8 α hinge and a CD8 α transmembrane domain.

3. A 5T4 specific CAR according to claim 1, wherein said structure V5 comprises an IgG1 hinge and a CD8 α transmembrane domain.

4. A 5T4 specific CAR according to claim 1, wherein said structure V1 comprises a Fc γ RIII α hinge and CD8 α transmembrane domain.

5. A 5T4 specific CAR according to claim 1, wherein said structure V2 comprises a Fc γ RIII α hinge and a 4-1BB transmembrane domain.

6. A 5T4 specific CAR according to claim 1, wherein said structure V4 comprises a CD8 α hinge and a 4-1BB transmembrane domain.

7. A 5T4 specific CAR according to claim 1, wherein said structure V6 comprises a IgG1 hinge and a 4-1BB transmembrane domain.

8. A 5T4 specific CAR according to any one of claims 1 to 7, wherein said VH and VL have at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with a polypeptide sequence selected from SEQ ID NO. 11 to 18.

9. A 5T4 specific CAR according to any one of claims 1 to 8, wherein said extra cellular ligand binding-domain comprises a VH chain comprising the CDRs from the mouse monoclonal antibody A1 of SEQ ID NO. 54 (CDR1), SEQ ID NO.55 (CDR2) and SEQ ID NO.56 (CDR3), and a VL chain comprising

the CDRs from the mouse monoclonal antibody A1 of NO. 57 (CDR1), SEQ ID NO.58 (CDR2) and SEQ ID NO:59 (CDR3).

10. A 5T4 specific CAR according to any one of claims 1 to 9, wherein said VH and VL have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:13 (A1-VH) and SEQ ID NO:14 (A1-VL).

11. A 5T4 specific CAR according to any one of claims 1 to 8, wherein said extra cellular ligand binding-domain comprises a VH chain comprising the CDRs from the mouse monoclonal antibody A2 of SEQ ID NO. 61 (CDR1), SEQ ID NO.61 (CDR2) and SEQ ID NO.63 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody A2 of SEQ ID NO. 64 (CD1), SEQ ID NO.65 (CD2) and SEQ ID NO:65 (CDR3).

12. A 5T4 specific CAR according to any one of claims 1 to 8 and 11, wherein said VH and VL have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:15 (A2-VH) and SEQ ID NO:16 (A2-VL).

13. A 5T4 specific CAR according to any one of claims 1 to 8, wherein said extra cellular ligand binding-domain comprises a VH chain comprising the CDRs from the mouse monoclonal antibody A3 of SEQ ID NO. 66 (CDR1), SEQ ID NO.67 (CDR2) and SEQ ID NO.68 (CDR3), and a VL chain comprising the CDRs from the mouse monoclonal antibody A3 of SEQ ID NO. 69 (CDR1), SEQ ID NO.70 (CDR2) and SEQ ID NO:71 (CDR3).

14. A 5T4 specific CAR according to any one of claims 1 to 8 and 13, wherein said VH and VL have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:17 (A3-VH) and SEQ ID NO:18 (A3-VL).

15. A 5T4 specific CAR according to any one of claims 1 to 8, wherein said extra cellular ligand binding-domain comprises a VH chain comprising the CDRs from the mouse monoclonal antibody H8 of SEQ ID NO. 48 (CDR1), SEQ ID NO.49 (CDR2) and SEQ ID NO.50 (CDR3), and a VL chain

comprising the CDRs from the mouse monoclonal antibody H18 of NO. 51 (CDR1), SEQ ID NO.52 (CDR2) and SEQ ID NO:53 (CDR3).

16. A 5T4 specific CAR according to any one of claims 1 to 8 and 15, wherein said VH and VL have at least 80%, preferably at least 90%, more preferably at least 95%, and even more preferably at least 99% sequence identity respectively with SEQ ID NO:11 (H18-VH) and SEQ ID NO:12 (H18-VL).

17. A 5T4 specific CAR according to any one of claims 1 to 16, wherein co-stimulatory domain from 4-1BB has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.8.

18. A 5T4 specific CAR according to any one of claims 1 to 17, wherein said CD3 zeta signaling domain has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO. 9.

19. A 5T4 specific CAR according to any one of claims 4, 5 or 8-18, wherein said FcγRIIIα hinge has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.3.

20. A 5T4 specific CAR according to any one of claims 2, 6 or 8-18, wherein said CD8α hinge has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.4.

21. A 5T4 specific CAR according to any one of claims 3, 7 or 8-18, wherein said IgG1 hinge has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.5.

22. A 5T4 specific CAR according to any one of claims 2 to 4 or 8-21, wherein said CD8α transmembrane domain has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.6.

23. A 5T4 specific CAR according to any one of claims 5 to 7 or 8-21, wherein said 4-1BB transmembrane domain has at least 80 %, preferably 90%, more preferably 95%, and even more preferably 99% identity with SEQ ID NO.7.

24. A 5T4 specific CAR according to any one of claims 1 to 23 further comprising another extracellular ligand binding domain which is not specific for 5T4.

25. A 5T4 specific CAR of structure V3 according to claim 2, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 21, SEQ ID NO.27, SEQ ID NO.33 and SEQ ID NO.39.

26. A 5T4 specific CAR of structure V5 according to claim 3, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 23, SEQ ID NO.29, SEQ ID NO.34 and SEQ ID NO.41.

27. A 5T4 specific CAR of structure V1 according to claim 4, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 19, SEQ ID NO.25, SEQ ID NO.31 and SEQ ID NO.37.

28. A 5T4 specific CAR of structure V2 according to claim 5, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 20, SEQ ID NO.26, SEQ ID NO.32 and SEQ ID NO.38.

29. A 5T4 specific CAR of structure V4 according to claim 6, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 22, SEQ ID NO.28, SEQ ID NO.34 and SEQ ID NO.40.

30. A 5T4 specific CAR of structure V6 according to claim 7, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 24, SEQ ID NO.30, SEQ ID NO.36 and SEQ ID NO.42.

31. A 5T4 specific CAR according to any one of claims 1 to 30, further comprising a signal peptide.
32. A 5T4 specific CAR according to claim 31, wherein said signal peptide has at least 80% sequence identity with SEQ ID NO.1 or SEQ ID NO.2.
33. A polynucleotide encoding a chimeric antigen receptor according to any one of claims 1 to 32
34. An expression vector comprising a nucleic acid of claim 33.
35. An engineered immune cell expressing at the cell surface membrane a 5T4 specific chimeric antigen receptor according to any one of claims 1 to 32.
36. An engineered immune cell according to claim 35, derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.
37. An engineered immune cell according to claim 35, wherein it is derived from a NK cell.
38. An engineered cell according to any one of claims 35 to 37 for use in therapy.
39. An engineered cell according to any one of claims 35 to 38 for use in human therapy.
40. An engineered cell according to any one of claims 35 to 39 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by 5T4-expressing cells.
41. An engineered cell according to any one of claims 35 to 40 for use in therapy, wherein the condition is a condition which is characterized by an overabundance of 5T4-expressing cells.
42. An engineered cell according to any one of claims 35 to 41 for use in therapy, wherein the condition is a haematological cancer condition.
43. An engineered cell according to any one of claims 35 to 42 for use in therapy, wherein the haematological cancer condition is leukemia.

44. An engineered cell according to any one of claims 35 to 43 for use in therapy, wherein the haematological cancer condition is childhood pre-B acute lymphoblastic leukemia.

45. An engineered cell according to any one of claims 35 to 41 for use in therapy, wherein the condition is a solid tumor.

46. An engineered cell according to claim 45 for use in therapy, wherein the tumor is a gastric, colorectal, prostate, breast, lung, renal or ovarian tumor.

47. An engineered cell according to any one of claims 35 to 46 wherein expression of TCR is suppressed in said immune cell.

48. An engineered cell according to any one of claims 35 to 47, wherein expression of at least one MHC protein, preferably $\beta 2m$ or HLA, is suppressed in said immune cell.

49. An engineered cell according to any one of claims 35 to 48, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.

50. A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to any one of claims 35 to 49 in an amount effective to cause impairment of said cancer cell.

51. A method of engineering an immune cell comprising:

- (a) Providing an immune cell,
- (b) Expressing at the surface of said cell at least one 5T4 specific chimeric antigen receptor according to any one of claims 1 to 32.

52. The method of engineering an immune cell of claim 51 comprising:

- (a) Providing an immune cell,
- (b) Introducing into said cell at least one polynucleotide encoding said 5T4 specific chimeric antigen receptor,
- (c) Expressing said polynucleotide into said cell.

53. The method of engineering an immune cell of claim 51 comprising:

- (a) Providing an immune cell,

(b) Introducing into said cell at least one polynucleotide encoding said 5T4 specific chimeric antigen receptor,

(c) Introducing at least one other chimeric antigen receptor which is not specific for 5T4.

54. A method of treating a subject in need thereof comprising:

(a) Providing a immune cell expressing at the surface a 5T4 specific Chimeric Antigen Receptor according to any one of claims 1 to 32;

(b) Administering said immune cells to said patient.

55. A method according to any one of claim 51 to 54, wherein said immune cell is provided from a donor.

56. A method according to any one of claim 51 to 54, wherein said immune cell is provided from the patient himself.

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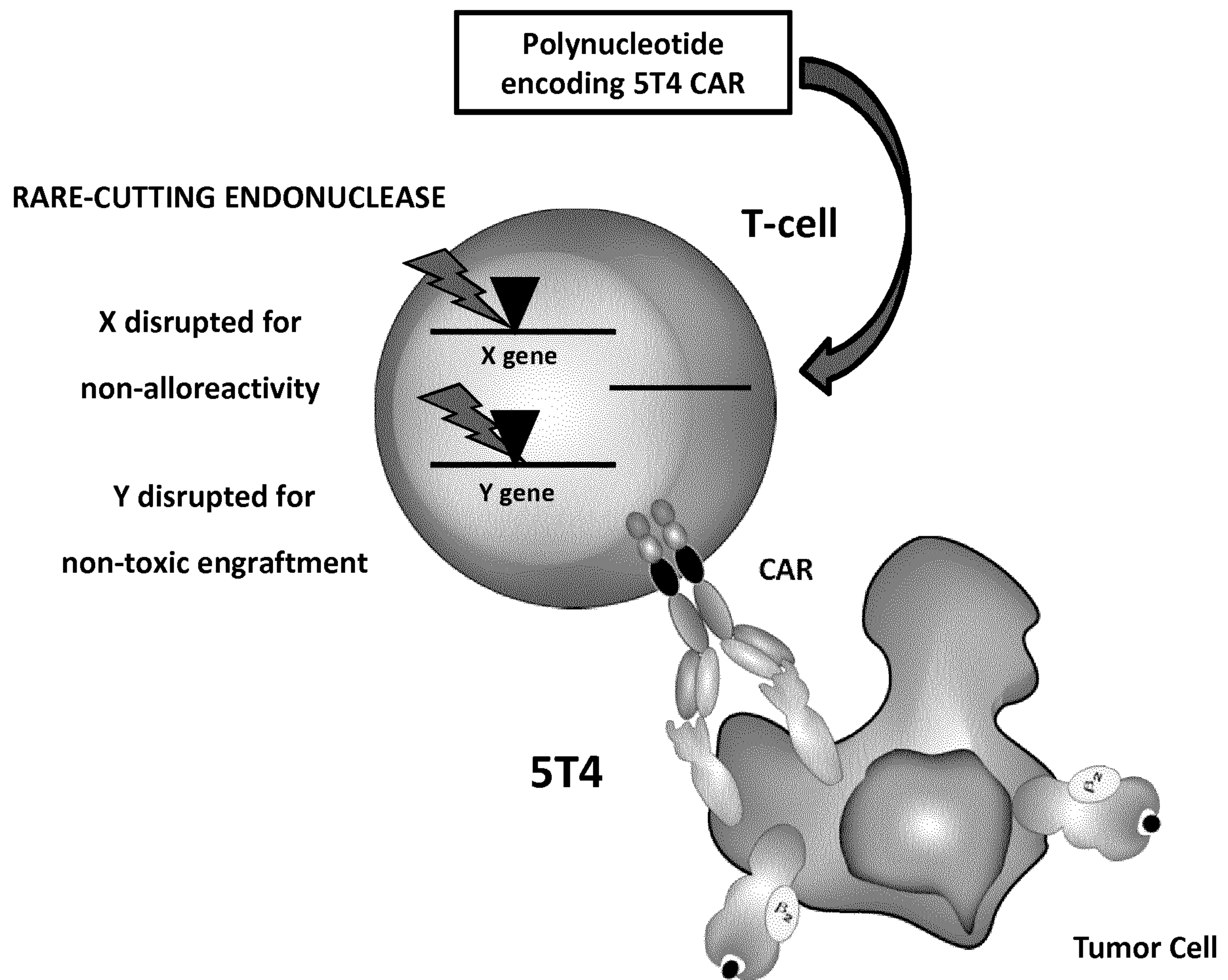


Figure 1

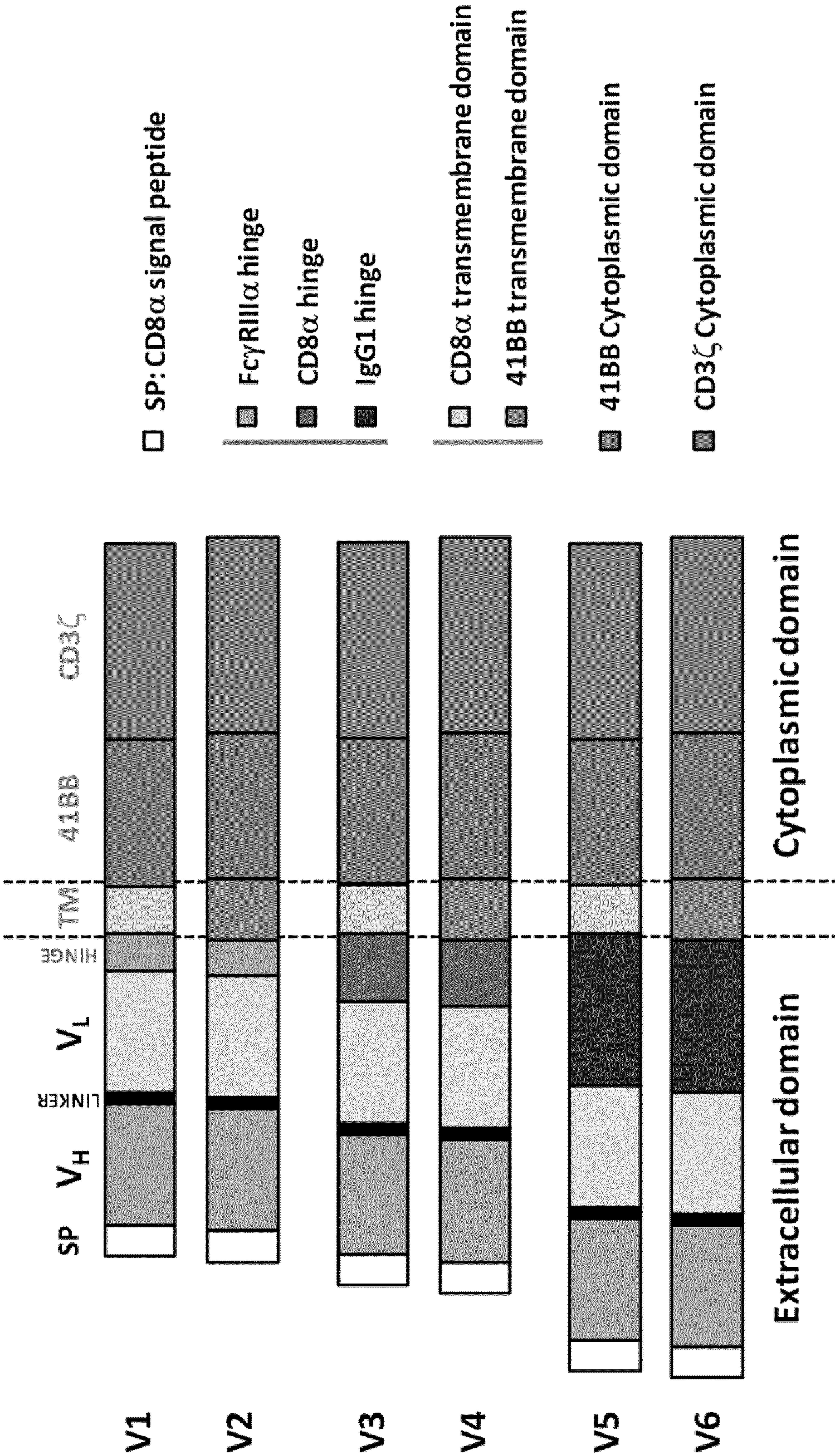


Figure 2

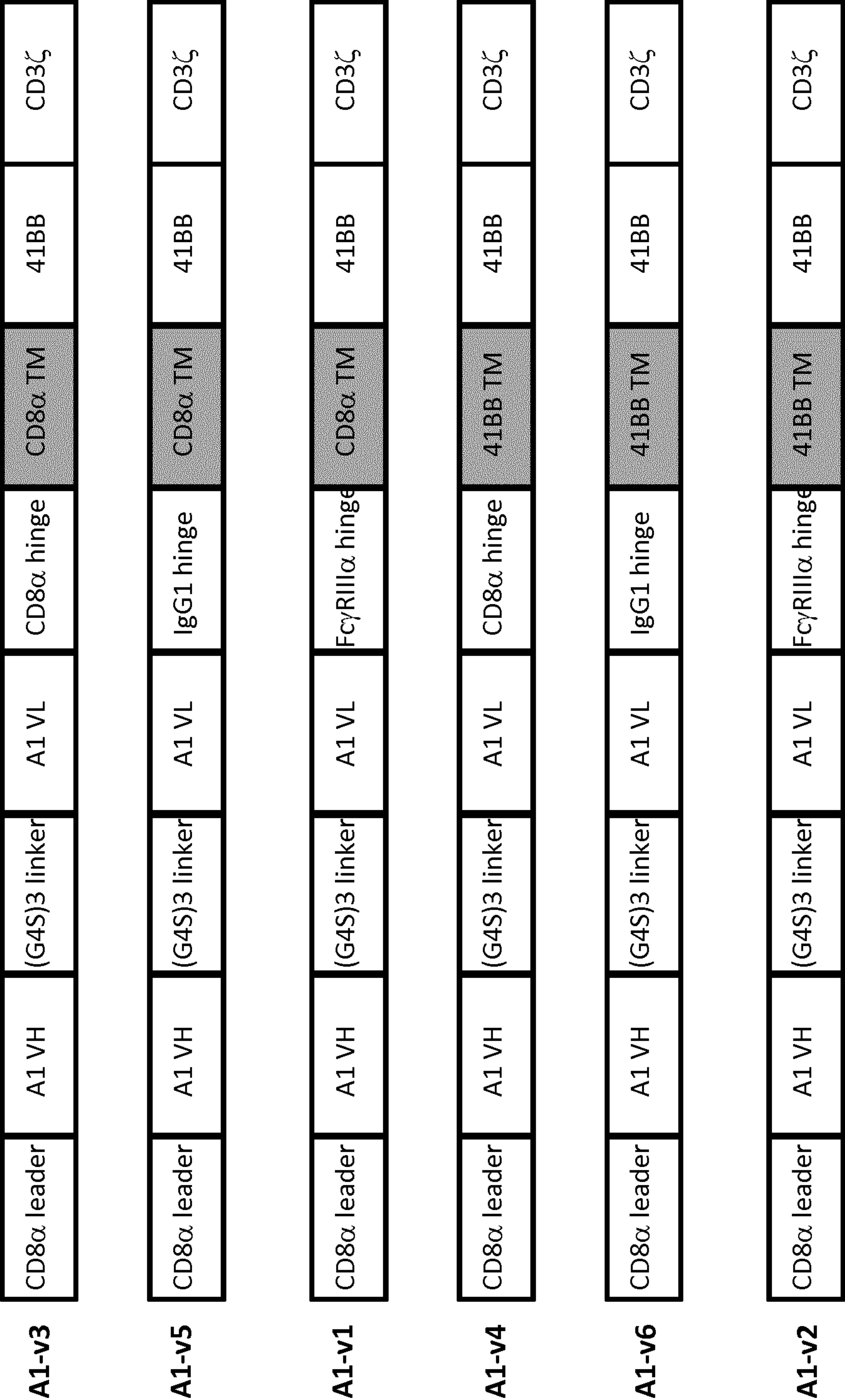


Figure 3

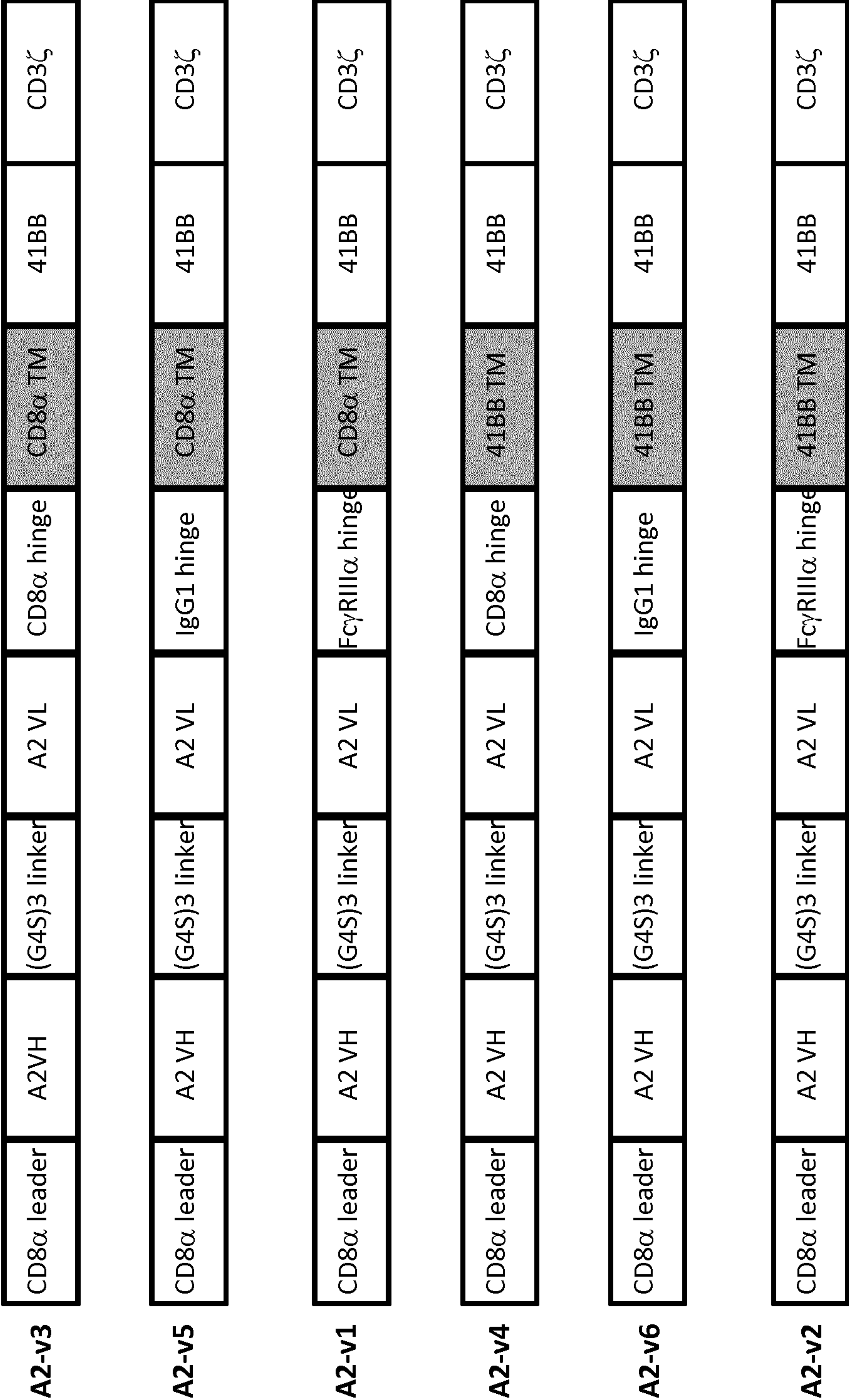


Figure 4

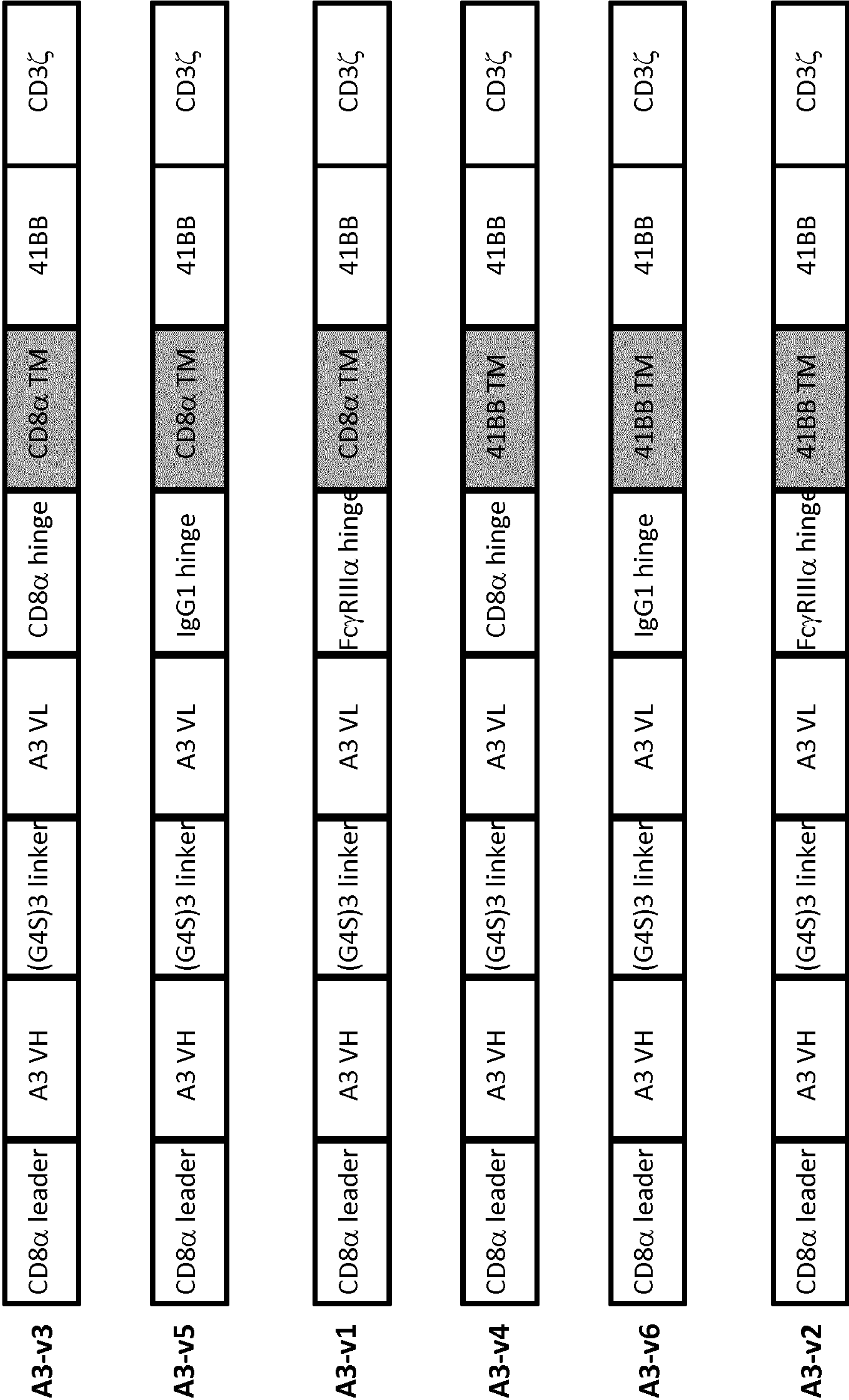


Figure 5

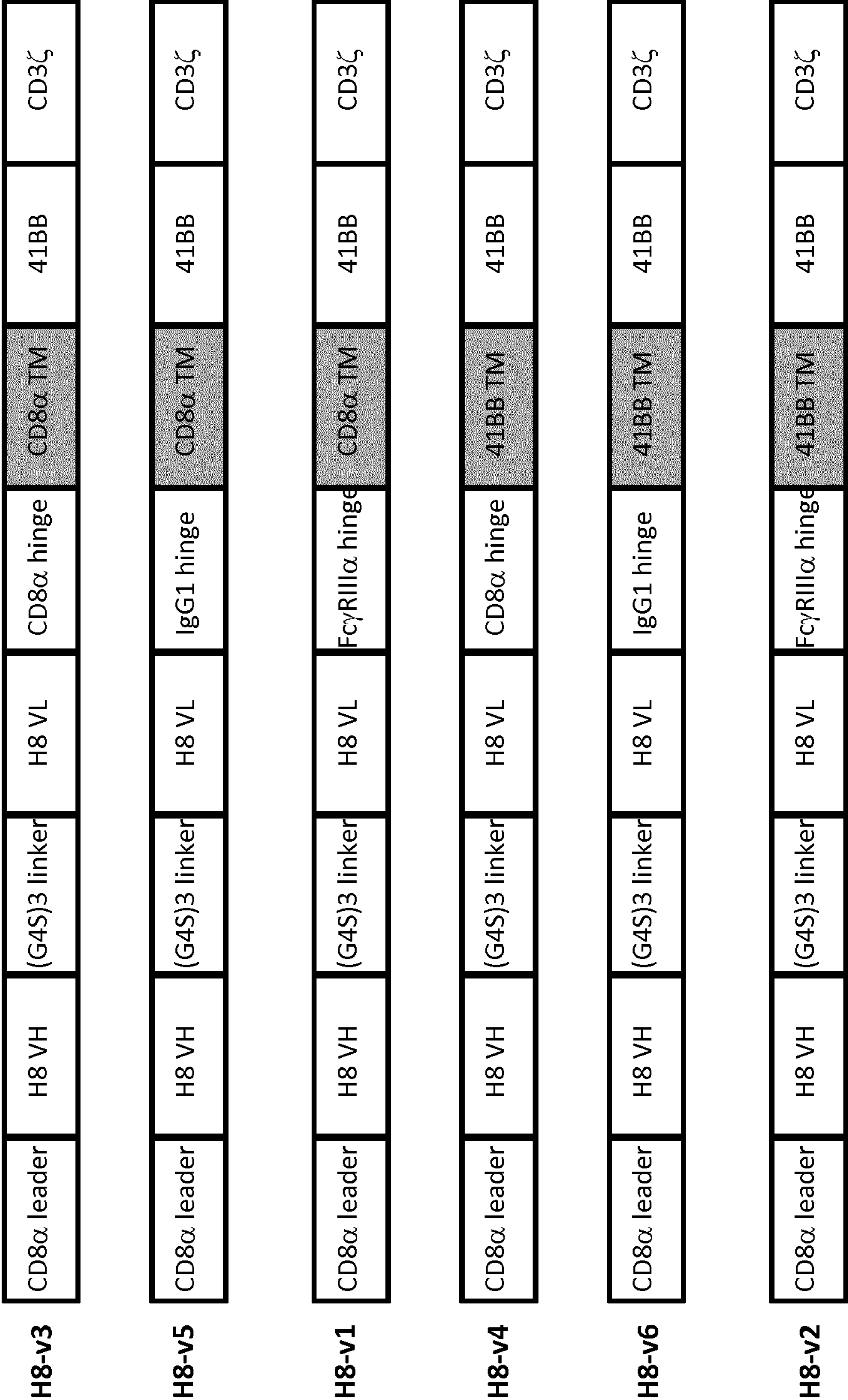


Figure 6

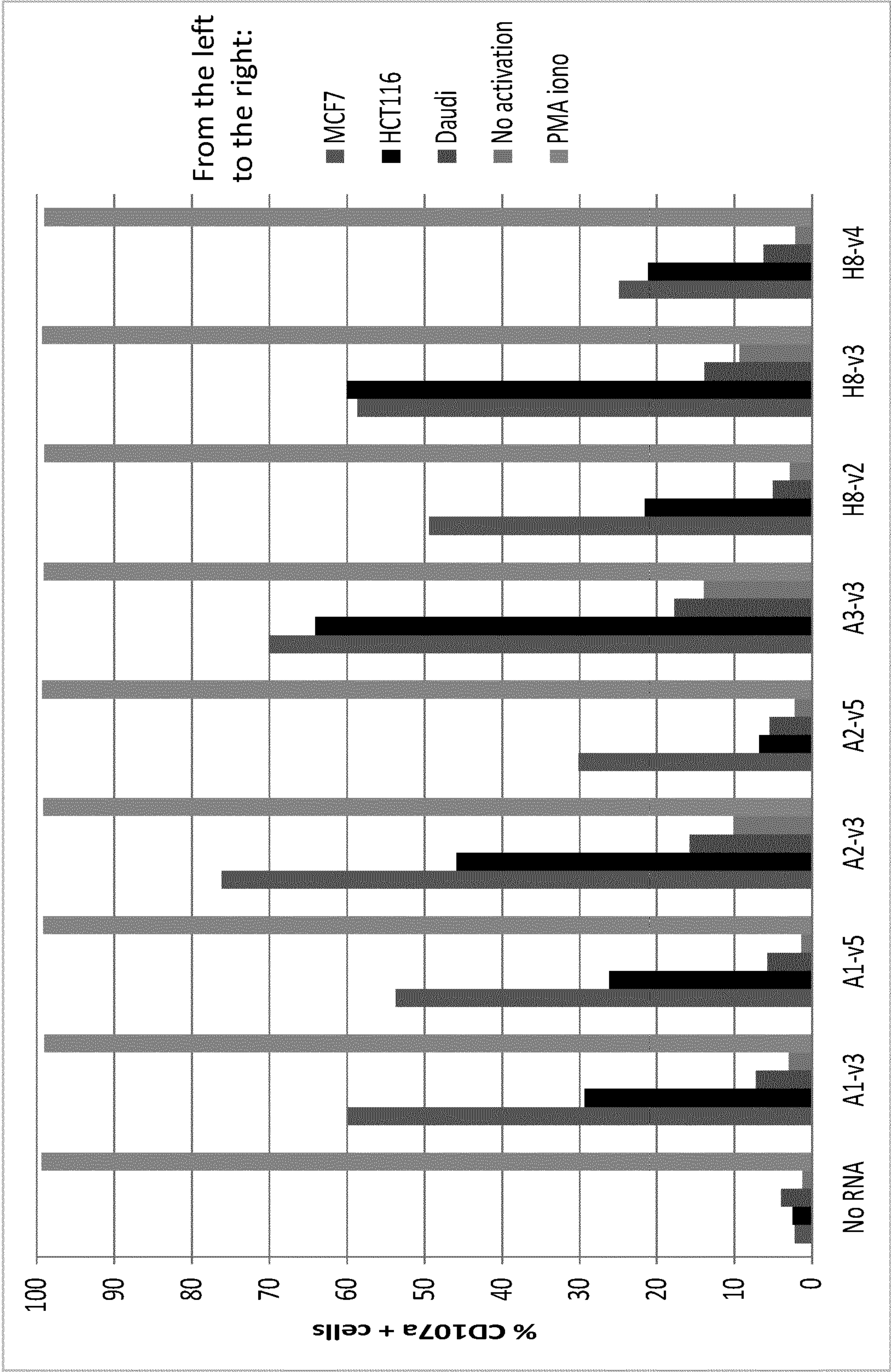


Figure 7

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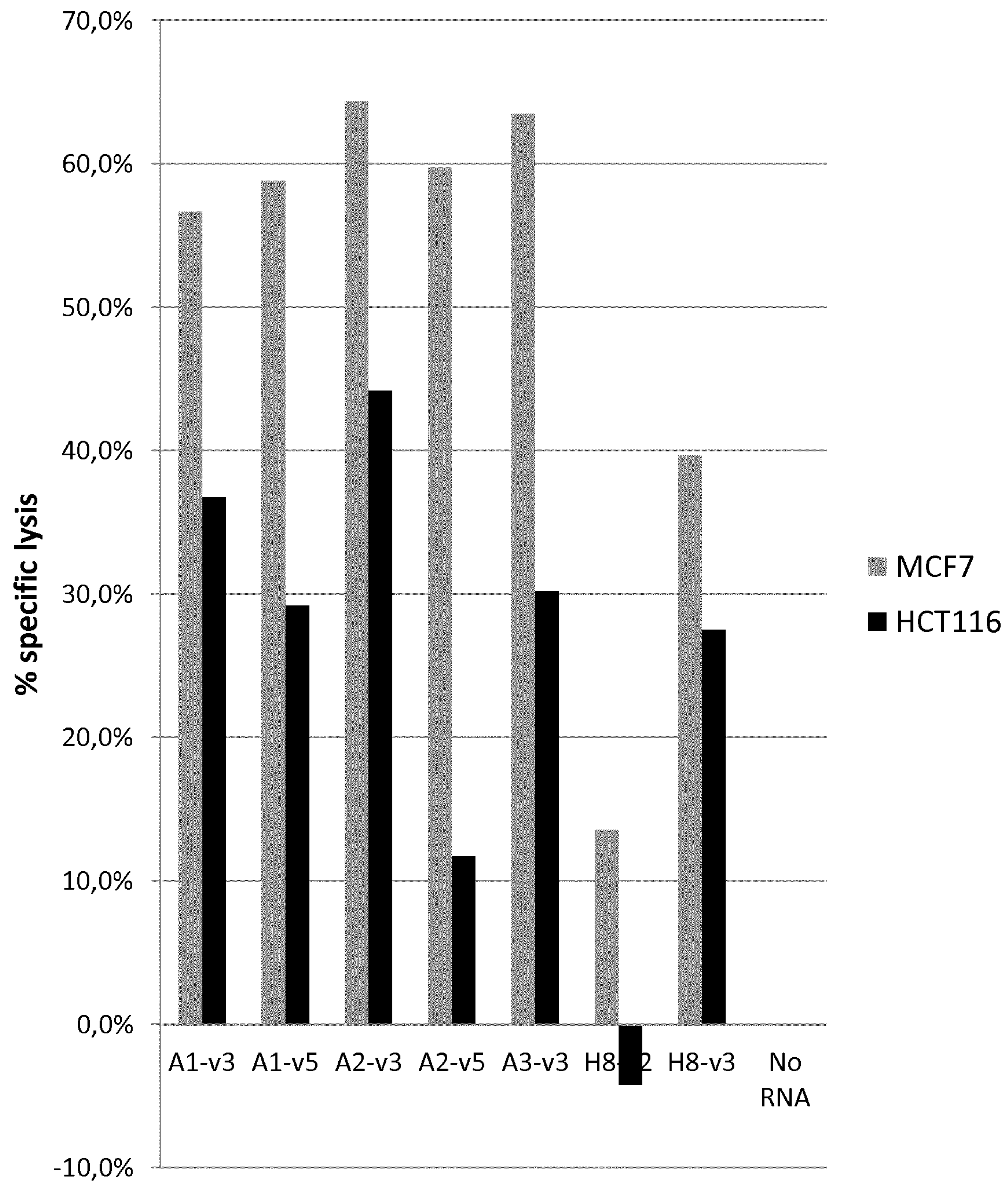


Figure 8

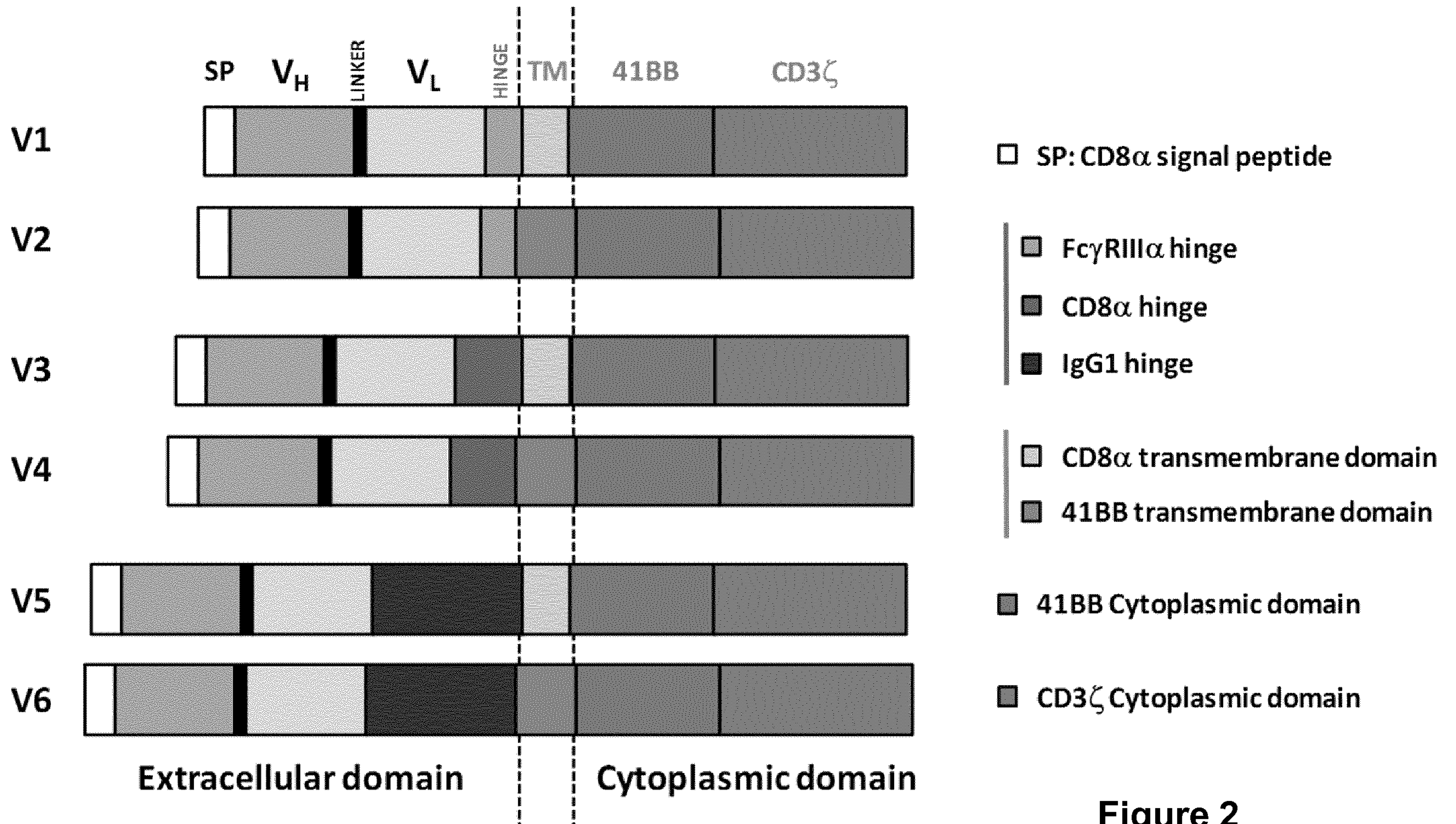


Figure 2