



(86) Date de dépôt PCT/PCT Filing Date: 2016/01/25
(87) Date publication PCT/PCT Publication Date: 2016/08/04
(85) Entrée phase nationale/National Entry: 2017/07/11
(86) N° demande PCT/PCT Application No.: EP 2016/051469
(87) N° publication PCT/PCT Publication No.: 2016/120218
(30) Priorité/Priority: 2015/01/26 (DK PA201570044)

(51) Cl.Int./Int.Cl. *C07K 19/00* (2006.01),
A61K 39/395 (2006.01), *C07K 14/705* (2006.01),
C07K 16/28 (2006.01), *C12N 15/62* (2006.01),
C12N 5/0783 (2010.01), *C12N 5/10* (2006.01)
(71) Demandeurs/Applicants:
CELLECTIS, FR;
RINAT NEUROSCIENCE CORPORATION, US
(72) Inventeurs/Inventors:
SMITH, JULIANNE, FR;
VALTON, JULIEN, US;
JUILLERAT, ALEXANDRE, US;
DUCHATEAU, PHILIPPE, FR;
SASU, BARBRA JOHNSON, US; ...

(54) Titre : RECEPTEURS D'ANTIGENES CHIMERIQUES MONOCATENAIRES SPECIFIQUES ANTI-CLL1 (SCCAR)
POUR UNE IMMUNOTHERAPIE ANTICANCEREUSE
(54) Title: ANTI-CLL1 SPECIFIC SINGLE-CHAIN CHIMERIC ANTIGEN RECEPTORS (SCCARS) FOR CANCER
IMMUNOTHERAPY

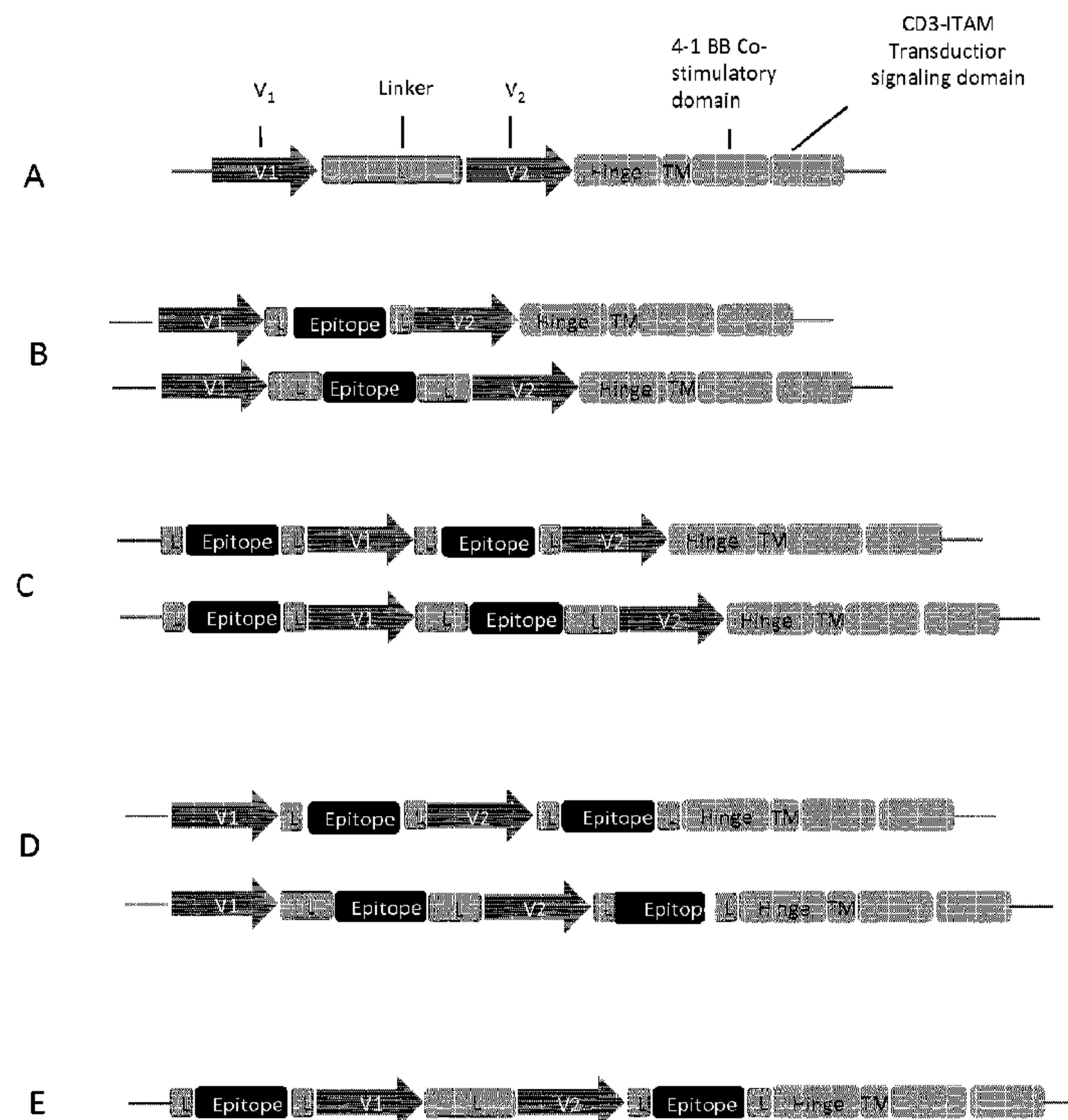


Figure 3A

(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 CLL1 positive cells. The engineered immune cells endowed with such CARs are particularly suited for immunotherapy for treating cancer, in particular leukemia.



(72) Inventeurs(suite)/Inventors(continued): RAJPAL, ARVIND, US

(74) Agent: BORDEN LADNER GERVAIS LLP

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

(19) World Intellectual Property
Organization
International Bureau(43) International Publication Date
4 August 2016 (04.08.2016)(10) International Publication Number
WO 2016/120218 A1(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)(21) International Application Number:
PCT/EP2016/051469(22) International Filing Date:
25 January 2016 (25.01.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA201570044 26 January 2015 (26.01.2015) DK(71) Applicants: **CELLECTIS** [FR/FR]; 8 Rue de la Croix Jarry, 75013 Paris (FR). **RINAT NEUROSCIENCE CORPORATION** [US/US]; 230 East Grand Avenue, South San Francisco, CA 94080 (US).(72) Inventors: **SMITH, Julianne**; 13 rue de la Ferme, 92350 Le Plessis-Robinson (FR). **VALTON, Julien**; 533 12th street, New York, New York 10028 (US). **JUILLERAT, Alexandre**; 444 East 82nd Street #5A, New York, New York 10028 (US). **DUCHATEAU, Philippe**; Bateau FAWEN, Quai des dames, 91210 Draveil (FR). **SASU,****Barbra Johnson**; 2125 Bryant St Apt 316, San Francisco, CA 94110 (US). **RAJPAL, Arvind**; 519 Foerster Street, San Francisco, CA 94127 (US).(74) Agent: **ZACCO DENMARK A/S**; Arne Jacobsens Allé 15, 2300 København S (DK).(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

(54) Title: ANTI-CLL1 SPECIFIC SINGLE-CHAIN CHIMERIC ANTIGEN RECEPTORS (scCARS) FOR CANCER IMMUNOTHERAPY

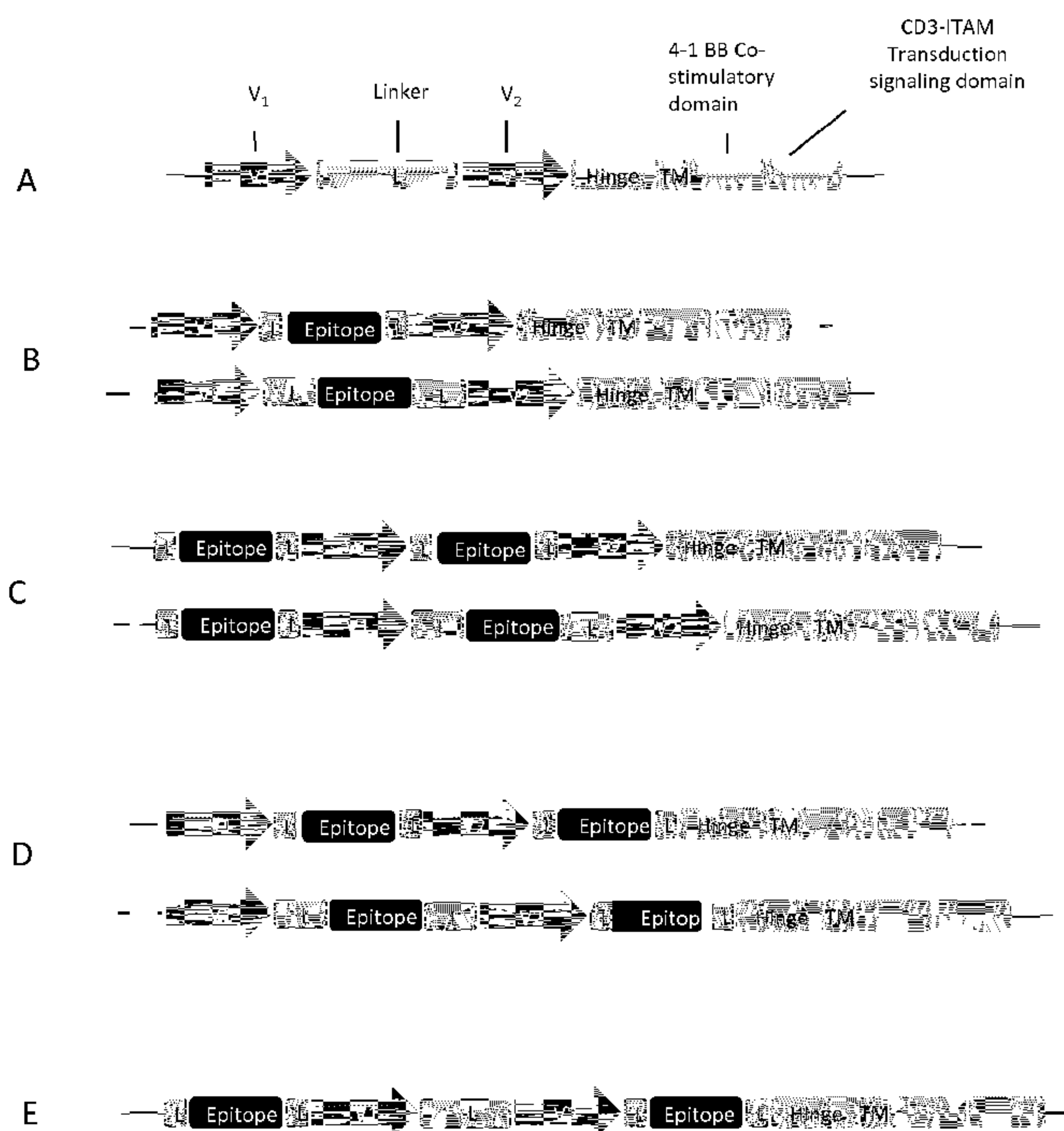


Figure 3A

(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 CLL1 positive cells. The engineered immune cells endowed with such CARs are particularly suited for immunotherapy for treating cancer, in particular leukemia.

WO 2016/120218 A1

LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, **Published:**
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, — *with international search report (Art. 21(3))*
GW, KM, ML, MR, NE, SN, TD, TG). — *with sequence listing part of description (Rule 5.2(a))*

ANTI-CLL1 SPECIFIC SINGLE-CHAIN CHIMERIC ANTIGEN RECEPTORS (scCARS) FOR CANCER
IMMUNOTHERAPY

Field of the invention

5 C-type lectin-like molecule-1 (CLL1) has been identified as being frequently over-expressed on most acute myeloid leukemia (AML) stem cells (LSC), compared to normal hematopoietic stem cells or other cell types. The present invention relates to methods to target CLL1 positive malignant cells using Chimeric Antigen Receptors (anti CLL1-CAR), which are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward selected membrane antigen CLL1.
10 These anti-CLL1 CAR more particularly comprise an extracellular ligand binding comprising a scFV derived from some specific anti-CLL1 monoclonal antibodies. The engineered immune cells endowed with such CARs confer adoptive immunity against CLL1 positive cell as part as various cell therapies for treating cancer, in particular hematologic cancers, with higher efficiency.

15 **Background of the invention**

Adoptive immunotherapy, which involves the transfer of antigen-specific T cells 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
20 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 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 (scCARS) (Jena, Dotti et al. 2010). scCARS are
25 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 scCAR consists of an antigen-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 scCARS are
30 derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. First generation scCARS have been shown to successfully redirect T cell cytotoxicity, however, they failed

to provide prolonged expansion and anti-tumor activity *in vivo*. Signaling domains from co-stimulatory molecules including CD28, OX-40 (CD134), and 4-1BB (CD137) have been added alone (second generation) or in combination (third generation) to enhance survival and increase proliferation of scCAR modified T cells. scCARs have successfully allowed T cells to be redirected
5 against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010).

Meanwhile, induction treatments for acute myeloid leukemia (AML) have remained largely unchanged for nearly 50 years and AML remains a disease of poor prognosis. AML is a disease characterized by the rapid proliferation of immature myeloid cells in the bone marrow resulting in
10 dysfunctional hematopoiesis. Although standard induction chemotherapy can induce complete remissions, many patients eventually relapse and succumb to the disease, calling for the development of novel therapeutics for AML. Recent advances in the immunophenotyping of AML cells have revealed several AML associated cell surface antigens that may act as targets for future therapies.

15 Among others, CLL1 (C-Type Lectin-Like Molecule-1) appears to be an interesting tumoral antigen target as it is expressed by leukemic blasts at diagnosis from 85-92% of AML patients analysed. It is a 75 kDa member of the group V C-type lectin-like receptor family of molecules. Group V molecules have a lectin-like domain that binds to non-sugar ligands. CLL1 is a 265 aminoacid type II transmembrane glycoprotein (Uniprot database: Q5QGZ9 for human protein encoded by gene
20 n°160364 in "Entrez Gene" database) that contains a 200 AA extracellular domain. CLL1 is also referred to in the literature and databases as MICL, CLEC12 and KLRL1.

Bakker et al, 2004 has shown that the CLL1 antigen is associated with AML stem cells. Like some other antigens (such as CD33), CLL1 is a cell surface protein that is specifically expressed on most malignant lymphoid stem cells (AML LSC), while not being expressed on normal HSC (Van
25 Rhenen et al, 2007). Meanwhile, CLL1 was revealed to be a diagnostic marker in AML (Larsen et al, 2012). Anti-CLL-1 antibodies enable both AML-specific stem-cell detection and possibly antigen-targeting as distinguishing malignant cells from normal stem cells both at diagnosis and in remission (van Rhenen et al, 2007). However, none of these antibodies have been reported to date as being tested in clinical trials as therapeutic antibodies.

30 Monoclonal antibodies have often been used to treat lymphomas, but their use in leukemias has been more limited. Gemtuzumab ozogamicin (Mylotarg[®]) is a monoclonal antibody with a cell poison attached to it. Previously approved to treat AML in older patients, it was withdrawn from the

market after studies found some toxicity associated with the product (press release of December 10, 2010 in PMLIVE “ASH: Pfizer eyes re-launch of Mylotarg”). Other monoclonal therapeutic antibodies have shown adverse effects over the last decade (Klastersky, J. (2006) “Adverse effects of the humanized antibodies used as cancer therapeutics” Current Opinion in Oncology. 18(4):316–320)

5 In the publication of Zhang et al (2011), micellar nanoparticles covalently decorated with CLL1-targeting peptides have been described for targeted drug delivery (daunorubicin); these "targeting nanomicelles" transport the drug load to the interior of cells expressing CLL1 and to LSCs isolated from clinical specimens in vitro. It was showed that CLL1-targeting nanomicelles had the potential to be used for targeted drug delivery to leukemia stem cells. However, no therapeutic
10 effects could be attributed to the CCL-1 targeting peptide *per se*.

In view of the above, the inventors have pursued a new approach to target CCL1 using immune cells endowed with specific chimeric antigen receptors based on anti-CLL1 monoclonal antibodies, which redirect immune cell specificity towards CLL1 positive cells

The engineered immune cells that they obtained using this approach have proven efficacy
15 to eliminate CLL1 positive malignant cells. In particular, they have appeared to be particularly useful in the context of the production of allogeneic TCR negative engineered immune cells, allowing a reduction of side effects, such as GvHD.

Thus, the present invention opens the way to treating patients affected with a condition characterized by an overabundance of CLL1-expressing cells using adoptive immunotherapy. Even
20 more, the present invention provides with engineered allogeneic immune cells that may be used as “off-the-shelf” allogeneic therapeutic products. As a further advantage of the invention, the CAR positive engineered cells can be made compatible (i.e. resistant) with chemotherapy or immunodepleting treatments, thereby enabling synergistic effects between chemotherapy and immunotherapy.

Summary of the invention

The inventors have generated CLL1 specific single-chain scCAR having different design and comprising different scFV derived from anti-CLL1 specific antibodies.

In particular, The Inventors have developed anti-CLL1 specific single-chain CAR (scCAR)
30 comprising VL and VL chains derived from SC02-357, SC02-378, SC02-161, M26, M31, G4, M22, M29,

M2, M5, G12, 21.26 and 1075.7 antibodies, with different architectures and identified highly specific and very selective scCARs constructions that bind to CLL1 expressing cells and selectively destroy CLL1 expressing cancer cells.

Following non-specific activation in vitro (e.g. with anti CD3/CD28 coated beads and recombinant IL2), primary T-cells from donors have been transformed with polynucleotides expressing these scCARs using viral transduction. In certain instances, the T-cells were further engineered to create less or non-alloreactive T-cells, more especially by disruption of a component of TCR ($\alpha\beta$ – T-Cell receptors) to prevent Graft versus host reaction.

T-cells were further engineered to create T cells resistant to anti-cancer drugs, to be used in combination with said classical anti-cancer drugs.

The resulting engineered T-cells displayed reactivity in-vitro against CLL1 positive cells to various extend, showing that the scCARs of the present invention contribute to antigen dependent activation, and also proliferation, of the T-cells, making them useful for immunotherapy.

The resulting engineered T-cells displayed reactivity in-vivo against CLL1 positive cells and significantly reduce the number of cancer cells in vivo.

The engineered T-cells of the invention are designed to display in-vivo reactivity against CLL1 positive cells, can be used in concomitance with anti-cancer drugs, are well tolerated. In a particular embodiment, the engineered T-cells of the invention remain efficient even after several administrations, making them useful for immunotherapy as a first treatment (induction), as a consolidation treatment, as a treatment in combination with classical anticancer chemotherapy. 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 acute myeloma leukemia (AML) treatments.

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 vector encoding CLL1-scCAR. This T-cell was 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 scCAR Architecture (V1 to V6) of the invention (anti-CLL1 scCAR) with the components presented in the following Table 1.

5 **Figure 3A and figure 3B:** Schematic representation of exemplary CLL1 specific CARs according to the invention involving different mAb-epitope tagging for T cell depletion, especially CD20 mimotope(s), which are designed to mitigate possible side effects associated with CAR positive cells injection.

10 (A) CLL1 specific CAR prototype according to the present invention not involving an epitope tagging sequence for sorting or depleting cells: V1 and v2 represents either VH or VL chain respectively of an antibody binding CLL1, TM: transmembrane domain, L: linker, TM: Transmembrane domain (preferably CD8 α transmembrane domain), 4-1BB: intracellular co-stimulatory domain, CD3 ITAM: activation domain.

15 (B) CLL1 specific CAR architectures according to the invention further including at least one epitope inserted in the extracellular ligand binding domain of the CAR, wherein said epitope is inserted between the VH and VL chains; said epitope being bordered by different linkers.;

(C): CLL1 specific CAR architectures according to the invention, where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; 20 the second epitope is inserted between the VH and VL chains, said 2nd epitope being also bordered by 2at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 2nd epitope.

(D): CLL1 specific CAR architectures according to the invention, where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the VH and VL chains; the other epitope is inserted between the VL chain and the hinge, each said epitope being 25 also bordered by at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 1st epitope.

(E): CLL1 specific CAR architecture according to the invention, where two epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the

CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VL chain and the hinge, said 2nd epitope being also bordered by such linkers.

(F): CLL1 specific CAR architectures according to the invention, where three epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VH and VL chains, said epitope being also bordered by such linkers, and the third epitope being inserted between the VL chain et the hinge. These two architectures differ by the linkers used bordering the 2nd epitope.

(G): CLL1 specific CAR architectures according to the invention, where at least two epitopes (preferably CD20 epitopes) are inserted in the extracellular ligand binding domain between the hinge and the anti CLL1 VH and VL chains. In the third exemplary architecture, one CD34 epitope is included between two CD20 epitopes. Further architectures may be considered where CD34 replaces any other previous CD20 epitopes.

(H): CLL1 specific CAR architectures according to the invention, where at least two epitopes are inserted at the extremity of the extracellular ligand binding domain.

Table 1 : Sequence of the different scCAR components excepted the scFvs

Functional domains	SEQ ID #	Raw amino acid sequence
CD8 α signal peptide	SEQ ID NO.1	MALPVTALLLPLALLLHAARP
Alternative signal peptide	SEQ ID NO.2	METDTLLLWVLLLWVPGSTG
Fc ϵ RIII α hinge	SEQ ID NO.3	GLAVSTISSFFPPGYQ
CD8 α hinge	SEQ ID NO.4	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
IgG1 hinge	SEQ ID NO.5	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
CD8 α transmembrane domain	SEQ ID NO.6	IYIWAPLAGTCGVLLLSLVITLYC
41BB transmembrane domain	SEQ ID NO.7	IISFFLALTSTALLFLLFFLTLRFSVV
41BB intracellular domain	SEQ ID NO.8	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
CD3 ζ intracellular domain	SEQ ID NO.9	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
Linker	SEQ ID NO.10	GGGGSGGGGSGGGGS

Table 2: Sequence of variable regions of exemplary anti-CLL1 VH and VL chains and their respective CDRs

ScFv sequences	SEQ ID #	Raw amino acid sequence
SC02-357 heavy chain variable region	SEQ ID NO.11	QVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLE WIGEIYHSGSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYSSS GGFFDYWGQGTLVTVSS
CDR1	SEQ ID NO.119	GSISSSNWWS
CDR2	SEQ ID NO.120	WIGEIYHSGSPDY
CDR3	SEQ ID NO.121	KVSTGGFFDY
SC02-378 heavy chain variable region	SEQ ID NO.12	QVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLE WIGEIYHSGSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCAR SSSGGFFDYWGQGTLVTVSS
CDR1	SEQ ID NO.122	GSISSSNWWS
CDR2	SEQ ID NO.123	WIGEIYHSGSPNY
CDR3	SEQ ID NO.124	RSSSGGFFDY
SC02-161 heavy chain variable region	SEQ ID NO.13	QVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLE WIGEIYHSGSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCAR QTTAGSFDYWGQGTLVTVSS
CDR1	SEQ ID NO.125	GSISSSNWWS
CDR2	SEQ ID NO.126	WIGEIYHSGSPNY
CDR3	SEQ ID NO.127	RQTTAGSFDY
SC02-357 & SC02-378 & SC02-161 light chain variable region	SEQ ID NO.14	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPPTFGQG TKVEIK
CDR1	SEQ ID NO.128	QSISSYLN
CDR2	SEQ ID NO.129	LLIYAASSLQS
CDR3	SEQ ID NO.130	QQSYSTPP
M26 heavy chain variable region	SEQ ID NO.15	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWI GFINPYNDGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRD DGYGYGYAMDYWGQGTSTVTVSS
CDR1	SEQ ID NO.131	GYTFTSYFIH
CDR2	SEQ ID NO.132	WIGFINPYNDGSKY
CDR3	SEQ ID	TRDDGYGYAMDY

	NO.133	
M26 light chain variable region	SEQ ID NO.16	DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYA ASTLD SGVPKRFSGNRS GSDYSLTISSESEDFADYYCLQYAIYPYTFGGG TKLEIKR
CDR1	SEQ ID NO.134	QELSGYLS
CDR2	SEQ ID NO.135	RLIYA ASTLDS
CDR3	SEQ ID NO.136	LQYAIYPY
M31 heavy chain variable region	SEQ ID NO.17	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLE WIGYINPYNDGTTYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFC ARPIYFDNDYFDYWGGQTTLKVSS
CDR1	SEQ ID NO.137	GYTFTSYVMH
CDR2	SEQ ID NO.138	WIGYINPYNDGTTY
CDR3	SEQ ID NO.139	ARPIYFDNDY
M31 light chain variable region	SEQ ID NO.18	TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPK LLIYLASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNYDP WTFGGG TKLEIK
CDR1	SEQ ID NO.140	ESVDSYGNSFMH
CDR2	SEQ ID NO.141	LLIYLASNLES
CDR3	SEQ ID NO.142	QQNNYDPW
G4 heavy chain variable region	SEQ ID NO.19	EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNHWKQSHEKNLEWI GPINPYNDGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDPVYYCAR TDDYDDYTMDYWGGQTSVTVSS
CDR1	SEQ ID NO.143	QQNNYDPW
CDR2	SEQ ID NO.144	WIGPINPYNDGTIY
CDR3	SEQ ID NO.145	ARTDDYDDYTMDY
G4 light chain variable region	SEQ ID NO.20	EIQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYT SRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGKTLLWTFGGG TKLEIK
CDR1	SEQ ID NO.146	HDISNYLN
CDR2	SEQ ID NO.147	LLIYYTSRLHS
CDR3	SEQ ID NO.148	QQGKTLLW
M22 heavy chain variable region	SEQ ID NO.21	QVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPGQGLE WIGNIDPSDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYY CAIYYGNPSYYAMDYWGGQTSVTVSS

CDR1	SEQ ID NO.149	GYTFTRYWMH
CDR2	SEQ ID NO.150	WIGNIDPSDTETHY
CDR3	SEQ ID NO.151	AIYYGNPSYYAMDY
M22 light chain variable region	SEQ ID NO.22	DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPG QPPKLLIWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYFCQND YSYPFTFGAGTKLELK
CDR1	SEQ ID NO.152	QNLLNSGNQKKYLN
CDR2	SEQ ID NO.153	LLIWASTRES
CDR3	SEQ ID NO.154	QNDYSYPF
M29 heavy chain variable region	SEQ ID NO.23	EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEW IGYINPYNDGTTYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCAR YYDYDYFDYWGGGTTTLTVSS
CDR1	SEQ ID NO.155	GYIFTSYVMY
CDR2	SEQ ID NO.156	WIGYINPY
CDR3	SEQ ID NO.157	ARYDYDYFDY
M29 light chain variable region	SEQ ID NO.24	DIQMTQSPSSLSASLGKVTITCKASQDINKYIAWYQHKGKGPRLLIHY TSTLQPGIPSRFSGSGGRDYSFISNLEPEDATYYCLQYDYLWTFGGGT KLEIK
CDR1	SEQ ID NO.158	QDINKYIA
CDR2	SEQ ID NO.159	LLIHYTSTLQP
CDR3	SEQ ID NO.160	LQYDYLW
M2 heavy chain variable region	SEQ ID NO.25	EVQLRQSGPELVKPGASVKMSCKASGYFTSYFMHWVKQKPGQGLEW IGFINPYNDGTTYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTR DDGYDYAMDYWGQGTSVTVSS
CDR1	SEQ ID NO.161	GYFTSYFMH
CDR2	SEQ ID NO.162	WIGFINPYNDGTTY
CDR3	SEQ ID NO.163	TRDDGYDYAMDY
M2 light chain variable region	SEQ ID NO.26	DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQQKPDGTIKRLIYAA STLD SGVPERFSGSRGSDYSLTISSEDFADYYCLQYASYPYTFGGGTK LEIKR
CDR1	SEQ ID NO.164	QEISVYLS

CDR2	SEQ ID NO.165	RLIYAASTLDS
CDR3	SEQ ID NO.166	LQYASYPY
M5 heavy chain variable region	SEQ ID NO.27	EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHVVKQRPEQGLEWI GWIDPEKGDYASKFQDKATITSDTSSNTAYLQLSSLTSEDYAVYYCTLT GRFDYWGQGTTTLTVSS
CDR1	SEQ ID NO.167	GFNIKDDYIH
CDR2	SEQ ID NO.168	WIGWIDPEKGDYAS
CDR3	SEQ ID NO.169	TLTGRFDY
M5 light chain variable region	SEQ ID NO.28	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLYSSNQKNNLAWYQQKPGQ SPKLLIWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQQYYS YRTFGGGTKLEIK
CDR1	SEQ ID NO.170	QSLYSSNQKNNLA
CDR2	SEQ ID NO.171	LLIWASTRES
CDR3	SEQ ID NO.172	QQYYSYR
G12 heavy chain variable region	SEQ ID NO.29	QVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLQTPGQGLE WIGVIYPGNGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFC ARVYNWHFDVWGAGTTVTVSS
CDR1	SEQ ID NO.173	GYTFPSSNIH
CDR2	SEQ ID NO.174	WIGVIYPGNGDTSY
CDR3	SEQ ID NO.175	AIYFVYNWHFDV
G12 light chain variable region	SEQ ID NO.30	NIVLTQSPASLAVSLGQRATISCRASESDGYGDIFMLWYQQKPGQP LLIYFASNLESGVPARFSGSGSRDFTLTIDPVEADDAATYYCQQNNEDP YTFGGGTKEIKR
CDR1	SEQ ID NO.176	ESVDGYGDIFML
CDR2	SEQ ID NO.177	LLIYFASNLES
CDR3	SEQ ID NO.178	QQNNEDPY
21.26 heavy chain variable region	SEQ ID NO.31	QVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWVKQRPGQGLE WIGMIHPSSGSTSYNEKVKNKATLTVDRSSTAYMQLSSLTSEDSAVYYC ARDGDYYYGTGDYWGQGTTTLTVSS
CDR1	SEQ ID NO.179	GYTFTRYWMH
CDR2	SEQ ID NO.180	MIHPSSGSTSYNEKVK

CDR3	SEQ ID NO.181	RDGDYYYGTGDY
21.26 light chain variable region	SEQ ID NO.32	QIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFA TSNLASGVPSRFSGSGSGTSSYSLTISRVEAEDAATYYCQQWRSDRALTFG AGTKLEL
CDR1	SEQ ID NO.182	RASSSINYMH
CDR2	SEQ ID NO.183	PWIFATSNLAS
CDR3	SEQ ID NO.184	QQWRSDRALT
1075.7 heavy chain variable region	SEQ ID NO.33	DIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWM GYISYDGRNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEG DYDVGNYYAMDYWGQGTSVTVSS
CDR1	SEQ ID NO.185	GYSITSAYYWN
CDR2	SEQ ID NO.186	YISYDGRNNYNPSLKN
CDR3	SEQ ID NO.187	AKEGDYDVGNYYAMDY
1075.7 light chain variable region	SEQ ID NO.34	ENVLTQSPAIMSASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLW IYSTSNLASGVPARFSGSGSGTSSYSLTISSVEAEDAATYYCQQYSGYPLTF GAGTKLEL
CDR1	SEQ ID NO.188	RASSNVISSYVH
CDR2	SEQ ID NO.189	LWIYSTSNLAS
CDR3	SEQ ID NO.190	QQYSGYPLT

Table 3: scCAR of structure V-1

scCAR Designation	scCAR Structure						
V-1	signal peptide	VH	VL	FcεRIIIα hinge	CD8α TM	41BB -IC	CD3ζ CD
V1-SCO2-357 scCAR (SEQ ID NO.35)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1-SCO2-378 scCAR (SEQ ID NO.41)	SEQ ID NO.1	SEQ ID NO.12	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1-SCO2-161 scCAR (SEQ ID NO. 47)	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
V1-M26 scCAR (SEQ ID NO.53)	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
V1- M31 scCAR (SEQ ID NO.59)	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
V1- G4 scCAR (SEQ ID NO.65)	SEQ ID NO.1	SEQ ID NO.19	SEQ ID NO.20	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- M22 scCAR (SEQ ID NO.71)	SEQ ID NO.1	SEQ ID NO.21	SEQ ID NO.22	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- M29 scCAR (SEQ ID NO.77)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- M2 scCAR (SEQ ID NO.83)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- M5 scCAR (SEQ ID NO.89)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- G12 scCAR (SEQ ID NO.95)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

V1- 21.26 scCAR (SEQ ID. NO.101)	SEQ ID NO.1	SEQ ID NO.31	SEQ ID NO.32	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V1- 1075.7 scCAR (SEQ ID NO.107)	SEQ ID NO.1	SEQ ID NO.33	SEQ ID NO.34	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 4: scCAR of structure V-2

scCAR Designation	scCAR Structure						
V-2	signal peptide	VH	VL	FcεRIIIα hinge	41BB-TM	41BB -IC	CD3ζ CD
V2-SCO2-357 scCAR (SEQ ID NO.36)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2-SCO2-378 scCAR (SEQ ID NO. 42)	SEQ ID NO.1	SEQ ID NO.12	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2-SCO2-161 scCAR (SEQ ID NO.48)	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
V2-M26 scCAR (SEQ ID NO. 54)	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
V2- M31 scCAR (SEQ ID NO. 60)	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
V2- G4 scCAR (SEQ ID NO.66)	SEQ ID NO.1	SEQ ID NO.19	SEQ ID NO.20	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- M22 scCAR (SEQ ID NO.72)	SEQ ID NO.1	SEQ ID NO.21	SEQ ID NO.22	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- M29 scCAR (SEQ ID NO.78)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

V2- M2 scCAR (SEQ ID NO.84)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- M5 scCAR (SEQ ID NO.90)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- G12 scCAR (SEQ ID NO. 96)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- 21.26 scCAR (SEQ ID NO.102)	SEQ ID NO.1	SEQ ID NO.31	SEQ ID NO.32	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V2- 1075.7 scCAR (SEQ ID NO.108)	SEQ ID NO.1	SEQ ID NO.33	SEQ ID NO.34	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Table 5: scCAR of structure V-3

scCAR Designation	scCAR Structure						
V-3	signal peptide	VH	VL	CD8 α hinge	CD8 α TM	41BB -IC	CD3 ζ CD
V3-SCO2-357 scCAR (SEQ ID NO.37)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3-SCO2-378 scCAR (SEQ ID NO.43)	SEQ ID NO.1	SEQ ID NO.12	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3-SCO2-161 scCAR (SEQ ID NO.49)	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
V3-M26 scCAR (SEQ ID NO.55)	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
V3- M31 scCAR (SEQ ID NO.61)	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

V3- G4 scCAR (SEQ ID NO.67)	SEQ ID NO.1	SEQ ID NO.19	SEQ ID NO.20	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- M22 scCAR (SEQ ID NO. 73)	SEQ ID NO.1	SEQ ID NO.21	SEQ ID NO.22	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- M29 scCAR (SEQ ID NO.79)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- M2 scCAR (SEQ ID NO. 85)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- M5 scCAR (SEQ ID NO.91)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- G12 scCAR (SEQ ID NO.97)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- 21.26 scCAR (SEQ ID NO.103)	SEQ ID NO.1	SEQ ID NO.31	SEQ ID NO.32	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V3- 1075.7 scCAR (SEQ ID NO.109)	SEQ ID NO.1	SEQ ID NO.33	SEQ ID NO.34	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 6: scCAR of structure V-4

scCAR Designation	scCAR Structure						
V-4	signal peptide	VH	VL	CD8 α hinge	41BB-TM	41BB -IC	CD3 ζ CD
V4-SCO2-357 scCAR (SEQ ID NO.38)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

V4-SCO2-378 scCAR (SEQ ID NO.44)	SEQ ID NO.1	SEQ ID NO.12	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V4-SCO2-161 scCAR (SEQ ID NO.50)	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
V4-M26 scCAR (SEQ ID NO.56)	SEQ ID NO.1	SEQ ID NO.16	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V4- M31 scCAR (SEQ ID NO.62)	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
V4- G4 scCAR (SEQ ID NO.68)	SEQ ID NO.1	SEQ ID NO.19	SEQ ID NO.20	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- M22 scCAR (SEQ ID NO.74)	SEQ ID NO.1	SEQ ID NO.21	SEQ ID NO.22	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- M29 scCAR (SEQ ID NO.80)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- M2 scCAR (SEQ ID NO.86)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- M5 scCAR (SEQ ID NO.92)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- G12 scCAR (SEQ ID NO.98)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- 21.26 scCAR (SEQ ID NO.104)	SEQ ID NO.1	SEQ ID NO.31	SEQ ID NO.32	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V4- 1075.7 scCAR (SEQ ID NO.110)	SEQ ID NO.1	SEQ ID NO.33	SEQ ID NO.34	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Table 7: scCAR of structure V-5

scCAR Designation	scCAR Structure						
V-5	signal peptide	VH	VL	IgG1 hinge	CD8 α TM	41BB -IC	CD3 ζ CD
V5-SCO2-357 scCAR (SEQ ID NO.39)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5-SCO2-378 scCAR (SEQ ID NO.45)	SEQ ID NO.1	SEQ ID NO.12	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5-SCO2-161 scCAR (SEQ ID NO.51)	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
V5-M26 scCAR (SEQ ID NO.57)	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
V5- M31 scCAR (SEQ ID NO.63)	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
V5- G4 scCAR (SEQ ID NO.69)	SEQ ID NO.1	SEQ ID NO.19	SEQ ID NO.20	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5- M22 scCAR (SEQ ID NO.75)	SEQ ID NO.1	SEQ ID NO.21	SEQ ID NO.22	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5- M29 scCAR (SEQ ID NO.81)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5- M2 scCAR (SEQ ID NO.87)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5- M5 scCAR (SEQ ID NO.93)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
V5- G12 scCAR (SEQ ID NO.99)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

(SEQ ID NO.76)	NO.1	NO.21	NO.22	NO.5	NO.7	NO.8	NO.9
V6- M29 scCAR (SEQ ID NO.82)	SEQ ID NO.1	SEQ ID NO.23	SEQ ID NO.24	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V6- M2 scCAR (SEQ ID NO.88)	SEQ ID NO.1	SEQ ID NO.25	SEQ ID NO.26	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V6- M5 scCAR (SEQ ID NO.94)	SEQ ID NO.1	SEQ ID NO.27	SEQ ID NO.28	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V6- G12 scCAR (SEQ ID NO.100)	SEQ ID NO.1	SEQ ID NO.29	SEQ ID NO.30	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V6- 21.26 scCAR (SEQ ID NO.106)	SEQ ID NO.1	SEQ ID NO.31	SEQ ID NO.32	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
V6- 1075.7 scCAR (SEQ ID NO. 112)	SEQ ID NO.1	SEQ ID NO.33	SEQ ID NO.34	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Detailed description of the invention

Unless specifically defined herein, all technical and scientific terms used have the same
5 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
10 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,
15 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).

The present invention provides:

1. A CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) comprising at least:

- an extra cellular ligand binding-domain anti-CLL1,
- a transmembrane domain, and
- a cytoplasmic signaling domain.

2. A CLL1 specific chimeric antigen receptor according to embodiment 1, further comprising a co-stimulatory domain.

3. A CLL1 specific chimeric antigen receptor according to embodiment 1, further comprising a CD28 or a 4-1BB co-stimulatory domain.

4. A CLL1 specific chimeric antigen receptor according to any one of embodiments 1 to 3, wherein said transmembrane domain is a CD8 α transmembrane domain.

5. A CLL1 specific chimeric antigen receptor according to any one of embodiments 1 to 4, further comprising a hinge.
6. A CLL1 specific chimeric antigen receptor according to any one of embodiments 1 to 5, wherein said cytoplasmic signaling domain is a T-cell activating domain.
- 5 7. A CLL1 specific chimeric antigen receptor according to any one of embodiments 1 to 6, wherein said chimeric antigen receptor is expressed under the form of a single polypeptide.
8. A CLL1 specific chimeric antigen receptor according to any one of embodiments 1 to 7, wherein said extra cellular ligand binding-domain is from a monoclonal anti-CLL1
10 antibody.
9. A CLL1 specific chimeric antigen receptor according to embodiment 8, wherein extra cellular ligand binding-domain comprises CDRs from VH and VL domains of monoclonal anti-CLL1 antibody(ies).
10. A CLL1 specific chimeric antigen receptor according to embodiment 9, wherein said CDRs
15 are selected from SEQ ID NO. 109 to 190.
11. A CLL1 specific scCAR according to any one of embodiments 1 to 10 having one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a hinge, a CD8 α transmembrane domain, a cytoplasmic
20 domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain.
12. A CLL1 specific scCAR according to any one of embodiments 1 to 11, wherein said structure V1 comprises a Fc γ RIII α hinge and CD8 α transmembrane domain.
13. A CLL1 specific scCAR according to any one of embodiments 1 to 11, wherein said structure V3 comprises a CD8 α hinge and a CD8 α transmembrane domain.

14. A CLL1 specific scCAR according any one of embodiments 1 to 11, wherein said structure V5 comprises an IgG1 hinge and a CD8 α transmembrane domain.
15. A CLL1 specific scCAR according to any one of embodiments 1 to 14, wherein said VH and VL have at least 80 % identity with a polypeptide sequence selected from SEQ ID NO. 11 to 34.
16. A CLL1 specific scCAR according to any one of embodiments 1 to 15, wherein co-stimulatory domain from 4-1BB has at least 80 % identity with SEQ ID NO.8.
17. A CLL1 specific scCAR according to any one of embodiments 1 to 16, wherein said CD3 zeta signaling domain has at least 80 % identity with SEQ ID NO. 9.
18. A CLL1 specific scCAR according to any one of embodiments 1 to 12 or 15 to 17, wherein said Fc γ RIII α hinge has at least 80 % identity with SEQ ID NO.3.
19. A CLL1 specific scCAR according to any one of embodiments 1 to 11, 13 or 15 to 17, wherein said CD8 α hinge has at least 80 % identity with SEQ ID NO.4.
20. A CLL1 specific scCAR according to any one of embodiments 1 to 11 or 14 to 19, wherein said IgG1 hinge has at least 80 % identity with SEQ ID NO.5.
21. A CLL1 specific scCAR according to any one of embodiments 1 to 20, wherein said CD8 α transmembrane domain has at least 80 % identity with SEQ ID NO.6.
22. A CLL1 specific scCAR according to any one of embodiments 1 to 21 further comprising another extracellular ligand binding domain which is not specific for CLL1.
23. A CLL1 specific scCAR of structure V1 according to any one of embodiments 1 to 12, 15 to 17 or 21 to 22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101 or 107.

24. A CLL1 specific scCAR of structure V3 according to any one of embodiments 1 to 11, 13, 15 to 17 or 21 to 22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103 or 109.
25. A CLL1 specific scCAR of structure V5 according to any one of embodiments 1 to 11, 14 to 19 or 21 to 22 which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO.39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105 or 111.
26. A CLL1 specific scCAR according to any one of embodiments 1 to 25 further comprising a signal peptide.
27. A CLL1 specific scCAR according to embodiment 26, wherein said signal peptide has at least 80 % sequence identity with SEQ ID NO.1 or SEQ ID NO.2.
28. The polypeptide according to any one of embodiments 1 to 27, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.
29. The polypeptide according to any one of embodiments 1 to 28, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.
30. The polypeptide according to any one of embodiments 1 to 29, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes
31. The polypeptide according to any one of embodiments 1 to 30, wherein the extracellular binding domain comprises the following sequence
- $V_1-L_1-V_2-(L)_x\text{-Epitope1-(L)}_x\text{-};$
- $V_1-L_1-V_2-(L)_x\text{-Epitope1-(L)}_x\text{-Epitope2-(L)}_x\text{-};$
- $V_1-L_1-V_2-(L)_x\text{-Epitope1-(L)}_x\text{-Epitope2-(L)}_x\text{-Epitope3-(L)}_x\text{-};$
- $(L)_x\text{-Epitope1-(L)}_x\text{-}V_1-L_1-V_2\text{-};$
- $(L)_x\text{-Epitope1-(L)}_x\text{-Epitope2-(L)}_x\text{-}V_1-L_1-V_2\text{-};$
- $\text{Epitope1-(L)}_x\text{-Epitope2-(L)}_x\text{-Epitope3-(L)}_x\text{-}V_1-L_1-V_2\text{-};$
- $(L)_x\text{-Epitope1-(L)}_x\text{-}V_1-L_1-V_2\text{-(L)}_x\text{-Epitope2-(L)}_x\text{-};$
- $(L)_x\text{-Epitope1-(L)}_x\text{-}V_1-L_1-V_2\text{-(L)}_x\text{-Epitope2-(L)}_x\text{-Epitope3-(L)}_x\text{-};$

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂;

5 V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂; or,

(L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂-(L)_x-Epitope3-(L)_x;

10 wherein,

V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L;

L₁ is a linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

x is 0 or 1 and each occurrence of x is selected independently from the others; and,

Epitope 1, Epitope 2 and Epitope 3 are mAb-specific epitopes and can be identical or different.

20 32. The polypeptide according to any one of embodiment 31, wherein the extracellular binding domain comprises the following sequence

V₁-L₁-V₂-L-Epitope1; V₁-L₁-V₂-L-Epitope1-L; V₁-L₁-V₂-L-Epitope1-L-Epitope2; V₁-L₁-V₂-L-

Epitope1-L-Epitope2-L; V₁-L₁-V₂-L-Epitope1-L-Epitope2-L-Epitope3; V₁-L₁-V₂-L-Epitope1-L-

Epitope2-L-Epitope3-L; V₁-L₁-V₂-Epitope1; V₁-L₁-V₂-Epitope1-L; V₁-L₁-V₂-Epitope1-L-Epitope2;

25 V₁-L₁-V₂-Epitope1-L-Epitope2-L; V₁-L₁-V₂-Epitope1-L-Epitope2-L-Epitope3; V₁-L₁-V₂-Epitope1-L-

Epitope2-L-Epitope3-L; Epitope1-V₁-L₁-V₂; Epitope1-L-V₁-L₁-V₂; L-Epitope1-V₁-L₁-V₂; L-

Epitope1-L-V₁-L₁-V₂; Epitope1-L-Epitope2-V₁-L₁-V₂; Epitope1-L-Epitope2-L-V₁-L₁-V₂; L-

Epitope1-L-Epitope2-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-V₁-L₁-V₂; Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂; Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂; V₁-L-Epitope1-L-V₂; L-Epitope1-L-V₁-L-Epitope2-L-V₂; V₁-L-Epitope1-L-V₂-L-Epitope2-L; V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3; V₁-L-Epitope1-L-V₂-L-Epitope2-Epitope3; V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3-Epitope4; L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L; Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L; L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-Epitope3, or Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3-Epitope4

wherein

V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L;

L₁ is any linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

epitope 1, epitope 2 and epitope 3 are mAb-specific epitopes and can be identical or different.

33. The polypeptide according to embodiment 31 or 32, wherein L₁ is a linker comprising Glycine and/or Serine.

34. The polypeptide according to embodiment 33, wherein L₁ is a linker comprising the amino acid sequence (Gly-Gly-Gly-Ser)_n or (Gly-Gly-Gly-Gly-Ser)_n, where n is 1, 2, 3, 4 or 5.

35. The polypeptide according to embodiment 34, wherein L₁ is a linker comprising the amino acid sequence (Gly₄Ser)₄ or (Gly₄Ser)₃.

36. The polypeptide according to any one of embodiments 31 to 35 wherein L is a linker comprising Glycine and/or Serine.

37. The polypeptide according to embodiment 36, wherein L is a linker having an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS,

GGGGGS, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGSGGGGS.

38. The polypeptide according to embodiment 37, wherein L is a SGGGG, GGGGS or SGGGGS.

39. The polypeptide according to any one of embodiments 31 to 38 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.

40. The polypeptide according to any one of embodiments 31 to 38 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes having an amino acid sequence of SEQ ID NO 113, SEQ ID NO 191, , SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 195, SEQ ID NO 195, SEQ ID NO 196, SEQ ID NO 197, SEQ ID NO 198 or SEQ ID NO 199.

41. The polypeptide according to any one of embodiments 31 to 40 wherein Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.

42. The polypeptide according to any one of embodiments 31 to 41 wherein Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.

43. The polypeptide according to any one of embodiments 31 to 42 wherein Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113, SEQ ID NO 198 or SEQ ID NO 199.

44. The polypeptide according to any one of embodiments 31 to 43 wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.

45. A polynucleotide encoding a chimeric antigen receptor according to any one of embodiments 1 to 44.

46. An expression vector comprising a nucleic acid of embodiment 39.
47. An engineered lymphoid immune cell expressing at the cell surface membrane an anti-CLL1 CAR according to any one of embodiments 1 to 44.
- 5 48. An engineered lymphoid immune cell according to embodiment 41 derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.
49. An engineered cell according to embodiment 47 or 48 for use in therapy.
50. An engineered cell according to any one of embodiments 41 to 43 for use in therapy, wherein the patient is a human.
- 10 51. An engineered cell according to any one of embodiments 47 to 50 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by CLL1-expressing cells.
52. An engineered cell according to any one of embodiments 47 to 51 for use in therapy, wherein the condition is a condition which is characterized by an overabundance of CLL1-expressing cells.
- 15 53. An engineered cell according to any one of embodiments 51 to 52 for use in therapy, wherein the condition is a hematological cancer condition.
54. An engineered cell according to embodiment 53 for use in therapy, wherein the hematological cancer condition is leukemia.
- 20 55. An engineered cell according to embodiment 54 for use in therapy, wherein the leukemia is acute myelogenous leukemia (AML).
56. An engineered cell according to any one of embodiments 47 to 55, wherein expression of TCR is suppressed in said immune cell.
- 25 57. An engineered cell according to any one of embodiments 47 to 55, wherein expression of at least one MHC protein, preferably $\beta 2m$ or HLA, is repressed or suppressed in said immune cell.

58. An engineered cell according to any one of embodiments 47 to 57, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.
59. A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to any one of embodiments 47 to 58 in an amount effective to cause impairment of said cancer cell.
60. A method of engineering an immune cell comprising:
- (a) Providing an immune cell,
 - (b) Expressing at the surface of said cell at least one CLL1 single-chain specific chimeric antigen receptor according to any one of embodiments 1 to 44.
61. The method of engineering an immune cell of embodiment 60 comprising:
- (a) Providing an immune cell,
 - (b) Introducing into said cell at least one polynucleotide encoding said CLL1 single-chain specific chimeric antigen receptor ,
 - (c) Expressing said polynucleotide into said cell.
62. The method of engineering an immune cell of embodiment 61 comprising:
- (a) Providing an immune cell,
 - (b) Introducing into said cell at least one polynucleotide encoding said anti-CLL1 single-chain specific chimeric antigen receptor ,
 - (c) Introducing at least one other chimeric antigen receptor which is not specific for CLL1.
63. A method of treating a subject in need thereof comprising:
- (a) Providing an immune cell expressing at the surface an anti-CLL1 single-chain specific chimeric antigen receptor according to any one of embodiments 1 to 44;
 - (b) Administering said immune cells to said patient.
64. A method according to embodiment 63, wherein said immune cell is provided from a donor.

65. A method according to embodiment 63, wherein said immune cell is provided from the patient himself.

CLL1 single-chain specific chimeric antigen receptors

5 The present invention relates to CLL1 specific chimeric antigen receptor comprising an extracellular ligand-binding domain specifically directed against one portion of the CLL1 antigen, a transmembrane domain and a signaling transducing domain.

By chimeric antigen receptor (CAR) is intended molecules that combine an extracellular binding domain directed against a component present on a target cell, for example an antibody-based specificity for a desired antigen (e.g., tumor antigen) with an immune cell receptor component to generate a chimeric protein that will transduce an activating or inhibitory signal toward cellular immune activity.

The present invention more particularly relates to a CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) comprising at least:

- 15
- an extracellular ligand binding-domain anti-CLL1,
 - a transmembrane domain, and
 - a cytoplasmic signaling domain.

Preferably, the CLL1 specific chimeric antigen receptor according to the invention further comprises a co-stimulatory domain, and more preferably a CD28 or a 4-1BB co-stimulatory domain as described for instance by Jena, B., G. Dotti, et al. (2010). It can also comprise a transmembrane domain which can be a CD8 α transmembrane domain, as well as an optional hinge.

25 The signal transducing domain or "cytoplasmic 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 or inhibition of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation or inactivation 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 "cytoplasmic signaling domain" refers to the

portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

The cytoplasmic signaling domain, which is preferably from a human protein involved in signal transduction pathway(s), determines whether anti-CLL1 CAR is a positive CAR (PCAR) or a negative CAR (NCAR) depending on the nature of the signaling. Respectively, the CAR is a PCAR when the signaling domain, such as CD3zeta from human TCR receptor, has the effect of stimulating the cellular immune activity of the immune cell when the extracellular ligand binding-domain is bound to CLL1. Conversely, the anti-CLL1 CAR is a NCAR or inhibitory CAR (iCAR) when the signaling domain has the effect of reducing the cellular immune activity, such as signaling domains of human immunoinhibitory receptors CTLA-4 and PD-1 (Federov et al., Sci Transl Med. 2013 Dec 11; 5 (215): 215ra172). Preferred examples of signal transducing domain for use in a anti-CLL1 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 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 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 anti-CLL1 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 % 97 % or 99 % or 100 % sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 9.

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. "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 co-stimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an
 5 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
 10 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
 15 ligand that specifically binds with CD83 and the like.

In a preferred embodiment, the co-stimulatory domain of the anti-CLL1 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 anti-CLL1 CAR of the present invention comprises amino acid sequence
 20 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.

An anti-CLL1 CAR according to the present invention generally further comprises a transmembrane domain (TM). 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
 25 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
 30 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.

5 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
10 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 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
15 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. According to one embodiment, the hinge can also be a human Ig (immunoglobulin) hinge, e.g., a PD-1 hinge, an IgG4 hinge.

 According to a preferred embodiment, the anti-CLL1 CAR according to the invention
20 comprises a transmembrane domain more particularly selected from CD8α and 4-1BB, showing identity with the polypeptides of SEQ ID NO. 6 or 7.

 An anti-CLL1 CAR according to the invention generally further comprises a transmembrane domain (TM) more particularly a TM selected from CD8α and 4-1BB, and even more particularly showing identity with the polypeptides of SEQ ID NO. 6 or 7.

25 In a preferred embodiment, a anti-CLL1 CAR according to the invention further comprises a TM domain from CD8α with SEQ ID NO. 6 or showing at least 90 %, 91%, 92%, 93%, 94%, 95 %, 96%, 97 %, 98% or 99 % sequence identity with SEQ ID NO. 6

 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
30 specific to target, the CLL1 specific anti-CLL1 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 placed on different transmembrane polypeptides composing the anti-CLL1 CAR. In another embodiment, the present invention relates to a population of anti-CLL1 CAR s 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 anti-CLL1 CAR each one comprising different extracellular ligand binding domains. In another particular 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 anti-CLL1 CAR each one comprising different extracellular ligand binding domains. By population of anti-CLL1 CAR s, it is meant at least two, three, four, five, six or more anti-CLL1 CAR s each one comprising different extracellular ligand binding domains. The different extracellular ligand binding 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 anti-CLL1 CAR s each one comprising different extracellular ligand binding domains.

CLL1 specific chimeric antigen receptors according to the invention can have different architectures, as they can be expressed, for instance, under a single-chain chimeric protein (scCAR) or under the form of several polypeptides (multi-chain) including at least one such chimeric protein. Such multi-chain CAR architectures are disclosed in WO2014/039523, especially in Figures 2 to 4, and from page 14 to 21, which are herein incorporated by reference.

In general, anti-CLL1 CAR comprises an extracellular single chain antibody (scFv Fc) fused to the intracellular signaling domain of T-cell antigen receptor complex zeta chain (scFv Fc:ζ), which has the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity.

The present application discloses several anti-CLL1 single chain CAR directed against CLL1 antigen, which comprise as non-limiting example the amino acid sequences : SEQ ID NO: 35 to 112.

CLL1 CAR of the present invention can also be “multi-chain CARs” as previously mentioned, which means that the extracellular binding domain and the signaling domains are preferably located on different polypeptide chains, whereas co-stimulatory domains may be located on the same or a third polypeptide. Such multi-chain CARs can be derived from FcεRI (Ravetch et al, 1989), by
 5 replacing the high affinity IgE binding domain of FcεRI alpha chain by an extracellular ligand-binding domain such as scFv, whereas the N and/or C-termini tails of FcεRI beta and/or gamma chains are fused to signal transducing domains and co-stimulatory domains respectively. The extracellular ligand binding domain has the role of redirecting T-cell specificity towards cell targets, while the signal transducing domains activate or reduce the immune cell response. The fact that the different
 10 polypeptides derive from the alpha, beta and gamma polypeptides from FcεRI are transmembrane polypeptides sitting in juxtamembrane position provides a more flexible architecture to CARs, improving specificity towards the targeted molecule and reducing background activation of immune cells as described in WO2014/039523.

Extracellular ligand-binding domain

15 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. It can be for instance binding domains derived from a ligand, a receptor, human or
 20 mice antibodies or antigen recognition domains derived from camels or cartilaginous fish.

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 CLL1 antibody joined by a flexible linker. Said V_L and V_H are preferably selected from the antibodies referred to in the literature as SCO02-357, SC02-378 and
 25 SC02-161 in WO2005/00894 (Applicant: Crucell Holland BV); M26, M31, G4, M22, M29, M2, M5, G12 in WO2013/169625 (Applicant: Cellerant Therapeutics); and 21.26, 1075.7 in WO2009/051974 (Applicant: Nuvelo Inc .), and more particularly as comprising CDRs from V_H and V_L domains of monoclonal anti-CLL1 antibodies selected from SEQ ID NO. 119 to 190 described below..

The CDR sequences of V_H chain from monoclonal anti-CLL1 antibody may be chosen among
 30 GSISSSNWWS (SEQ ID NO 119), WIGEIYHSGSPDY (SEQ ID NO 120), KVSTGGFFDY (SEQ ID NO 121), and

GSISSSNWWWS (SEQ ID NO 122), WIGEIYHSGSPNY (SEQ ID NO 123), RSSSGGFFDY (SEQ ID NO 124), and GSISSSNWWWS (SEQ ID NO 125), WIGEIYHSGSPNY (SEQ ID NO 126), RQTTAGSFDY (SEQ ID NO 127), and GYTFTSYFIH (SEQ ID NO 131), WIGFINPYNDGSKY (SEQ ID NO 132), TRDDGYYGYAMDY (SEQ ID NO 133), and GYTFTSYVMH (SEQ ID NO 137), WIGYINPYNDGTKY (SEQ ID NO 138), ARPIYFDNDY (SEQ ID NO 139), and QQNNYDPW (SEQ ID NO 143), WIGPINPYNDGTI (SEQ ID NO 144), ARTDDYDDYTMDY (SEQ ID NO 145), and GYTFTRYWMH (SEQ ID NO 149), WIGNIDPSDTETHY (SEQ ID NO 150), AIYYGNPSYYAMDY (SEQ ID NO 151), and GYIFTSYVMY (SEQ ID NO 155), WIGYINPY (SEQ ID NO 156), ARYYDYDYFFDY (SEQ ID NO 157), and GYTFTSYFMH (SEQ ID NO 161), WIGFINPYNDGTKY (SEQ ID NO 162), TRDDGYYDYAMDY (SEQ ID NO 163), and GFNIKDDYIH (SEQ ID NO 167), WIGWIDPEKGDTAYA (SEQ ID NO 168), TLTGRFDY (SEQ ID NO 169), and GYTFPSSNIH (SEQ ID NO 173), WIGVIYPGNGDTSY (SEQ ID NO 174), AIYFVYNWHFDV (SEQ ID NO 175), and GYTFTRYWMH (SEQ ID NO 179), MIHPSSGSTSYNEKVK (SEQ ID NO 180), RDGDYYYGTGDY (SEQ ID NO 181), and GYSITSAYYWN (SEQ ID NO 185), YISYDGRNNYNPSLKN (SEQ ID NO 186) and AKEGDYDVGNYAMDY (SEQ ID NO 187).

Similarly, the CDR sequences of VL chain from monoclonal anti-CLL1 antibody may be chosen among: QSISSYLN (SEQ ID NO 128), LLIYAASSLQS (SEQ ID NO 129), QQSYSTPP (SEQ ID NO 130), and QELSGYLS (SEQ ID NO 134), RLIYAASTLDS (SEQ ID NO 135), LQYAIYPY (SEQ ID NO 136), and ESVDSYGNSFMH (SEQ ID NO 140), LLIYLASNLES (SEQ ID NO 141), QQNNYDPW (SEQ ID NO 142), HDISNYLN (SEQ ID NO 146), LLIYTSRLHS (SEQ ID NO 147), QQGKTLLW (SEQ ID NO 148), and QNLLNSGNQKKYLN (SEQ ID NO 152), LLIYWASTRES (SEQ ID NO 153), QNDYSYPF (SEQ ID NO 154), and QDINKYIA (SEQ ID NO 158), LLIHYTSTLQP (SEQ ID NO 159), LQYDYLW (SEQ ID NO 160), and QEISVYLS (SEQ ID NO 164), RLIYAASTLDS (SEQ ID NO 165), LQYASYPY (SEQ ID NO 166), and QSLLYSSNQKNNLA (SEQ ID NO 170), LLIYWASTRES (SEQ ID NO 171), QQYYSYR (SEQ ID NO 172), and ESVDGYGDIFML (SEQ ID NO 176), LLIYFASNLES (SEQ ID NO 177), QQNNEDPY (SEQ ID NO 178), and RASSSINYMH (SEQ ID NO 182), PWIFATSNLAS (SEQ ID NO 183), QQWRSDRALT (SEQ ID NO 184), and RASSNVISSYVH (SEQ ID NO 188), LWIYSTSNLAS (SEQ ID NO 189) and QQYSGYPLT (SEQ ID NO 190).

The extracellular domain and the transmembrane domain are preferably linked together by a flexible linker comprising the sequence SEQ ID NO.10. In other words, said anti-CLL1 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: 34 (see Table 2).

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. 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.

The present invention discloses a CLL1 specific single-chain chimeric antigen receptor (anti-CLL1 scCAR) as described 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 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 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).

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 CLL1 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. Current methodology applied for humanization is according to Lefranc MP et al (Lefranc, MP, Ehrenmann F , Ginestoux C, Giudicelli V , Duroux P "Use of IMGT (®) databases and tools for

antibody engineering and humanization", Methods Mol Biol. 2012; 907: 3-37). In these four alignments are indicated.

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 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; and Roguska et al., 1994, PNAS, 91:969-973, 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 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, Tan et al., J. Immunol., 169: 1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16): 10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8): 1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959- 73 (1994), 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 Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.).

Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine),

beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a anti-CLL1 CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered anti-CLL1 CAR can be tested for the ability to bind CLL1 using the functional assays
5 described herein.

An anti-CLL1 CAR of the invention optionally includes a suicide domain, which is intended to deplete the immune cells endowed with the CAR in the event these later would cause adverse effects in vivo. Such a suicide domain can be obtained, for instance, by including two copies of a CD20 mimotope, preferably of sequence CPYSNPSLCS (SEQ ID NO. 113), into the CAR polypeptide
10 sequence. Said two copies of a CD20 mimotope can be linked to each other and also to the V_L by a linker. They can also be inserted between the anti-CLL1 scFv and the hinge (such as CD8alpha), by using an optional linker. The CD20 mimetopes can be bound by anti-CD20 antibodies, such as Rituximab (McLaughlin P, et al. 1998). The anti-CLL1 CAR of the present invention may thus comprise a V_H and a V_L chains which are able to bind to CLL1 cell surface antigen, optionally
15 humanized, a linker L, a suicide domain, a hinge or part of it, a transmembrane domain, a co-stimulatory domain and a stimulatory domain.

In a preferred embodiment, the present invention discloses an anti-CLL1 specific single-chain chimeric antigen receptor ("anti-CLL1 scCAR" or "scCAR") having one of the polypeptide structure selected from V1 to V6, as illustrated in Figure 2 and Tables 3-8, said structure comprising an extra
20 cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CLL1 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a signaling domain and a co-stimulatory domain.

In a more preferred embodiment, the present invention discloses a CLL1 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises a FcγRIIIα, CD8 alpha or IgG1 hinge
25 and a CD8 alpha transmembrane domain.

In another more preferred embodiment, said CLL1 specific scCAR comprises the co-stimulatory domain 4-1BB or the CD28, or more preferably the 4-1BB co-stimulatory domain.

The present invention discloses a CLL1 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises a FcγRIIIα, CD8 alpha or IgG1 hinge and a 4-1BB transmembrane
30 domain.

The present invention discloses a CLL1 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises a FcγRIIIα, CD8 alpha or IgG1, a 4-1BB cytoplasmic domain and a CD8 alpha transmembrane domain.

5 According to a preferred embodiment, the anti-CLL1 scCAR of the invention has 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-CLL1 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain, said CD3 zeta signaling domain preferably having a sequence SEQ ID NO.9.

10 According to another preferred embodiment, the anti-CLL1 scCAR of the invention has 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-CLL1 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain, said 4-1BB co-stimulatory domain preferably having a sequence SEQ ID NO.8.

15 The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a FcγRIIIα hinge, a CD8α transmembrane domain, preferably having SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

20 The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a CD8α hinge, a CD8α transmembrane domain, preferably having SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

25 The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a IgG1 hinge, a CD8α transmembrane domain, preferably having SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a FcγRIIIα hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO.7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a CD8α hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO.7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

The present invention discloses anti-CLL1 scCAR 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-CLL1 antibody, a IgG1 hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO.7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

In a particular aspect, the present invention discloses an anti-CLL1 specific scCAR having a V1 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a FcγRIIIα hinge preferably with SEQ ID NO.3, a CD8α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8.

More specifically, the present invention discloses an anti-CLL1 specific scCAR having a V1 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a FcγRIIIα hinge preferably with SEQ ID NO.3, a CD8α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity with SEQ ID NO.11-13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34.

In another particular aspect, the present invention discloses an anti-CLL1 specific scCAR having a V3 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a CD8 α hinge preferably with SEQ ID NO.4, a CD8 α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8.

More specifically, the present invention discloses an anti-CLL1 specific scCAR having a V3 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a CD8 α hinge preferably with SEQ ID NO.4, a CD8 α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity with SEQ ID NO.11-13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL chain having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34.

In still another particular aspect, the present invention discloses an anti-CLL1 specific scCAR having a V5 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a IgG1 hinge preferably with SEQ ID NO.5, a CD8 α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8.

More specifically, the present invention discloses an anti-CLL1 specific scCAR having a V5 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a IgG1 hinge preferably with SEQ ID NO.5, a CD8 α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity with SEQ ID NO.11-13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL chain having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34.

The present invention discloses an anti-CLL1 specific scCAR having a V1 polypeptide structure, as illustrated in Figure 2, said polypeptide having at least 80% identity with SEQ ID NO. 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101 or 107.

In particular, said anti-CLL1 specific scCAR having a V1 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a FcγRIIIα hinge preferably with SEQ ID NO.3, a CD8α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity with SEQ ID NO.11,13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34, and wherein said polypeptide has at least 80% identity with SEQ ID NO. 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101 or 107.

The present invention discloses an anti-CLL1 specific scCAR of structure V3, as illustrated in Figure 2, said polypeptide having at least 80% identity with SEQ ID NO. 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103 or 109.

More specifically, the present invention discloses an anti-CLL1 specific scCAR having a V3 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a CD8α hinge preferably with SEQ ID NO.4, a CD8α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity with SEQ ID NO.11,13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL chain having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34, and wherein said polypeptide has at least 80% identity with SEQ ID NO. 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103 or 109.

The present invention discloses an anti-CLL1 specific scCAR of structure V5, as illustrated in Figure 2, said polypeptide having at least 80% identity with SEQ ID NO.39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105 or 111.

More specifically, the present invention discloses an anti-CLL1 specific scCAR having a V5 polypeptide structure, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a IgG1 hinge preferably with SEQ ID NO.5, a CD8α transmembrane domain, preferably with SEQ ID NO.6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO.9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO.8, wherein said VH chain having at least 80% identity

with SEQ ID NO.11,13, 15, 17, 19, 21, 23, 25, 27, 29, 31 or 33 and said VL chain having at least 80% identity with SEQ ID NO.14, 16, 18, 20, 22, 24, 26, 28, 30, 32 or 34, and wherein said polypeptide having at least 80% identity with SEQ ID NO.39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105 or 111.

The present invention more particularly discloses a CLL1 single-chain specific chimeric antigen receptor (scCAR) having a polypeptide structure V1, V3 or V5 as illustrated in Figure 2, and described above said structure comprising an extra cellular ligand binding-domain VH from a monoclonal anti-CLL1 antibody comprising the following CDR sequences: GSISSSNWWS (SEQ ID NO 119), WIGEIHSGSPDY (SEQ ID NO 120),KVSTGGFFDY (SEQ ID NO 121), and GSISSSNWWS (SEQ ID NO 122), WIGEIHSGSPNY (SEQ ID NO 123), RSSSGGFFDY (SEQ ID NO 124), and GSISSSNWWS (SEQ ID NO 125), WIGEIHSGSPNY (SEQ ID NO 126), RQTTAGSFDY (SEQ ID NO 127), and GYTFTSYFIH (SEQ ID NO 131), WIGFINPYNDGSKY (SEQ ID NO 132), TRDDGYGYAMDY (SEQ ID NO 133), and GYTFTSYVMH (SEQ ID NO 137), WIGYINPYNDGTKY (SEQ ID NO 138), ARPIYFDNDY (SEQ ID NO 139), and QQNNYDPW (SEQ ID NO 143), WIGPINPYNDGTI (SEQ ID NO 144), ARTDDYDDYTMDY (SEQ ID NO 145), and GYTFTRYWMH (SEQ ID NO 149), WIGNIDPSDTETHY (SEQ ID NO 150), AIYYGNPSYYAMDY (SEQ ID NO 151), and GYIFTSYVMY (SEQ ID NO 155), WIGYINPY (SEQ ID NO 156), ARYYDYDYYFDY (SEQ ID NO 157), and GYTFTSYFMH (SEQ ID NO 161), WIGFINPYNDGTKY (SEQ ID NO 162),TRDDGYDYAMDY (SEQ ID NO 163), and GFNIKDDYIH (SEQ ID NO 167), WIGWIDPEKGDTAYA (SEQ ID NO 168), TLTGRFDY (SEQ ID NO 169), and GYTFPSSNIH (SEQ ID NO 173), WIGVIYPGNGDTSY (SEQ ID NO 174), AIYFVYNWHFDV (SEQ ID NO 175), and GYTFTRYWMH (SEQ ID NO 179), MIHPSSGSTSYNEKVK (SEQ ID NO 180), RDGDYYYGTGDY (SEQ ID NO 181), and GYSITSAYYWN (SEQ ID NO 185), YISYDGRNNYNPSLKN (SEQ ID NO 186), AKEGDYDVGNYAMDY (SEQ ID NO 187), and preferably

an extra cellular ligand binding-domain VL from a monoclonal anti-CLL1 antibody comprising the following CDR sequences: QSISSYLN (SEQ ID NO 128), LLIYAASSLQS (SEQ ID NO 129), QQSYSTPP (SEQ ID NO 130), and QELSGYLS (SEQ ID NO 134), RLIYAASTLDS (SEQ ID NO 135), LQYAIYPY (SEQ ID NO 136), and ESVDSYGNSFMH (SEQ ID NO 140), LLIYLASNLES (SEQ ID NO 141), QQNNYDPW (SEQ ID NO 142), HDISNYLN (SEQ ID NO 146), LLIYTSRLHS (SEQ ID NO 147), QQGKTLLW (SEQ ID NO 148), and QNLLNSGNQKKYLN (SEQ ID NO 152), LLIYWASTRES (SEQ ID NO 153), QNDYSYPF (SEQ ID NO 154), and QDINKYIA (SEQ ID NO 158), LLIHYTSTLQP (SEQ ID NO 159), LQYDYLW (SEQ ID NO 160), and QEISVYLS (SEQ ID NO 164), RLIYAASTLDS (SEQ ID NO 165), LQYASYPY (SEQ ID NO 166), and QSLLYSSNQKNNLA (SEQ ID NO 170), LLIYWASTRES (SEQ ID NO 171), QQYYSYR (SEQ ID NO 172), and ESVDGYGDIFML (SEQ ID NO 176), LLIYFASNLES (SEQ ID NO 177), QQNNEDPY (SEQ ID NO 178), and RASSSINYMH (SEQ

ID NO 182), PWIFATSNLAS (SEQ ID NO 183), QQWRSDRALT (SEQ ID NO 184), and RASSNVISSYVH (SEQ ID NO 188), LWIYSTSNLAS (SEQ ID NO 189), QQYSGYPLT (SEQ ID NO 190),

- and wherein said structure generally comprising :

a hinge, a transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling
5 domain and a co-stimulatory domain from 4-1BB.

The present invention discloses an anti-CLL1 single-chain specific chimeric antigen receptor (anti-CLL1 scCAR) as above, wherein said extra cellular ligand binding-domain VH and VL is humanized.

10 The present invention discloses a CLL1 single-chain specific chimeric antigen receptor (scCAR) as described above, wherein said extra cellular ligand binding-domain VH from a monoclonal anti-CLL1 antibody comprise at least one of the following sequences:

- QVQLQESGPGGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEIIYHSGSPNYNP
SLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYSSSGGFFDYWGQGTLVTVSS

15 (corresponding to SC02-357),

- QVQLQESGPGGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEIIYHSGSPNYNP
SLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSS

(corresponding to SC02-378),

- QVQLQESGPGGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEIIYHSGSPNYNP
20 SLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSS

(corresponding to SC02-161),

- EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYNDGSKYNE
KFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSVTVSS

(corresponding to M26),

- EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPYNDGTKYN
25 EKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLVKSS

(corresponding to M31),

- EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYNDGTIYNP
NFKGKATLTVDKASSTAYMELLSTSDDPVYYCARTDDYDDYTMDYWGQGTSVTVSS
(corresponding to G4),
- 5 - QVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIGNIDPSDTETHY
NQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSS
(corresponding to M22),
- EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWKQKPGQGLEWIGYINPYNDGTKYN
EKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGQGTTTLTVSS
(corresponding to M29),
- 10 - EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPYNDGTKYN
EKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSVTVSS
(corresponding to M2),
- EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEKGDYAYAS
KFQDKATITSDTSSNTAYLQLSSLTSEDTAVYYCTLTGRFDYWGQGTTTLTVSS (corresponding
15 to M5),
- QVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLQKTPGQGLEWIGVIYPGNGDTSYN
QKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSS
(corresponding to G12),
- QVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIGMIHPSSGSTSY
20 NEKVKNKATLTVDRSSTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSS
(corresponding to 21.26),
- DIQLQESGPGLVKPSQSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDGRNNYNPS
LKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGTSVTVSS
(corresponding to 1075.7),
- 25 and VL from a monoclonal anti-CLL1 antibody comprise at least one of the following
sequences:
- DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSG
SGTDFTLTISLQPEDFATYYCQQSYSTPPTFGQGTKVEIK (corresponding to SC02-357,
SC02-378 and SC02161),

- DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASTLD SGV PKRFSGNR
SGSDYSLTISSELESEDFADYYCLQYAIYPYTFGGG TKLEIKR (corresponding to M26),
- TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGV PAR
FSGSGSRTDFTLTIDPVEADDAATYYCQQNNYDPWTFGGG TKLEIK (corresponding to
5 M31),
- EIQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSG
SGTDYSLTISNLEQEDIATYFCQQGKTLLWTFGGG TKLEIK (corresponding to G4),
- DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIYWASTRESG
VPDRFTGSGSGTDFTLTISSVQAEDLAVYFCQNDYSYPFTFGAGTKLELK (corresponding to
10 M22),
- DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKPGKGPRLLIHYTSTLQPGIPSRFSGSG
SGRDYSFSISNLEPEDIATYYCLQYDYLWTFGGG TKLEIK (corresponding to M29),
- DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQQKPDGTIKRLIYAASTLD SGV PERFSGSRS
GSDYSLTISSELESEDFADYYCLQYASYPYTFGGG TKLEIKR (corresponding to M2),
- DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNNLAWYQQKPGQSPKLLIYWASTRESGV
PDRFTGSGSGTDFTLTISSVQAEDLAVYYCQQYYSYRTFGGG TKLEIK (corresponding to M5),
15
- NIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGV PARF
SGSGSRTDFTLTIDPVEADDAATYYCQQNNEDPYTFGGG TKLEIKR (corresponding to G12),
- QIVLSQSPAILSASPGKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSG
SGTSYSLTISRVEAEDAATYYCQQWRS DRALTFGAGTKLEL (corresponding to 21.26),
20
- DIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDGRNNYNPSL
KNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNY YAMDYWGQGTSVTVSS
(corresponding to 1075.7)

25 The present invention also discloses a CLL1 specific scCAR as previously defined, further comprising another extracellular ligand binding domain which is not specific for CLL1, such as CD33 antigen, CD44 antigen, CD47 antigen, CD123 antigen, CD96 antigen and T-cell immunoglobulin mucin-3 (TIM-3).

The present invention discloses a CLL1 specific scCAR as above, further comprising a signal peptide, preferably of SEQ ID NO 1 or SEQ ID NO 2, in order to help the CAR polypeptide to reach the immune cell's membrane.

5 The present invention discloses a CLL1 specific scCAR as above, wherein a linker of SEQ ID NO 10 is inserted between VH and VL.

Polynucleotides, vectors:

10 The present invention also relates to polynucleotides and vectors allowing heterologous expression into cells of the anti-CLL1 CAR according to the invention, encoding the polypeptides sequences which have been previously detailed.

The polynucleotides may be included 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).

15 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
20 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
25 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
30 transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading

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 the amino acid sequence SEQ ID NO: 1 and 2 or at least 90 %, 95 % 97 % or 99 % sequence identity with SEQ ID NO: 1 and/or 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 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.

Delivery methods

The present invention encompasses the different means to express the anti-CLL1 Chimeric Antigen Receptor (CAR) described herein in immune cells

Methods for introducing a polynucleotide construct into cells are known in the art and include as non-limiting examples stable transformation methods wherein the polynucleotide construct encoding said CAR 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 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, cell fusion. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells. Said plasmid vector can comprise a selection marker which provides for identification and/or selection of cells which received said vector.

Different transgenes can be included in one vector. Said vector can comprise 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 et al., J. of General Virology 82: 1013-1025 (2001); Donnelly et al., J. of Gen. Virology 78: 13-21 (1997); Doronina et al., Mol. And. Cell. Biology 28(13): 4227-4239 (2008); Atkins et al., RNA 13: 803-810 (2007)).

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.

In a more preferred embodiment of the invention, polynucleotides encoding polypeptides according to the present invention can be mRNA which is introduced directly into the cells, for example by electroporation. The inventors determined the optimal condition for mRNA electroporation in T-cell. The inventor used the cytoPulse technology which allows, by the use of pulsed electric fields, to transiently permeabilize living cells for delivery of material into the cells. The technology, based on the use of PulseAgile (BTX Havard Apparatus, 84 October Hill Road, Holliston, MA 01746, USA) electroporation waveforms grants the precise control of pulse duration, intensity as well as the interval between pulses (U.S. patent 6,010,613 and International PCT application WO2004083379). All these parameters can be modified in order to reach the best conditions for high transfection efficiency with minimal mortality. Basically, the first high electric field pulses allow pore formation, while subsequent lower electric field pulses allow moving the polynucleotide into the cell.

The different methods described above involve introducing scCAR into a cell. As non-limiting example, said scCAR 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

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 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.

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 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

(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-cysteine and 2-mercaptoethanol. 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 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 cells. Said cells can also be expanded *in vivo*, for example in the subject's blood after administering said cell into the subject.

Engineered immune cells

A "Cell" according to the present invention generally refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptive immune response. Cell according to the present invention is preferably an isolated immune cell, and more preferably a T-cell obtained from a donor. Said immune cell according to the present invention can also 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 primary T-cells, endowed with a CLL1 CAR as described above, that do not express functional TCR and that are reactive towards CLL1 positive cells, for their allogeneic transplantation into patients.

As a more preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a CLL1 scCAR and that are reactive towards CLL1 positive cells as described above, that do not express a functional TCR and are resistant to a selected drug, for their allogeneic transplantation into patients treated with said selected drug. The present invention encompasses the method of preparing engineered immune cells for immunotherapy comprising introducing *ex-vivo* into said immune cells the polynucleotides or vectors encoding the CLL1 CAR according to transformation methods as previously described in WO2014/130635, WO2013/176916, WO2013/176915 and incorporated herein by reference.

In a preferred embodiment, said polynucleotides are introduced into the immune cells by means of retroviral vectors in view of being stably integrated into the cell genome.

Methods of engineering immune cells endowed with the CARs according to the invention

The present invention also aims to produce immune cells endowed with anti CLL1 CAR, which are less or non-alloreactive, which can be used in allogeneic treatments (i.e. with reduced risk of inducing Graft versus host reaction) and/or made resistant to various standard of care treatments).

As further described in this specification, said methods may further comprise the step of
5 genetically modifying said immune cell by using at least one endonuclease.

- 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 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
10 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.

Preferably, the methods according to the present invention involve a rare cutting endonuclease. Rare-cutting endonucleases can for example be a homing endonuclease (Paques and
15 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 TALE-nuclease, a Cas9 endonuclease from CRISPR system as described below (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
20 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 conjugates of orthophenanthroline, a DNA cleaving molecule, and triplex-forming oligonucleotides (TFOs), known to bind specific DNA sequences (Kalish and Glazer 2005).. Rare-cutting endonucleases can be used for
25 inactivating genes at a locus or to integrate transgenes by homologous recombination (HR) i.e. by inducing DNA double-strand breaks (DSBs) at a locus and insertion of exogenous DNA at this locus by gene repair mechanism (Perrin, Buckle et al. 1993; Rouet, Smih et al. 1994; Choulika, Perrin et al. 1995; Pingoud and Silva 2007).

- By “TALE-nuclease” (TALEN) is intended a fusion protein consisting of a nucleic acid-binding
30 domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease 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). Engineered TAL-nucleases are available under the trade name TALENTM (Cellestis, 8 rue de la Croix Jarry, 75013 Paris, France) and can be ordered from manufacturers, such as Life Technologies (Carlsbad, California, USA).

Preferred TALE-nucleases recognizing and cleaving the target sequence are described in PCT/EP2014/075317. In particular, additional catalytic domain can be further introduced into the cell with said rare-cutting endonuclease to increase mutagenesis in order to enhance their capacity to inactivate targeted genes. More particularly, said additional catalytic domain is a DNA end processing enzyme. Non limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5' flap endonucleases, helicases, phosphatase, hydrolases and template-independent DNA polymerases. Non limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivate of the protein domain selected from the

group consisting of hExoI (EXO1_HUMAN), Yeast ExoI (EXO1_YEAST), E.coli ExoI, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In a preferred embodiment, said additional catalytic domain has a 3'-5'-exonuclease activity, and in a more preferred embodiment, said additional catalytic domain is TREX, more preferably TREX2 catalytic domain (WO2012/058458). In another preferred embodiment, said catalytic domain is encoded by a single chain TREX2 polypeptide. Said additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein according to the invention optionally by a peptide linker.

- By "Cas9 endonuclease". is meant any genome engineering tool 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 protospacer 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 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). The use of Cas9 in immune cells, especially in T- Cells, has been previously described in WO2014191128.

Modifying T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component

According to one aspect, T-cell endowed with anti-CLL1 CAR of the present invention can be made less alloreactive, for instance, by inactivating at least one gene expressing one or more component of T-cell receptor (TCR) as described in WO 2013/176915. This inactivation can be combined with that of another gene, such as of a gene encoding or regulating HLA or β 2m protein expression. Accordingly, the risk of graft versus host syndrome and graft rejection is significantly reduced.

Methods of making cells less allogenic can comprise the step of inactivating at least one gene encoding a T-Cell Receptor (TCR) component, in particular TCRalpha and/or TCRbeta genes.

Methods disclosed in WO2013/176915 to prepare CAR expressing immune cell suitable for allogeneic transplantation, by inactivating one or more component of T-cell receptor (TCR), are all incorporated herein by reference.

The present invention encompasses an anti-CLL1 CAR expressing immune cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) has been inactivated. Thus, the present invention provides an anti-CLL1 CAR expressing T cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated.

By inactivating a TCR gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. The nucleic acid strand breaks caused by the rare-cutting endonuclease are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). However, NHEJ is an imperfect repair process that often results in changes to the DNA sequence at the site of the cleavage. Mechanisms involve rejoining of what remains of the two DNA ends through direct re-ligation (Critchlow and Jackson 1998) or via the so-called microhomology-mediated end joining (Betts, Brenchley et al. 2003; Ma, Kim et al. 2003). Repair via non-homologous end joining (NHEJ) often results in small insertions or deletions and can be used for the creation of specific gene knockouts. Said modification may be a substitution, deletion, or addition of at least one nucleotide. Cells in which a cleavage-induced mutagenesis event, i.e. a mutagenesis event consecutive to an NHEJ event, has occurred can be identified and/or selected by well-known method in the art. In a particular embodiment, the step of inactivating at

least a gene encoding a component of the T-cell receptor (TCR) into the cells of each individual sample comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one gene encoding a component of the T-cell receptor (TCR). In a more particular embodiment, said cells of each individual sample are transformed with nucleic acid encoding a rare-cutting endonuclease
5 capable of disrupting at least one gene encoding a component of the T-cell receptor (TCR), and said rare-cutting endonuclease is expressed into said cells.

In a preferred embodiment said method of further engineer the immune cells involves introducing into said T cells polynucleotides, in particular mRNAs, encoding specific rare-cutting endonuclease to selectively inactivate the genes mentioned above by DNA cleavage. In a more
10 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.

According to the invention, anti-CLL1 CAR immune cells with one or more component of T-
15 cell receptor (TCR) inactivated are intended to be used as a medicament.

Drug Resistant T-cells

According to another aspect, anti-CLL1 CAR expressing immune cells of the invention can be further genetically engineered to make them resistant to immunosuppressive drugs or
20 chemotherapy treatments, which are used as standard care for treating cancer associated with CLL1 positive malignant cell, especially AML..

Several cytotoxic agents (anti-cancer drug)s) such as anti-metabolites, alkylating agents, anthracyclines, DNA methyltransferase inhibitors, platinum compounds and spindle poisons have been developed to kill cancer cells. However, the introduction of these agents with novel therapies,
25 such as immunotherapies, is problematic. For example, chemotherapy agents can be detrimental to the establishment of robust anti-tumor immunocompetent cells due to the agents' non-specific toxicity profiles. Small molecule-based therapies targeting cell proliferation pathways may also hamper the establishment of anti-tumor immunity. If chemotherapy regimens that are transiently effective can be combined with novel immunocompetent cell therapies then significant improvement
30 in anti-neoplastic therapy might be achieved (for review (Dasgupta, McCarty et al. 2011)).

To improve cancer therapy and selective engraftment of allogeneic immune cells, drug resistance is conferred to said allogeneic cells to protect them from the toxic side-effects of chemotherapy agents. The drug resistance of immune cells also permits their enrichment *in* or *ex vivo*, as T-cells which express the drug resistance gene will survive and multiply relative to drug sensitive cells.

Methods for engineering immunecells resistant to chemotherapeutic agents are disclosed in PCT/EP2014/075317 which is fully incorporated by reference herein.

In particular, the present invention relates to a method of engineering allogeneic cells suitable for immunotherapy wherein at least one gene encoding a T-cell receptor (TCR) component is inactivated and one gene is modified to confer drug resistance comprising:

- Providing an anti-CLL1 scCAR expressing T-cell; expressing T cell,
- Modifying said anti- CLL1 scCAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;
- Modifying said anti-CLL1 scCAR expressing T-cell, preferably humanized CLL1 scCAR, to confer drug resistance to said anti-CLL1 scCAR expressing T-cell;
- Expanding said engineered anti-CLL1 scCAR expressing T-cell in the presence of said drug.

Alternatively, the present invention relates to a method comprising:

- Providing an anti-CLL1 scCAR expressing T-cell; preferably humanized CLL1 scCAR;
- Modifying said anti-CLL1 scCAR expressing T-cell to confer drug resistance to said anti-CLL1 scCAR expressing T-cell;
- Modifying said anti-CLL1 scCAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;
- Expanding said engineered anti-CLL1 scCAR expressing T-cell in the presence of said drug.

In particular, the present invention also relates to a method of engineering allogeneic cells suitable for immunotherapy wherein at least one gene encoding a T-cell receptor (TCR) component is inactivated and one gene is modified to confer drug resistance comprising:

- Providing an anti-CLL1 scCAR expressing T-cell; preferably humanized CLL1 scCAR;
- Modifying said anti-CLL1 scCAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;
- Modifying said anti-CLL1 scCAR expressing T-cell to confer drug resistance to said anti-CLL1 scCAR expressing T-cell;
- Expanding said engineered anti-CLL1 scCAR expressing T-cell in the presence of said drug.

Alternatively, the present invention relates to a method comprising:

- Providing an anti-CLL1 scCAR expressing T-cell; preferably humanized CLL1 scCAR;
- Modifying said anti-CLL1 scCAR expressing T-cell to confer drug resistance to said anti-CLL1 scCAR expressing T-cell;
- Modifying said anti-CLL1 scCAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;
- Expanding said engineered anti-CLL1 scCAR expressing T-cell in the presence of said drug.

Expression of drug resistance genes in anti-CLL1 scCAR-expressing immune cells

In a particular embodiment, said drug resistance can be conferred to the T-cell by the expression of at least one drug resistance gene. Said drug resistance gene refers to a nucleic acid sequence that encodes "resistance" to an agent, such as a chemotherapeutic agent (e.g. methotrexate). In other words, the expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent to a greater extent than the proliferation of a corresponding cell without the drug resistance gene. The expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent and does not affect its activity. A drug resistance gene of the invention can encode resistance to anti-metabolite, methotrexate, vinblastine, cisplatin, alkylating agents, anthracyclines, cytotoxic antibiotics, anti-immunophilins, their analogs or derivatives, and the like.

In one embodiment, a drug resistance gene of the invention can confer resistance to a drug (or an agent), in particular an anti-cancer drug selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16),

arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

Several drug resistance genes have been identified that can potentially be used to confer
5 drug resistance to targeted cells (Takebe, Zhao et al. 2001; Sugimoto, Tsukahara et al. 2003; Zielske, Reese et al. 2003; Nivens, Felder et al. 2004; Bardenheuer, Lehmborg et al. 2005; Kushman, Kabler et al. 2007).

One example of drug resistance gene can also be a mutant or modified form of Dihydrofolate reductase (DHFR). DHFR is an enzyme involved in regulating the amount of tetrahydrofolate in the
10 cell and is essential to DNA synthesis. Folate analogs such as methotrexate (MTX) inhibit DHFR and are thus used as anti-neoplastic agents in clinic. Different mutant forms of DHFR which have increased resistance to inhibition by anti-folates used in therapy have been described. In a particular embodiment, the drug resistance gene according to the present invention can be a nucleic acid sequence encoding a mutant form of human wild type DHFR (GenBank: AAH71996.1) which
15 comprises at least one mutation conferring resistance to an anti-folate treatment, such as methotrexate. In particular embodiment, mutant form of DHFR comprises at least one mutated amino acid at position G15, L22, F31 or F34, preferably at positions L22 or F31 (Schweitzer, Dicker et al. 1990); International application WO94/24277; US patent US6,642,043). In a particular embodiment, said DHFR mutant form comprises two mutated amino acids at position L22 and F31.
20 Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type DHFR polypeptide set forth in GenBank: AAH71996.1. In a particular embodiment, the serine residue at position 15 is preferably replaced with a tryptophan residue. In another particular embodiment, the leucine residue at position 22 is preferably replaced with an amino acid which will disrupt binding of the mutant DHFR to antifolates,
25 preferably with uncharged amino acid residues such as phenylalanine or tyrosine. In another particular embodiment, the phenylalanine residue at positions 31 or 34 is preferably replaced with a small hydrophilic amino acid such as alanine, serine or glycine.

As used herein, "antifolate agent" or "folate analogs" refers to a molecule directed to interfere with the folate metabolic pathway at some level. Examples of antifolate agents include,
30 e.g., methotrexate (MTX); aminopterin; trimetrexate (Neutrexin™); edatrexate; N10-propargyl-5,8-

dideazafolic acid (CB3717); ZD1694 (Tumodex), 5,8-dideazaisofolic acid (IAHQ); 5,10-dideazatetrahydrofolic acid (DDATHF); 5-deazafolic acid; PT523 (N alpha-(4-amino-4- deoxypteroyl)-N delta-hemiphthaloyl-L-ornithine); 10-ethyl-10-deazaaminopterin (DDATHF, lomatrexol); piritrexim; 10-EDAM; ZD1694; GW1843; Pemetrexate and PDX (10-propargyl-10- deazaaminopterin).

5 Another example of drug resistance gene can also be a mutant or modified form of ionisine-5'- monophosphate dehydrogenase II (IMPDH2), a rate-limiting enzyme in the *de novo* synthesis of guanosine nucleotides. The mutant or modified form of IMPDH2 is an IMPDH inhibitor resistance gene. IMPDH inhibitors can be mycophenolic acid (MPA) or its prodrug mycophenolate mofetil (MMF). The mutant IMPDH2 can comprises at least one, preferably two mutations in the MAP
10 binding site of the wild type human IMPDH2 (NP_000875.2) that lead to a significantly increased resistance to IMPDH inhibitor. The mutations are preferably at positions T333 and/or S351 (Yam, Jensen et al. 2006; Sangiolo, Lesnikova et al. 2007; Jonnalagadda, Brown et al. 2013). In a particular embodiment, the threonine residue at position 333 is replaced with an isoleucine residue and the serine residue at position 351 is replaced with a tyrosine residue. Correspondence of amino acid
15 positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human IMPDH2 polypeptide set forth in NP_000875.2.

Another drug resistance gene is the mutant form of calcineurin. Calcineurin (PP2B), an ubiquitously expressed serine/threonine protein phosphatase that is involved in many biological processes and which is central to T-cell activation. Calcineurin is a heterodimer composed of a
20 catalytic subunit (CnA; three isoforms) and a regulatory subunit (CnB; two isoforms). After engagement of the T-cell receptor, calcineurin dephosphorylates the transcription factor NFAT, allowing it to translocate to the nucleus and active key target gene such as IL2. FK506 in complex with FKBP12, or cyclosporine A (CsA) in complex with CyPA block NFAT access to calcineurin's active site, preventing its dephosphorylation and thereby inhibiting T-cell activation (Brewin, Mancao et al.
25 2009). The drug resistance gene of the present invention can be a nucleic acid sequence encoding a mutant form of calcineurin resistant to calcineurin inhibitor such as FK506 and/or CsA. In a particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer a at positions: V314, Y341, M347, T351, W352, L354, K360, preferably double mutations at positions T351 and L354 or V314 and Y341. In a particular embodiment, the
30 valine residue at position 341 can be replaced with a lysine or an arginine residue, the tyrosine residue at position 341 can be replaced with a phenylalanine residue; the methionine at position 347

can be replaced with the glutamic acid, arginine or tryptophane residue; the threonine at position 351 can be replaced with the glutamic acid residue; the tryptophane residue at position 352 can be replaced with a cysteine, glutamic acid or alanine residue, the serine at position 353 can be replaced with the histidine or asparagines residue, the leucine at position 354 can be replaced with an alanine residue; the lysine at position 360 can be replaced with an alanine or phenylalanine residue of a sequence corresponding to GenBank: ACX34092.1. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer a polypeptide set forth in (GenBank: ACX34092.1).

In another particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer b at positions: V120, N123, L124 or K125, preferably double mutations at positions L124 and K125. In a particular embodiment, the valine at position 120 can be replaced with a serine, an aspartic acid, phenylalanine or leucine residue; the asparagine at position 123 can be replaced with a tryptophan, lysine, phenylalanine, arginine, histidine or serine; the leucine at position 124 can be replaced with a threonine residue; the lysine at position 125 can be replaced with an alanine, a glutamic acid, tryptophan, or two residues such as leucine-arginine or isoleucine-glutamic acid can be added after the lysine at position 125 in the amino acid sequence coresponding to GenBank: ACX34095.1. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer b polypeptide set forth in (GenBank: ACX34095.1).

Another drug resistance gene is 0(6)-methylguanine methyltransferase (MGMT) encoding human alkyl guanine transferase (hAGT). AGT is a DNA repair protein that confers resistance to the cytotoxic effects of alkylating agents, such as nitrosoureas and temozolomide (TMZ). 6-benzylguanine (6-BG) is an inhibitor of AGT that potentiates nitrosourea toxicity and is co-administered with TMZ to potentiate the cytotoxic effects of this agent. Several mutant forms of MGMT that encode variants of AGT are highly resistant to inactivation by 6-BG, but retain their ability to repair DNA damage (Maze, Kurpad et al. 1999). In a particular embodiment, AGT mutant form can comprise a mutated amino acid of the wild type AGT position P140, in the amino acid sequence such as disclosed in the database Uniprot under the reference P16455. In a preferred embodiment, said proline at position 140 is replaced with a lysine residue.

Another drug resistance gene can be multidrug resistance protein 1 (MDR1) gene. This gene encodes a membrane glycoprotein, known as P-glycoprotein (P-GP) involved in the transport of metabolic byproducts across the cell membrane. The P-Gp protein displays broad specificity towards several structurally unrelated chemotherapy agents.

5 Overexpressing multidrug resistance protein 1 has been described to confer resistance to drugs such as Mitoxantrone (Charles S. Morrow, Christina Peklak-Scott, Bimjhana Bishwokarma, Timothy E. Kute, Pamela K. Smitherman, and Alan J. Townsend. Multidrug Resistance Protein 1 (MRP1, ABCC1) Mediates Resistance to Mitoxantrone via Glutathione-Dependent Drug Efflux *Mol Pharmacol* April 2006 69:1499-1505).

10 Thus, drug resistance can be conferred to cells by the expression of nucleic acid sequence that encodes MDR-1 (NP_000918).

Still another way of preparing drug resistant cells is to prepare cells with specific mutation (s) such as mutations at Arg486 and Glu571 in the Human Topoisomerase II gene, to confer resistance to amsacrine (S. PATEL, B. A. KELLER, and L. M. FISHER. 2000. MOLECULAR PHARMACOLOGY. Vol 57: 15 p784 –791 (2000).

Still another way of preparing drug resistant cells is to prepare cells overexpressing microRNA-21 to confer resistance to Daunorubicine (Involvement of miR-21 in resistance to daunorubicin by regulating PTEN expression in the leukaemia K562 cell line Bai, Haitao et al. FEBS Letters , Volume 585 , Issue 2 , 402 – 408).

20 In a preferred embodiment, cells bearing such a drug resistance conferring mRNA or protein also comprise an inhibitory mRNA or a gene the expression of which is conditioned, allowing the selective destruction of said drug resistant cells in the presence of said drug or upon administration of said drug.

Drug resistance gene can also confer resistance to cytotoxic antibiotics, and can be *ble* gene 25 or *mcrA* gene. Ectopic expression of *ble* gene or *mcrA* in an immune cell gives a selective advantage when exposed to the chemotherapeutic agent, respectively the bleomycine or the mitomycin C.

The most practical approach to gene therapy is the addition of a gene to engineer T-cell by using efficient gene delivery with vectors, preferably viral vector. Thus, in a particular embodiment,

said drug resistance gene can be expressed in the cell by introducing a transgene preferably encoded by at least one vector into a cell.

In one embodiment, cells bearing a drug resistance gene or a modified gene conferring resistance to a drug also comprise an inducible suicide gene – the induction of which provokes cell death- allowing their selective destruction.

The random insertion of genes into the genome may lead to the inappropriate expression of the inserted gene or the gene near the insertion site. Specific gene therapy using homologous recombination of exogenous nucleic acid comprising endogenous sequences to target genes to specific sites within the genome can allow engineering secure T-cells. As described above, the genetic modification step of the method can comprise a step of introduction into cells of an exogenous nucleic acid comprising at least a sequence encoding the drug resistance gene and a portion of an endogenous gene such that homologous recombination occurs between the endogenous gene and the exogenous nucleic acid. In a particular embodiment, said endogenous gene can be the wild type “drug resistance” gene, such that after homologous recombination, the wild type gene is replaced by the mutant form of the gene which confers resistance to the drug.

Endonucleolytic breaks are known to stimulate the rate of homologous recombination. Thus, in a particular embodiment, the method of the invention further comprises the step of expressing in the cell a rare-cutting endonuclease which is able to cleave a target sequence within an endogenous gene. Said endogenous gene can encode for examples DHFR, IMPDH2, calcineurin or AGT. Said rare-cutting endonuclease can be a TALE-nuclease, a Zinc finger nuclease, a CRISPR/Cas9 endonuclease, a MBBBD-nuclease or a meganuclease.

Inactivation of drug sensitizing genes in anti-CLL1 CAR-expressing immune cells

In another particular embodiment, said drug resistance can be conferred to the cell of the invention (anti-CLL1 CAR expressing immune cell,) by the inactivation of a drug sensitizing gene.

The inventor sought to inactivate potential drug sensitizing gene to engineer T-cell for immunotherapy, in particular to engineer anti-CLL1 CAR expressing immune cell that can be used in combination with a therapeutic agent (anti-cancer drug).

By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiment, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. In a particular embodiment, the step of inactivating at least one drug sensitizing gene comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one drug sensitizing gene. In a more particular embodiment, said cells are transformed with nucleic acid encoding a rare-cutting endonuclease capable of disrupting a drug sensitizing gene, and said rare-cutting endonuclease is expressed into said cells. Said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease, CRISPR/Cas9 nuclease, A MBBBD-nuclease or a TALE-nuclease. In a preferred embodiment, said rare-cutting endonuclease is a TALE-nuclease.

In a preferred embodiment, drug sensitizing gene which can be inactivated to confer drug resistance to the T-cell is the human deoxycytidine kinase (dCK) gene. This enzyme is required for the phosphorylation of the deoxyribonucleosides deoxycytidine (dC), deoxyguanosine (dG) and deoxyadenosine (dA). Purine nucleotide analogs (PNAs) are metabolized by dCK into mono-, di- and tri-phosphate PNA. Their triphosphate forms and particularly clofarabine triphosphate compete with ATP for DNA synthesis, acts as proapoptotic agent and are potent inhibitors of ribonucleotide reductase (RNR) which is involved in trinucleotide production.

Preferably, the inactivation of dCK in T cells is mediated by TALE nuclease. To achieve this goal, several pairs of dCK TALE-nuclease have been designed, assembled at the polynucleotide level and validated by sequencing. Examples of TALE-nuclease pairs which can be used according to the invention are depicted in PCT/EP2014/075317. .

This dCK inactivation in T cells confers resistance to purine nucleoside analogs (PNAs) such as clofarabine, fludarabine or decitabine (Dacogen).

In another preferred embodiment, the dCK inactivation in T cells is combined with an inactivation of TRAC genes rendering these double knock out (KO) T cells both resistant to drug such as clofarabine and less allogeneic. This double features is particularly useful for a therapeutic goal, allowing "off-the-shelf" allogeneic cells for immunotherapy in conjunction with chemotherapy to treat patients with cancer. This double KO inactivation dCK/TRAC can be performed simultaneously

or sequentially. One example of TALE-nuclease dCK/TRAC pairs which gave success in the invention is described in PCT/EP2014/075317, in particular, the target sequences in the 2 loci (dCK and TRAC).

Another example of enzyme which can be inactivated is human hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene (Genbank: M26434.1). In particular HPRT can be inactivated
5 in engineered T-cells to confer resistance to a cytostatic metabolite, the 6-thioguanine (6TG) which is converted by HPRT to cytotoxic thioguanine nucleotide and which is currently used to treat patients with cancer, in particular leukemias (Hacke, Treger et al. 2013). Guanines analogs are metabolized by HPRT transferase that catalyzes addition of phosphoribosyl moiety and enables the formation of TGMP. Guanine analogues including 6 mercaptopurine (6MP) and 6 thioguanine (6TG) are usually
10 used as lymphodepleting drugs to treat leukemias. They are metabolized by HPRT (hypoxanthine phosphoribosyl transferase that catalyzes addition of phosphoribosyl moiety and enables formation TGMP. Their subsequent phosphorylations lead to the formation of their triphosphorylated forms that are eventually integrated into DNA. Once incorporated into DNA, thio GTP impairs fidelity of DNA replication via its thiolate groupment and generate random point mutation that are highly
15 deleterious for cell integrity.

Thus, the present invention provides an anti-CLL1 scCAR expressing cell, in particular an anti-CLL1 scCAR expressing T cell wherein the scCAR has a polypeptide sequence according to SEQ ID NO.35 to 112 (optionally humanized) and wherein the dCK gene is inactivated.

In another embodiment, the inactivation of the CD3 normally expressed at the surface of the
20 T-cell can confer resistance to anti-CD3 antibodies such as teplizumab.

Multiple drug resistance of anti-CLL1 scCAR-expressing immune cells

In another particular embodiment, the inventors sought to develop an “off-the shelf” immunotherapy strategy, using allogeneic T-cells, in particular allogeneic anti-CLL1 scCAR expressing T-cell resistant to multiple drugs to mediate selection of engineered T-cells when the patient is
25 treated with different drugs. The therapeutic efficiency can be significantly enhanced by genetically engineering multiple drug resistance allogeneic T-cells. Such a strategy can be particularly effective in treating tumors that respond to drug combinations that exhibit synergistic effects. Moreover multiple resistant engineered T-cells can expand and be selected using minimal dose of drug agents.

Thus, the method according to the present invention can comprise modifying T-cell to confer multiple drug resistance to said T-cell. Said multiple drug resistance can be conferred by either expressing more than one drug resistance gene or by inactivating more than one drug sensitizing gene. In another particular embodiment, the multiple drug resistance can be conferred to said T-cell
5 by expressing at least one drug resistance gene and inactivating at least one drug sensitizing gene. In particular, the multiple drug resistance can be conferred to said T-cell by expressing at least one drug resistance gene such as mutant form of DHFR, mutant form of IMPDH2, mutant form of calcineurin, mutant form of MGMT, the *ble* gene, and the *mcrA* gene and inactivating at least one drug sensitizing gene such as HPRT gene. In a preferred embodiment, multiple drug resistance can be conferred by
10 inactivating HPRT gene and expressing a mutant form of DHFR; or by inactivating HPRT gene and expressing a mutant form of IMPDH2; or by inactivating HPRT gene and expressing a mutant form of calcineurin; by inactivating HPRT gene and expressing a mutant form of MGMT; by inactivating HPRT gene and expressing the *ble* gene; by inactivating HPRT gene and expressing the *mcrA* gene.

In one embodiment, the present invention provides allogenic anti-CLL1 scCAR expressing T-
15 cell expressing more than one drug resistance gene or wherein more than one drug sensitizing gene is inactivated.

Suicide genes in anti-CLL1 scCAR-expressing immune cells

In some instances, since engineered T-cells can expand and persist for years after administration, it can be desirable to include a safety mechanism to allow selective deletion of
20 administrated T-cells. Thus, in some embodiments, the method of the invention can comprises the transformation of said T-cells with a recombinant suicide gene. Said recombinant suicide gene is used to reduce the risk of direct toxicity and/or uncontrolled proliferation of said T-cells once administrated in a subject (Quintarelli C, Vera F, blood 2007; Tey SK, Dotti G. , Rooney CM, boil blood marrow transplant 2007). Suicide genes enable selective deletion of transformed cells *in vivo*. In
25 particular, the suicide gene has the ability to convert a non-toxic pro-drug into cytotoxic drug or to express the toxic gene expression product. In other words, "Suicide gene" is a nucleic acid coding for a product, wherein the product causes cell death by itself or in the presence of other compounds.

A representative example of such a suicide gene is one which codes for thymidine kinase of herpes simplex virus. Additional examples are thymidine kinase of varicella zoster virus and the
30 bacterial gene cytosine deaminase which can convert 5-fluorocytosine to the highly toxic compound 5-fluorouracil. Suicide genes also include as non limiting examples caspase-9 or caspase-8 or cytosine

deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID). Suicide genes can also be polypeptides that are expressed at the surface of the cell and can make the cells sensitive to therapeutic monoclonal antibodies. As used herein "prodrug" means any compound useful in the methods of the present invention that can be converted to a toxic product. The prodrug is converted to a toxic product by the gene product of the suicide gene in the method of the present invention. A representative example of such a prodrug is ganciclovir which is converted in vivo to a toxic compound by HSV-thymidine kinase. The ganciclovir derivative subsequently is toxic to tumor cells. Other representative examples of prodrugs include acyclovir, FIAU [1-(2-deoxy-2-fluoro- β -D-arabinofuranosyl)-5-iodouracil], 6-methoxypurine arabinoside for VZV-TK, and 5-fluorocytosine for cytosine deaminase.

One preferred suicide gene system employs a recombinant antigenic polypeptide comprising antigenic motif recognized by the anti-CD20 mAb Rituximab, especially QBen10, such as in the so-called RQR8 polypeptide described in WO2013153391, which is expressed independently from the anti-CLL1 CAR. Rituximab, an authorized antibody drug, can then be used for cell depletion when needed.

In one embodiment, the present invention provides allogenic anti-CLL1 scCAR expressing T-cell expressing more than one drug resistance gene or wherein more than one drug sensitizing gene is inactivated, and a suicide gene allowing said cells to be destroyed.

In particular, the present invention relates to an allogeneic T-cell, in particular an allogeneic anti-CLL1 scCAR expressing T-cell, and preferably an allogeneic anti-CLL1 scCAR expressing T-cell comprising a peptide having 80% to 100% identity with scfv from SC02-357, SC02-378, SC02-161, M26, M31, G4, M22, M29, M2, M5, G12, 21.26 or 1075.7 antibodies, said allogeneic anti-CLL1 scCAR expressing T-cell comprising a peptide having 80% to 100% identity with scfv from SC02-357, SC02-378, SC02-161, M26, M31, G4, M22, M29, M2, M5, G12, 21.26 and 1075.7 antibodies is more particularly resistant to a drug, and specifically suitable for immunotherapy.

The resistance of a drug can be conferred by inactivation of drug sensitizing genes or by expression of drug resistance genes. Some examples of drugs which suit to the invention are the purine nucleoside analogues (PNAs) such as clofarabine or fludarabine, or other drugs such as 6-Mercaptopurine (6MP) and 6 thio-guanine (6TG).

In one aspect, the present invention provides methods for engineering immune cells to make them resistant to purine nucleotide analogs (PNA), such a clorofarabine or fludarabine, so that they can be used in cancer immunotherapy treatments in patients pre-treated with these conventional chemotherapies.

5 The resistance to drugs can be conferred to the T-cells by inactivating one or more gene(s) responsible for the cell's sensitivity to the drug (drug sensitizing gene(s)), such as the dck and/or HPRT genes.

According to another aspect, the resistance to drugs can be conferred to a T-cell by expressing a drug resistance gene. Variant alleles of several genes such as dihydrofolate reductase (DHFR), inosine monophosphate dehydrogenase 2 (IMPDH2), calcineurin or methylguanine transferase (MGMT) have been identified to confer drug resistance to a cell according to the invention.

For instance, CD52 and glucocorticoid receptors (GR), which are drug targets of Campath® (alemtuzumab) or rituximab and glucocorticoids treatments, 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 anti-CLL1 scCARs. 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 for the treatment of acute lymphoblastic leukemia.

Immune checkpoints engineered cells

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 the following gene selected from CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBR2, TGFBR1, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3,

GUCY1B2, GUCY1B3, preferably, said gene is PDCD1 or CTLA-4. Examples of genes, which expression could be reduced or suppressed are also indicated in Table 9.

The present invention also provides allogeneic T-cells expressing an anti- CLL1 scCAR, in particular an anti-CLL1, wherein at least one gene expressing one or more component of T-cell
 5 receptor (TCR) is inactivated and /or one gene selected from the genes CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBR2, TGFBR1, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, is inactivated as
 10 referred to in WO2014/184741.

In one embodiment said gene is a gene that acts as a regulator of T-cells activation coding the beta 2 microglobulin protein.

According to a further aspect of the invention, the anti-CLL1 scCAR-immune cells of the invention can be further manipulated to make them resistant to a drug, in particular to a drug used
 15 during chemotherapy against cancer, in particular a CLL1-expressing cell-mediated cancer such as AML. This can be achieved by introducing a gene conferring resistance to said drug. This same gene may be turned on and off by using a gene inducible inhibition/expression system as previously described (Garcia EL, Mills AA (2002) Getting around lethality with inducible Cre-mediated excision. Semin Cell Dev Biol 13:151–8 , Lewandoski M (2001) Conditional control of gene expression in the
 20 mouse. Nat Rev Genet 2:743–55 ; Scharfenberger L, Hennerici T, Kirly G *et al.* (2014) Transgenic mouse technology in skin biology: Generation of complete or tissue-specific knockout mice. J Invest Dermatol 134:e16 ; Schwenk F, Kuhn R, Angrand PO *et al.* (1998) Temporally and spatially regulated somatic mutagenesis in mice. Nucleic Acids Res 26:1427–32

Thus, anti-CLL1 scCAR-expressing, drug resistant immune cell, wherein (i) at least one gene
 25 expressing one or more component of T-cell receptor (TCR) is inactivated (ii) at least one gene conferring resistance to a drug is incorporated or a gene conferring sensitivity to said drug is deleted or mutated to be inactivated (iii) optionally another gene selected from the gene disclosed in the following table 9 is inactivated - is an object of the present invention.

The present invention encompasses the isolated anti-CLL1 scCAR-immune cells or cell lines obtainable by the method of the invention, more particularly isolated cells comprising any of the proteins, polypeptides, allelic variants, altered or deleted genes or vectors described herein.

5 The immune cells of the present invention or cell lines can further comprise exogenous recombinant polynucleotides, in particular scCARs or suicide genes or they can comprise altered or deleted genes coding for checkpoint proteins or ligands thereof that contribute to their efficiency as a therapeutic product, ideally as an “off the shelf” product. In another aspect, the present invention concerns the method for treating or preventing cancer in the patient by administrating at least once an engineered immune cell obtainable by the above methods.

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
----------------------------	---------------------------------	------------------------------------

CLL1+/luc+ drug resistant Daudi cells for testing the cytotoxicity of drug resistant allogenic scCAR T cells

5 The present invention encompasses also a method for manufacturing target cells which express both a surface receptor specific to the scCAR T cells and a resistance gene. These target cells are particularly useful for testing the cytotoxicity of scCAR T cells. These cells are readily resistant to clinically relevant dose of clofarabine and harbor luciferase activity. This combination of features enable traking them *in vivo* in a mice model or destroy them when required.

10 More particularly, they can be used to assess the cytotoxicity properties drug resistant T cells in mice in the presence of clofarabine or other PNAs. Clofarabine resistant Daudi cells mimick the physiological state of acute myeloma leukemia (AML) patients relapsing form induction therapy, that harbor drug resistant B cell malignancies. Thus, these cells are of great interest to evaluate the reliability and cytotoxicity of drug resistant scCAR T cells. Preferably, these target cells are CLL1+

15 Luciferase+ Daudi cells.

Insertion of at least one epitope in the extracellular domain of the anti-CLL1-single chain CAR

An anti-CLL1 CAR of the invention may include at least the insertion of at least one epitope in the extracellular domain of said CAR. This is intended to temptatively deplete the immune cells endowed with the CAR in the event of in vivo adverse effects such as a cytokine storm. Moreover,

20 such insertion of epitope or “epitope-tagging” may be useful to sort or purify the engineered immune cells in-vitro during their manufacturing process Said at least one epitope may be any antigenic peptide which is enough immunogenic to be bound by a specific antibody recognizing such peptide. For instance, this can be obtained, for instance, by inserting at least one, and preferably two copies of a CD20 mimotope, preferably of sequence CPYSNPSLCS (SEQ ID NO.113), into the CAR

25 polypeptide sequence. For purpose of simplication hereafter, the order of the scFvs from the N terminal end to the C terminal end is presented as follows: the VH chain and then the VL chain.

However, it can be envisioned in the scope of the present invention that this order is inversed: VL chain and then the VL chain.

Different positions of the at least one CD20 mimotope within the anti-CLL1 CAR of the invention are schematized in Figure 3. Said two copies of a CD20 mimotope can be linked to each other and also to the V_L by a linker. They can also be inserted between the anti-CLL1 scFv and the hinge (such as CD8alpha), by using an optional linker. The CD20 mimotopes can be bound by anti-CD20 antibodies, such as Rituximab (McLaughlin P, et al. 1998).

Accordingly, the anti-CLL1 CAR of the present invention may comprise VH and a VL chains which are able to bind to CLL1 cell surface antigen, optionally humanized, a linker L, a suicide domain, a hinge or part of it, a transmembrane domain, a co-stimulatory domain and a stimulatory domain.

According to another embodiment, the epitope is a mimotope. As a macromolecule, often a peptide, which mimics the structure of an epitope, the mimotope has the advantage to be smaller than conventional epitope, and therefore may be beneficial for a non-conformational sequence and easier to reproduce in a long polypeptide such a CAR. Mimotopes are known for several pharmaceutically-approved mAb such as two 10 amino acid peptides for cetuximab (Riemer et al., 2005), or a 24 aa for palivizumab (Arbiza et al, 1992). As these mimotopes can be identified by phage display, it is possible to try several of them in order to obtain a sequence which does not perturb the scFv for the same mAb. Furthermore, their use can enhance a complement-dependent cytotoxicity (CDC).

Several examples of such epitopes and mimotopes with their corresponding binding mAb are presented in the following Table 10.

Table 10: Mimotopes and epitope with their corresponding mAb

Rituximab		
Mimotope	SEQ ID NO 113	CPYSNPSLC
Palivizumab		
Epitope	SEQ ID NO 191	NSELLSLINDMPITNDQKKLMSNN
Cetuximab		
Mimotope 1	SEQ ID NO 192	CQFDLSTRRLKC
Mimotope 2	SEQ ID NO 193	CQYNLSSRALKC
Mimotope 3	SEQ ID NO 194	CVWQRWQKSYVC
Mimotope 4	SEQ ID NO 195	CMWDRFSRWYKC
Nivolumab		
Epitope A	SEQ ID NO 196	SFVLNWYRMSPSNQTDKLAAPEDR
Epitope B	SEQ ID NO 197	SGTYLCGAISLAPKAQIKE

In a preferred embodiment, the epitope introduced within the chimeric scFv is the CD20
5 mimotope (SEQ ID NO.113) and the infused mAb presenting an affinity to this mimotope -for sorting
and/or depletion purpose(s)- is rituximab.

In one embodiment, said at least one epitope is inserted between the VH and VL chains of
the anti-CLL1.1 CAR, optionally linked to said VH and VL chains by one linker.

In some embodiment, the term "linker" as used in the context of a scFv refers to a peptide
10 linker that consists of amino acids such as glycine and/or serine residues used alone or in
combination, to link variable heavy and variable light chain regions together. In one embodiment, the
flexible polypeptide linker is a Glycine/Serine linker and comprises the amino acid sequence (Gly-Gly-
Gly-Ser)_n or (Gly-Gly-Gly-Gly-Ser)_n, where n is a positive integer equal to or greater than 1. For
example, n=1, n=2, n=3, n=4, n=5, n=6, n=7, n=8, n=9 and n=10. In one embodiment, the flexible
15 polypeptide linkers include, but are not limited to, (Gly₄Ser)₄ or (Gly₄Ser)₃. In another embodiment,
the linkers include multiple repeats of (Gly_xSer)_n, where x=1, 2, 3, 4 or 5 and n is 1, 2, 3, 4, 5, 6, 7, 8, 9
or 10, such as multiple repeat of (GlySer), (Gly₂Ser) or (Gly₅Ser). Also included within the scope of the
invention are linkers described in WO2012/138475, incorporated herein by reference.

In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has
20 one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, wherein one
CD20 mimotope is inserted between the VH and VL chains of the anti-CLL1.1 CAR, optionally linked to
said VH and VL chains by one linker.

In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one CD20 mimotope is
5 inserted between the VH and VL chains of the anti-CLL1.1 CAR, optionally linked to said VH and VL chains by one linker.

In another embodiment, said at least one epitope is inserted at the N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N terminal end of the CAR by one linker.

10 In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted at the N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N
15 terminal end of the CAR by one linker.

In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted at the
20 N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N terminal end of the CAR by one linker.

In another embodiment, said at least one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

25 In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted
 5 between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

In a preferred embodiment, at least two epitopes are inserted in the extracellular domain of the anti-CLL1 CAR of the present invention.

In an embodiment, CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the
 10 polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and two CD20 mimotopes,

said extra-binding domain comprising VH and VL chains directed against CLL1 and a Fc γ RIII α or CD8 α or IgG1 hinge;

15 wherein said 2 epitopes being inserted in tandem between the scFvs and said hinge, and optionally

a linker (SEQ ID NO.10) being interspaced between the 2 epitopes and/or between the VH and the 2 epitopes.

In an embodiment, CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the
 20 polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and two CD20 mimotopes,

said extra-binding domain comprising VH and VL chains directed against CLL1 and a Fc γ RIII α or CD8 α or IgG1 hinge;

25 wherein said 2 epitopes being inserted in tandem upfront the scFvs i.e. at the N terminal end of the CAR-

and optionally, a linker (SAQ ID NO.10) being interspaced between the 2 epitopes and/or at the N terminal end of the CAR.

According to one embodiment, at least two epitopes are inserted in the extracellular domain in such a way that the VH is located between them, all these components being optionally interspaced by at least one linker.

5 In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH is located between them, all these components being optionally interspaced by at least one linker.

10 In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components
15 being optionally interspaced by at least one linker.

According to another embodiment, two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has
20 one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

25 In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain , CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components
30 being optionally interspaced by at least one linker.

According to another embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) comprises an extracellular binding domain wherein at least two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In another embodiment, three epitopes are inserted in the extracellular domain of the anti-CLL1 CAR of the present invention.

According to a particular embodiment, said CLL1 specific CAR of the invention contains an extracellular binding domain wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a more preferred embodiment, said CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure of version V3 as illustrated in Figure 2, said structure comprising at least an extracellular ligand binding-domain anti-CLL1, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In another embodiment, CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and three CD20 epitopes,

said extra-binding domain comprising VH and VL chains directed against CLL1 and a Fc γ RIII α or CD8 α or IgG1 hinge;

wherein said 3 epitopes being inserted in tandem between the scFvs and said hinge, and optionally

a linker (SEQ ID NO.10) being interspaced between the 3 epitopes and/or between the VH and the 3 epitopes.

In another embodiment, CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising at least an anti-CLL1 extra cellular ligand binding-domain, CD8 α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, two CD20 epitopes, and one CD34 epitope;

said extra-binding domain comprising VH and VL chains directed against CLL1 and a Fc γ RIII α or CD8 α or IgG1 hinge;

said 2 epitopes being inserted in tandem between the scFvs and said hinge,

and said CD34 epitope being inserted between the said 2 CD20 epitopes, all components being interspaced between them by a linker (SEQ ID NO.10) and a linker between the epitope and and between the VH and the 3 epitopes.

In all the above embodiments relating to the epitope-containing anti-CLL1 CARs, the VH and VL chains which are used as extracellular binding domain are binding preferably to human membrane CLL1-1.

In a preferred embodiment, said above anti-CLL1 CARs comprising at least an extra cellular
5 ligand binding-domain including VH and VL chains derived from anti-CLL1 monoclonal antibodies.

More specifically, the epitopes can be included into the CAR of the present invention such as follows:

In some embodiments, the extracellular binding domain comprises at least 1, 2, 3, 4, 5, 6, 7,
10 8, 9 or 10 mAb-specific epitopes.

In some embodiments, the extracellular binding domain comprises at least 1, 2 or 3 mAb-specific epitopes.

In some embodiments, when the extracellular binding domain comprises several mAb-specific epitopes, all the mAb-specific epitopes are identical.

15 In some embodiments, when the extracellular binding domain comprises several mAb-specific epitopes, the mAb-specific epitopes are not identical. For example, the extracellular binding domain can comprises three mAb-specific epitopes, two of them being identical and the third one being different.

20 In some embodiments, the extracellular binding domain comprises a VH, a VL, one or more mAb-specific epitopes, preferably 1, 2 or 3, more preferably 2 or 3 mAb-specific epitopes.

In some embodiments, the extracellular binding domain comprises the following sequence (Nterm is located on the left hand side):

$V_1-L_1-V_2-(L)_x$ -Epitope1-(L)_x;

25 $V_1-L_1-V_2-(L)_x$ -Epitope1-(L)_x-Epitope2-(L)_x;

$V_1-L_1-V_2-(L)_x$ -Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂;

Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-V₁-L₁-V₂;

5 (L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-V₁-L₁-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x;

(L)_x-Epitope1-(L)_x-Epitope2-(L)_x-V₁-L₁-V₂-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

10

V₁-(L)_x-Epitope1-(L)_x-V₂;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x;

V₁-(L)_x-Epitope1-(L)_x-V₂-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

15 (L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂;

(L)_x-Epitope1-(L)_x-V₁-(L)_x-Epitope2-(L)_x-V₂-(L)_x-Epitope3-(L)_x;

V₁-L₁-V₂-L-Epitope1;

V₁-L₁-V₂-L-Epitope1-L;

20 V₁-L₁-V₂-L-Epitope1-L-Epitope2;

V₁-L₁-V₂-L-Epitope1-L-Epitope2-L;

V₁-L₁-V₂-L-Epitope1-L-Epitope2-L-Epitope3;

V₁-L₁-V₂-L-Epitope1-L-Epitope2-L-Epitope3-L;

V₁-L₁-V₂-Epitope1;

25 V₁-L₁-V₂-Epitope1-L;

V₁-L₁-V₂-Epitope1-L-Epitope2;

V₁-L₁-V₂-Epitope1-L-Epitope2-L;

V₁-L₁-V₂-Epitope1-L-Epitope2-L-Epitope3;

V₁-L₁-V₂-Epitope1-L-Epitope2-L-Epitope3-L;

5

Epitope1-V₁-L₁-V₂;

Epitope1-L-V₁-L₁-V₂;

L-Epitope1-V₁-L₁-V₂;

L-Epitope1-L-V₁-L₁-V₂;

10

Epitope1-L-Epitope2-V₁-L₁-V₂;

Epitope1-L-Epitope2-L-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-L-V₁-L₁-V₂;

Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂;

15

Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂;

V₁-L-Epitope1-L-V₂;

20

L-Epitope1-L-V₁-L-Epitope2-L-V₂;

V₁-L-Epitope1-L-V₂-L-Epitope2-L;

V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3;

V₁-L-Epitope1-L-V₂-L-Epitope2-Epitope3;

V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3-Epitope4;

25

L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L;

85

Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L;

L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3;

L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L;

5 L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3;

L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-Epitope3, or,

Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3-Epitope 4.

wherein,

10 V₁ and V₂ are V_H and V_L of an ScFv (i.e , V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L);

L₁ is any linker suitable to link the VH chain to the VL chain in an ScFv;

L is a linker, preferably comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

15 x is 0 or 1 and each occurrence of x is independently from the others; and,

epitope 1, epitope 2 and epitope 3 are mAb-specific epitopes and can be identical or different.

In some embodiments, the extracellular binding domain comprises the following sequence (Nterm is located on the left hand side):

20 V_H-L₁-V_L-L-Epitope1-L-Epitope2-L;

L-Epitope1-L-V_H-L-Epitope2-L-V_L-L-Epitope3-L;

V_L-L¹-V_H-L-Epitope1-L-Epitope2-L; or,

L-Epitope1-L-V_L-L-Epitope2-L-V_H-L-Epitope3-L.

wherein L, L₁, epitope 1, epitope 2 and epitope 3 are as defined above.

25

In some embodiments, L_1 is a linker comprising Glycine and/or Serine. In some embodiment, L_1 is a linker comprising the amino acid sequence $(\text{Gly-Gly-Gly-Ser})_n$ or $(\text{Gly-Gly-Gly-Gly-Ser})_n$, where n is 1, 2, 3, 4 or 5. In some embodiments L_1 is $(\text{Gly}_4\text{Ser})_4$ or $(\text{Gly}_4\text{Ser})_3$.

5 In some embodiment, L is a flexible linker, preferably comprising Glycine and/or Serine. In some embodiments, L has an amino acid sequence selected from SGG, GGS, SGGs, SSGGS, GGGG, SGGGG, GGGGS, SGGGGs, GGGGGs, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGs, SGGGGGGG, SGGGGGGGS, or SGGGGSGGGGS preferably SGG, SGGs, SSGGS, GGGG, SGGGS, SGGGGs, SGGGGG, GSGGGGS or SGGGGSGGGGS. In some embodiment, when the extracellular binding
10 domain comprises several occurrences of L , all the L s are identical. In some embodiments, when the extracellular binding domain comprises several occurrences of L , the L s are not all identical. In some embodiments, L is SGGGS. In some embodiments, the extracellular binding domain comprises several occurrences of L and all the L s are SGGGS.

15 In some embodiments, Epitope 1, Epitope 2 and Epitope 3 are identical or different and are selected from mAb-specific epitopes having an amino acid sequence of anyone of SEQ ID NO 33 to SEQ ID NO 42.

In some embodiments, Epitope 1, Epitope 2 and Epitope 3 are identical or different and are selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-
20 CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.

25

In some embodiment, Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 33.

In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 35.

In some embodiment, Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 36.

In some embodiment, Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 37.

5 In some embodiment, Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 38.

10 In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 33 and Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 41 or 42.

In some embodiment, one of Epitope 1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID 41 or 42. In some embodiment, one of Epitope1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID 41 or 42 and the other mAb specific epitopes are CD20 mimotopes, preferably mimotope of SEQ ID NO 33.

15

Method for depleting CAR-expressing immune cells

20 The immune cells expressing the CLL1 sepcific CAR according to the present invention may comprise epitope(s) in their extracellular domain such as described above, so that they can be depleted in a patient in the event of adverse or too acute immune response (e.g. cytokine storm) by administering to said patient an antibody -preferably monoclonal- specific to said epitope (s).

25 By “*in vivo* depletion” is meant in the present invention the administration of a treatment to a mammalian organism aiming to stop the proliferation of CAR-expressing immune cells by inhibition or elimination.

One aspect of the invention is related to a method for *in vivo* depleting an engineered immune cell expressing a CAR comprising an m-Ab specific epitope as previously described, comprising

contacting said engineered immune cell or said CAR-expressing immune cell with at least one epitope-specific mAbs. Another aspect of the invention relates to a method for *in vivo* depleting immune CAR-expressing immune cell which comprises the above chimeric scFv (formed by insertion of a mAb-specific epitope) by contacting said engineered immune cell with epitope-specific
5 antibodies.

Preferably, said immune cells are T-cells and/or the antibodies are monoclonal.

According to one embodiment, the *in vivo* depletion of immune engineered cell is performed on engineered immune cell which has been previously sorted using the *in vitro* method of the present invention. In this case, this will be the same infused mAb used.

10 According to a preferred embodiment, the mAb-specific antigen is CD20 antigen and the epitope-specific mAb is rituximab.

In some embodiments, the invention relates to a method for *in vivo* depleting an engineered immune cell expressing a CAR comprising an mAb-specific epitope (CAR-expressing immune cell) as previously described, in a patient comprising contacting said CAR-expressing immune cell with at
15 least one epitope-specific mAbs.

In some embodiment, said mAb-specific epitope is a CD20 epitope or mimotope, preferably SEQ ID NO 35 and the epitope-specific mAbs is rituximab.

In some embodiments, the step of contacting said engineered immune cell or said CAR-expressing immune cell with at least one epitope-specific mAb comprises infusing the patient with
20 epitope-specific mAb, preferably rituximab.

In some embodiment, when immune cells expressing a CAR comprising an mAb-specific epitope (CAR-expressing immune cells) are depleted in a CDC assay using epitope specific mAb, the amount of viable CAR-expressing immune cells decreases, preferably by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90%. Preferably the CDC assay is the assay disclosed in Example 3, Example 4
25 or Example 7.4. In some embodiment, said mAb-specific epitope is a CD20 epitope or mimotope, preferably SEQ ID NO 35 and the epitope-specific mAbs is rituximab.

Besides the possibility of in-vivo depleting the immune cells according to the invention, the epitopes inserted into the extracellular domain of the CARs may be useful to the steps of sorting or purifying the immune cells expressing said CARs, as part of the method for producing them.

5 Isolated cells

The resulting cells are engineered immune cell expressing at the cell surface membrane a CLL1 specific chimeric antigen receptor as previously described, in particular engineered immune cells derived from primary T-lymphocytes, optionally resistant to an anti-cancer drug, and bearing a deletion in a gene coding for an alpha TCR or a beta TCR.

10 The present invention discloses an engineered immune cell as above, wherein expression of TCR is suppressed.

15 The present invention discloses an engineered immune cell as above, wherein expression of at least one MHC protein, preferably $\beta 2m$ or HLA, is reduced or suppressed in said engineered immune cell. $\beta 2m$ stands for beta 2 microglobulin and HLA for *human leukocyte antigen*. The MHC protein is a MHC protein of Class I or of class II.

The present invention discloses an engineered immune cell as above, wherein said engineered immune cell is mutated to confer resistance to at least one immune suppressive drug, chemotherapy drug, or anti-cancer drug.

The present invention discloses an engineered immune cell as above for use in therapy.

20 The present invention discloses an engineered immune cell for use in therapy as above, wherein the patient is a human.

The present invention discloses an engineered immune cell for use in therapy as above, wherein the condition is a pre-malignant or malignant cancer condition characterized by CLL1-expressing cells.

25 The present invention discloses an engineered immune cell for use in therapy as above, wherein the condition is a condition which is characterized by an overabundance of CLL1-expressing cells.

The present invention discloses an engineered immune cell for use in therapy as above, wherein the malignant cancer condition is a hematological cancer condition.

The present invention discloses an engineered immune cell for use in therapy as above, wherein the hematological cancer condition is leukemia or malignant lymphoproliferative disorders.

5 The present invention discloses an engineered immune cell for use in therapy as above, wherein said leukemia is selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

10 The present invention discloses an engineered immune cell for use in therapy as above, wherein the leukemia is acute myelogenous leukemia (AML).

The present invention discloses an engineered immune cell for use in therapy as above, wherein said hematologic cancer is a malignant lymphoproliferative disorder.

The present invention discloses an engineered immune cell for use in therapy as above, wherein said malignant lymphoproliferative disorder is lymphoma.

15 The present invention discloses an engineered immune cell for use in therapy as above, wherein said lymphoma is selected from the group consisting of multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

20 The present invention discloses a method of impairing a hematologic cancer cell comprising contacting said hematologic cancer cell with an engineered cell, which at least expresses anti-CLL1 CAR such as exposed above, in an amount effective to cause impairment of said cancer cell.

The present invention thus discloses a method of engineering an immune cell comprising:

(a) Providing an immune cell,

(b) Expressing at the surface of said cell at least one CLL1 single-chain specific chimeric antigen receptor such as previously exposed.

25 The present invention discloses a method of engineering an immune cell as above comprising:

(a) Providing an immune cell,

(b) Introducing into said cell at least one polynucleotide encoding said CLL1 single-chain specific chimeric antigen receptor ,

(c) Expressing said polynucleotide into said cell.

5 The present invention discloses a method of engineering an immune cell as above comprising:

(a) Providing an immune cell,

(b) Introducing into said cell at least one polynucleotide encoding said CLL1 single-chain specific chimeric antigen receptor ,

10 (c) Introducing at least one other chimeric antigen receptor which is not specific for CLL1.

The present invention discloses a method of treating a subject in need thereof comprising:

(a) Providing an immune cell expressing at the surface a CLL1 single-chain specific chimeric antigen receptor such as exposed above.

(b) Administrating said immune cells to said patient.

15 The present invention discloses a method of treating a subject in need thereof as above, wherein said immune cell is provided from a donor.

The present invention discloses a method of treating a subject in need thereof as above, wherein said immune cell is provided from the patient himself.

20

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.

25 In another embodiment, said medicament can be used for treating cancer, particularly for the treatment of 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.

5 In a particular embodiment, an anti-CLL1 CAR expressing T cell is provided as a medicament for the treatment of AML, of an AML subtype, of an AML-related complication, of an AML-related condition.

In another embodiment, said medicament can be used for treating a CLL1-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a CLL1-expressing cell.

10 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:

(a) providing an immune-cell obtainable by any one of the methods previously described;

(b) Administrating said transformed immune cells to said patient,

15 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.

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.

20 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 CLL1-expressing cells, especially by an overabundance of CLL1-expressing cells. Such conditions are found in hematologic cancers, such as leukemia.

In one embodiment, the present invention provides a composition for its use in the treatment of a CLL1 expressing cells-mediated disease, in particular a CLL1 expressing cells – mediated hematologic cancer, said composition comprising said anti-CLL1 scCAR expressing T cell of the invention.

30

Any other CLL1-mediating or CLL1-involving malignant lymphoproliferative disorders disclosed herein may be improved with the anti-CLL1 CAR-expressing cells of the present invention.

In a preferred embodiment, the cancer that may be treated using the anti-CLL1 CAR -expressing cells of the present invention is leukemia, a disease associated to leukemia or a
5 complication thereof.

Leukemias that can be treated using the anti-CLL1 CAR -expressing cells of the present invention can be acute myelogenous leukemia (AML). AML or AML subtypes that may be treated using the anti-CLL1 scCAR-expressing cells of the present invention may be in particular, acute myeloblastic leukemia, minimally differentiated acute myeloblastic leukemia, acute myeloblastic
10 leukemia without maturation, acute myeloblastic leukemia with granulocytic maturation, promyelocytic or acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia (M5a) or acute monocytic leukemia (M5b), acute erythroid leukemias, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b), acute megakaryoblastic leukemia, acute basophilic
15 leukemia, acute panmyelosis with myelofibrosis, whether involving CLL1-positive cells.

Subtypes of AML also include, hairy cell leukemia, philadelphia chromosome-positive acute lymphoblastic leukemia. AML may be classified as AML with specific genetic abnormalities. Classification is based on the ability of karyotype to predict response to induction therapy, relapse risk, survival.

20 Accordingly, AML that may be treated using the anti-CLL1 CAR-expressing cells of the present invention may be AML with a translocation between chromosomes 8 and 21, AML with a translocation or inversion in chromosome 16, AML with a translocation between chromosomes 9 and 11, APL (M3) with a translocation between chromosomes 15 and 17, AML with a translocation between chromosomes 6 and 9, AML with a translocation or inversion in chromosome 3, AML
25 (megakaryoblastic) with a translocation between chromosomes 1 and 22 .

The present invention is particularly useful for the treatment of AML associated with these particular cytogenetic markers.

The present invention also provides an anti-CLL1 CAR expressing T cell for the treatment of patients with specific cytogenetic subsets of AML, such as patients with t(15;17)(q22;q21) identified

using all-*trans* retinoic acid (ATRA)16-19 and for the treatment of patients with t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) identified using repetitive doses of high-dose cytarabine.

Preferably, the present invention provides an anti-CLL1 CAR expressing T cell for the treatment of patients with aberrations, such as -5/del(5q), -7, abnormalities of 3q, or a complex
5 karyotype, who have been shown to have inferior complete remission rates and survival.

The terms “therapeutic agent”, “chemotherapeutic agent”, or “drug” or “anti-cancer drug” as used herein refers to a medicament, preferably a compound or a derivative thereof that can interact with a cancer cell, thereby reducing the proliferative status of the cell and/or killing the cell. Examples of chemotherapeutic agents or “anti-cancer drug” include, but are not limited to, alkylating
10 agents (e.g., busulfan, carboplatine, chlorambucil, cisplatin, cyclophosphamide, ifosfamide, melphalan, mechlorethamine, oxaliplatin, uramustine, temozolomide, fotemustine), metabolic antagonists (e.g., purine nucleoside antimetabolite such as clofarabine, fludarabine or 2'-deoxyadenosine, methotrexate (MTX), 5-fluorouracil or derivatives thereof, azathioprine, capecitabine, cytarabine, floxuridine, fluorouracil, gemcitabine, methotrexate, pemetrexed),
15 antitumor antibiotics (e.g., mitomycin, adriamycin, bleomycin, daunorubicin, doxorubicin, epirubicin, hydroxyurea, idarubicin, mitomycin C, mitoxantrone), plant-derived antitumor agents (e.g., vincristine, vindesine, taxol, vinblastine, vinorelbine, docetaxel, paclitaxel), topoisomerase inhibitor (irinotecan, topotecan, etoposide).

In a preferred embodiment, a therapeutic agent, a chemotherapy drug as used herein refers
20 to a compound or a derivative thereof that may be used to treat cancer, in particular to treat a hematopoietic cancer cell and more particularly AML, thereby reducing the proliferative status of the cancer cell and/or killing the cancer cell. Examples of chemotherapeutic agents include, but are not limited to aracytine, Cytosine arabinoside, amsacrine, daunorubicin, idarubicin, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxide, transretinoic acid, mechlorethamine,
25 procarbazine, chlorambucil, and combination thereof.

In other embodiments of the present invention, cells of the invention are administered to a patient in conjunction with a drug (or an agent) selected from aracytine, cytosine arabinoside, amsacrine, daunorubicin, idarubicin, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxide, transretinoic acid, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea,
30 prednisone, and combination thereof.

Such agents may further include, but are not limited to, the anti-cancer agents TRIMETHOTRIMATE™ (TMTX), TEMOZOLOMIDE™, RALTRITREXED™, S-(4-Nitrobenzyl)-6-thioinosine (NBMPR), 6-benzylguanidine (6-BG), bis-chloronitrosourea (BCNU) and CAMPTOTHECIN™, or a therapeutic derivative of any thereof.

5 In a more preferred embodiment an anti-CLL1 scCAR expressing T cell, is administered to a patient, in combination with at least one therapeutic agent selected from aracytine, Cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxide, transretinoic acid and combination thereof.

10 As used herein, a cell which is "resistant or tolerant" to an agent means a cell which has been genetically modified so that the cell proliferates in the presence of an amount of an agent that inhibits or prevents proliferation of a cell without the modification.

Group of patients

In a preferred embodiment, the invention provides a treatment for AML in patients over 60 years or in patients of less than 20 years.

15 In a more preferred embodiment, the present invention provides a pediatric treatment, in particular a pediatric treatment against AML, or AML-related diseases or complications.

In still another preferred embodiment, the present invention is used as a treatment in AML patients with low, poor or unfavorable status that is to say with a predicted survival of less than 5 years survival rate. In this group, patients suffering AML with the following cytogenetic characteristics : -5; 5q; -7; 7q; 11q23; non t(9;11); inv(3); t(3;3); t(6;9); t(9;22) is associated with poor-risk status (Byrd J.C. et al., December 15, 2002; Blood: 100 (13) and is especially contemplated to be treated according to the present invention or with an object of the present invention.

20

In one embodiment, the anti-CLL1 CAR expressing T cell of present invention may be used as induction therapy, as post remission therapy of AML or as a consolidation therapy in patient with AML.

25

In one embodiment, the anti-CLL1 CAR expressing T cell of the present invention may be used in case of AML relapse, or in case of refractory or resistant AML, and more preferably, in combination with at least one other anti-cancer drug

In another preferred embodiment, at least one anti-CLL1 CAR expressing cell of the invention is used for preventing cancer cells development occurring in particular after anti-cancer treatment, during bone marrow depletion or before bone marrow transplantation, after bone marrow destruction.

5

AML complications

In one particular embodiment the invention provides a medicament that improves the health condition of a patient, in particular a patient undergoing a complication related to AML. More preferably, said engineered anti-CLL1 CAR expressing T cell of the invention is expressing at least one anti-CLL1 CAR of the invention and is used as a medicament for the treatment of a complication related to AML.

A complication or disease related to AML may include a preceding myelodysplasia phase, secondary leukemia, in particular secondary AML, high white blood cell count, and absence of Auer rods. Among others, leukostasis and involvement of the central nervous system (CNS), hyperleukocytosis, residual disease, are also considered as a complication or disease related to AML.

AML associated diseases

In one embodiment, the present invention also provides an anti-CLL1 CAR expressing T cell for the treatment of a pathological condition related to AML.

The present invention provides a therapy for AML related myeloid neoplasms, for acute myeloid leukemia and myelodysplastic syndrome, a treatment of relapsed or refractory acute myeloid leukemia, a treatment of relapsed or refractory acute promyelocytic leukemia in adults, a treatment for acute promyeloid leukaemia, a treatment of acute myeloid leukemia in adults over 60 years .

According to another aspect, the present invention provides a composition for the treatment of AML associated diseases, in particular hematologic malignancy related to AML.

Hematologic malignancy related to AML conditions include myelodysplasia syndromes (MDS, formerly known as "preleukemia") which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

Other pathological conditions or genetic syndromes associated with the risk of AML can be improved with the adequate use of the present invention, said genetic syndromes include Down syndrome, trisomy, Fanconi anemia, Bloom syndrome, Ataxia-telangiectasia, Diamond-Blackfan anemia, Schwachman-Diamond syndrome, Li-Fraumeni syndrome, Neurofibromatosis type 1, Severe
5 congenital neutropenia (also called Kostmann syndrome).

Other CLL1-mediated pathological conditions

According to another aspect, the present invention provides a composition for the treatment of CLL1+cell-mediated diseases. These CLL1+cell mediated diseases include inflammation, such as
10 rheumatoid arthritis.

In particular, the present invention can be used for the treatment of CLL1+cell mediated diseases such as inflammation and more particularly rheumatoid arthritis.

Compositions

15 The present invention also provides a composition comprising an engineered T cells according to the invention for its use or a method for treating a disease.

In one aspect, the disease is a hematologic cancer, in particular a stem cell cancer including but is not limited to leukemia (such as acute myelogenous leukemia (AML) or a complication thereof.

The present invention also provides a composition for its use or a method for inhibiting the
20 proliferation or reducing a CLL1-expressing cell population or activity in a patient. An exemplary method includes contacting a population of cells comprising a CLL1-expressing cell with an anti-CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell.

In a more specific aspect, the present invention provides a composition for its use or a method for inhibiting the proliferation or reducing the population of cancer cells expressing CLL1 in a
25 patient, the methods comprising contacting the CLL1-expressing cancer cell population with an anti-CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell, binding of an anti-CLL1 CAR cell, and in particular scCART, of the invention to the CLL1-expressing cancer cell resulting in the destruction of the CLL1-expressing cancer cells

In certain aspects, the anti-CLL1 CART cell, and in particular scCART, of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% (to undetectable level) in a subject with or animal model for myeloid leukemia or another cancer
5 associated with CLL1-expressing cells, relative to a negative control.

The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disorder or condition associated with CLL1-expressing cells (e.g., associated with a hematologic cancer), the methods comprising administering to a subject in need an anti-CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell.
10 In one aspect, the subject is a human. Non-limiting examples of disorders associated with CLL1-expressing cells include inflammatory disorders (such as rheumatoid arthritis) and cancers (such as hematological cancers, in particular AML or AML complications).

The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disease associated with CLL1-expressing cells, the method comprising
15 administering to a subject in need an anti-CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell. In one aspect, the subject is a human. Non-limiting examples of diseases associated with CLL1-expressing cells include in particular Acute Myeloid Leukemia (AML).

The present invention provides a composition for its use or a method for treating or preventing relapse of cancer associated with CLL1-expressing cells, the method comprising
20 administering to a subject in need thereof an anti-CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell. In another aspect, the methods comprise administering to the subject in need thereof an effective amount of an anti CLL1 CART cell, and in particular scCART, of the invention that binds to the CLL1-expressing cell in combination with an effective amount of another therapy.

25 In one aspect, CLL1 is considered to be a "cancer stem cell" marker in AML. Therefore, an anti-CLL1 CART cell, and in particular scCART, of the invention can prevent relapse of AML, or even treat AML that is mostly CLL1-negative but with a "stem" population of CLL1+ cells (a CLL1-expressing cells).

In one aspect, the invention provides compositions and methods for treating subjects that have undergone treatment for a disease or disorder associated with elevated expression levels of CD 19, and exhibits a disease or disorder associated with elevated levels of CLL1.

5 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.

10 Preferably, the treatment with the engineered immune cells according to the invention may be administered in combination (e.g., before, simultaneously or following) with one or more therapies against cancer selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), *arsenic trioxyde*, *transretinoic acid*, *combination of arsenic trioxyde*, *transretinoic acid*, mechlorethamine, procarbazine, chlorambucil, and combination thereof.

15 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.

20 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, 25 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 30 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 efaliztimab 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, mycoplienolic 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.

In certain embodiments of the present invention, anti-CLL1 scCAR expressing cells are administered to a patient in conjunction (e.g., before, simultaneously or following) with a drug selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), *arsenic trioxide*, *transretinoic acid*, *combination of arsenic trioxide*, *transretinoic acid*, mechlorethamine, procarbazine, chlorambucil, and combination thereof. In these embodiments anti-CLL1 scCAR expressing cells may be resistant to the particular drug or combination of drugs that is (are) administered in conjunction with anti-CLL1 scCAR expressing cells.

In other embodiments of the present invention, anti-CLL1 scCAR expressing cells are administered to a patient in conjunction with a drug selected from cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

Other definitions

- Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.- 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 “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, 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.

- 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 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.

5 - "similarity" describes the relationship between the amino acid sequences of two or more polypeptides. BLASTP may also be used to identify an amino acid sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to a reference amino acid sequence using a similarity matrix such as BLOSUM45, BLOSUM62 or BLOSUM80. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent
10 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
15 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. For example, a functional variant of pTalpha can have 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to the amino acid sequence of SEQ ID NO: 107 such as disclosed in
20 WO2013176916. A polynucleotide encoding such a functional variant would be produced by reverse translating its amino acid sequence using the genetic code.

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

25 The term "relapsed" refers to a situation where a subject or a mammal, who has had a remission of cancer after therapy has a return of cancer cells.

The term "refractory or resistant" refers to a circumstance where a subject or a mammal, even after intensive treatment, has residual cancer cells in his body.

30 The term "drug resistance" refers to the condition when a disease does not respond to the treatment of a drug or drugs. Drug resistance can be either intrinsic (or primary resistance), which means the disease has never been responsive to the drug or drugs, or it can be acquired, which

means the disease ceases responding to a drug or drugs that the disease had previously responded to (secondary resistance). In certain embodiments, drug resistance is intrinsic. In certain embodiments, the drug resistance is acquired.

5 The term "hematologic malignancy" or "hematologic cancer" refers to a cancer of the body's blood- bone marrow and/or lymphatic tissue. Examples of hematological malignancies include, in particular, acute myeloid leukemia (AML), AML with trilineage myelodysplasia (AML/TMDS), mixed lineage leukemia (MLL), and other AM- related pathologies.

The term "leukemia" refers to malignant neoplasms of the blood-forming tissues, including, in particular to acute myeloid leukemia or acute myelogenous leukemia (AML).

10 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.

15 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
20 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.

25 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

GENERAL METHODS

Primary T-cell cultures

T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium. The PBMC layer was recovered and T cells were purified using a commercially available T-cell enrichment kit. Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2, 5% Human, and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies).

scCAR mRNA transfection

Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15µg of mRNA encoding the different scCAR constructs. scCAR mRNAs were produced using T7 mRNA polymerase transfections done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4cm gap cuvettes in a final volume of 200µl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media and incubated at 37°C with 5% CO₂. IL-2 was added 2h after electroporation at 20ng/mL.

Degranulation assay (CD107a mobilization)

T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing various levels of the CLL1 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 6 hours at 37°C with 5% CO₂. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody at the beginning of the co-culture, together with 1µg/ml of anti-CD49d, 1µg/ml of anti-CD28, and 1x Monensin solution. After the 6h incubation period, cells were stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24h after mRNA transfection.

IFN gamma release assay

T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing various levels of the CLL1 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 24 hours at 37°C with 5% CO₂. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFN

gamma detection in the cell culture supernatants was done by ELISA assay. The IFN gamma release assays were carried by starting the cell co-cultures 24h after mRNA transfection.

Cytotoxicity assay

5 T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing CLL1) and 10,000 control (CLL1neg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet) before co-culturing them with scCAR+ T-cells. The co-cultures were incubated for 4 hours at 37°C with 5% CO₂. After this incubation period, cells were labelled with a fixable viability dye and analyzed by flow cytometry. Viability of
10 each cellular population (target cells or CLL1neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48h after mRNA transfection.

T-cell transduction

Transduction of T-cells with recombinant lentiviral vectors expression the scCAR was carried
15 out three days after T-cell purification/activation. scCAR detection at the surface of T-cells was done using a recombinant protein consisting on the fusion of the extracellular domain of the human CLL1 protein, together with a murine IgG1 Fc fragment. Binding of this protein to the scCAR molecule was detected with a fluorochrome-conjugated secondary antibody targeting the mouse Fc portion of the protein, and analyzed by flow cytometry.

20

Anti-tumor mouse model

Immunodeficient NOG mice were intravenously (iv) injected with (CLL1 expressing_MOLM13-Luciferase cells as an AML xenograft mouse model. Optionally, mice received an anti-cancer treatment. Mice were then iv injected (either 2 or 7 days after injection of the tumor cell line) with
25 different doses of scCAR+ T-cells to be tested, or with T-cells that were not transduced with the scCAR lentiviral vector. Bioluminescent signals were determined at the day of T-cell injection (D0), at D7, 14, 21, 28 and 40 after T-cell injection in order to follow tumoral progression on the different animals.

Example 1: Proliferation of TCRalpha inactivated cells expressing a CLL1-scCAR

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	Repeat sequence	Half TALE-nuclease
TRAC_T01	TTGTCCCACAGATATCC Agaaccctgaccctg CCGTGTACCAGCTGAGA (SEQ ID NO: 114)	Repeat TRAC_T01-L (SEQ ID NO: 115)	TRAC_T01-L TALEN (SEQ ID NO: 117)
		Repeat TRAC_T01-R (SEQ ID NO: 116)	TRAC_T01-R TALEN (SEQ ID NO: 118)

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.

Purified T cells preactivated during 72 hours with antiCD3/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 anti-CLL1 scCAR previously described (SEQ ID NO: 35 to 112). 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).

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 CLL1 scCARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

To investigate whether the human T cells expressing the CLL1-scCAR display activated state, the expression of the activation marker CD25 are analyzed by FACS 7 days post transduction. The purified cells transduced with the lentiviral vector encoding CLL1 scCAR 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.

Example 2: Construction of CLL1 scCAR using various anti-CLL1 antibody fragments*Primary T-cell cultures*

5 T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium (Ficoll Paque PLUS / GE Healthcare Life Sciences). The PBMC layer was recovered and T cells were purified using a commercially available T-cell enrichment kit (Stem Cell Technologies). Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2 (Miltenyi Biotec), 5% Human Serum (Sera
10 Laboratories), and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies). After activation cells were grown and maintained in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2 (Miltenyi Biotec) and 5% Human Serum (Sera Laboratories)

scCAR mRNA transfection

15 Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15µg of mRNA encoding the different scCAR constructs. scCAR mRNAs were produced using the mMESSAGE mMACHINE T7 Kit (Life Technologies) and purified using RNeasy Mini Spin Columns (Qiagen). Transfections were done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4cm gap
20 cuvettes in a final volume of 200µl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media (Lonza) and incubated at 37°C with 5% CO₂. IL-2 (from Miltenyi Biotec was added 2h after electroporation at 20ng/mL.

Degranulation assay (CD107a mobilization)

25 T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing or not the CLL1 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 6 hours at 37°C with 5% CO₂. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody (APC conjugated, from Miltenyi Biotec) at the beginning of the co-culture, together with 1µg/ml of anti-CD49d (BD Pharmingen),
30 1µg/ml of anti-CD28 (Miltenyi Biotec), and 1x Monensin solution (eBioscience). After the 6h incubation period, cells were stained with a fixable viability dye (eFluor 780, from eBioscience) and fluorochrome-conjugated anti-CD8 (PE conjugated Miltenyi Biotec) and analyzed by flow cytometry.

The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24h after mRNA transfection.

5 *IFNgamma release assay*

T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing or not the CLL1 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 24 hours at 37°C with 5% CO₂. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFN
10 gamma detection in the cell culture supernatants was done by ELISA assay (Human IFN-gamma Quantikine ELISA Kit, from R&D Systems). The IFN gamma release assays were carried by starting the cell co-cultures 24h after mRNA transfection.

Cytotoxicity assay

15 T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing CLL1) and 10,000 control (CLL1neg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet, from Life Technologies) before co-culturing them with scCAR+ T-cells. The co-cultures were incubated for 4 hours at 37°C with 5% CO₂. After this incubation period, cells were labelled with a fixable viability dye (eFluor 780, from
20 eBioscience) and analyzed by flow cytometry. Viability of each cellular population (target cells or CLL1neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48h after mRNA transfection.

Exemplary anti-CLL1 single chain Chimeric Antigen Receptors

25

sc SC02-357-CAR-v1 (SEQ ID NO.1 + SEQ ID NO.35)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWWSVVRQPPGKGLEWIGEIYHS
GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFLTIS
30 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc SC02-357-CAR-v2 (SEQ ID NO.1 + SEQ ID NO.36)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSSGGGGSGGGGSGG
 5 GGS¹DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLL
 YIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
 DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR

10 **sc SC02-357-CAR-v3** (SEQ ID NO.1 + SEQ ID NO.37)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS¹DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 15 WAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQ
 GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR

sc SC02-357-CAR-v4 (SEQ ID NO.1 + SEQ ID NO. 38)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS¹DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIIS
 FFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ
 25 QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGLYQGLSTATKDTYDALHMQALPPR

sc SC02-357-CAR-v5 (SEQ ID NO.1 + SEQ ID NO.39)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 30 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS¹DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKTHTCPAPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT
QEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

5

sc SC02-357-CAR-v6 (SEQ ID NO.1 + SEQ ID NO.40)

MALPVTALLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSSISSNWWSWVRQPPGKGLEWIGEYHS
GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
10 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQT
TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
15 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc SC02-378 CAR-v1 (SEQ ID NO.1 + SEQ ID NO. 41)

MALPVTALLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSSISSNWWSWVRQPPGKGLEWIGEYHS
GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
20 GGSDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

25 **sc SC02-378 CAR-v2** (SEQ ID NO.1 + SEQ ID NO.42)

MALPVTALLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSSISSNWWSWVRQPPGKGLEWIGEYHS
GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVITICRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
30 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLL
YIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc SC02-378 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.43)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
 GGS⁵DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQ
 GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG
 LYQGLSTATKDTYDALHMQALPPR

sc SC02-378 CAR-v4 (SEQ ID NO.1 + SEQ ID NO. 44)

10 MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
 GGS¹⁵DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIIS
 FFLALTSTALLFLLFLLTLRFSSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ
 15 QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
 GLYQGLSTATKDTYDALHMQALPPR

sc SC02-378 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.45)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 20 GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
 GGS²⁵DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
 25 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT
 QEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc SC02-378 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.46)

30 MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARSSSGGFFDYWGQGTLLTVSSGGGGSGGGGSGG
 GGS³⁵DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQT
TQEEDGCSCRFPEEEEEGGCEL RVKF SR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

5

sc SC02-161 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.47)

MALPVTALLPLALLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
10 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKF SR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP
EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

15 **sc SC02-161 CAR-v2** (SEQ ID NO.1 + SEQ ID NO. 48)

MALPVTALLPLALLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLL
20 YIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKF SR SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
DP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc SC02-161 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.49)

MALPVTALLPLALLHAARPQVQLQESGPGLVKPSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
25 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
GGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
WAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKF SR SADAPAYQQ
GQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDG
30 LYQGLSTATKDTYDALHMQALPPR

sc SC02-161 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.50)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIIS
 5 FFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQ
 QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHD
 GLYQGLSTATKDTYDALHMQALPPR

sc SC02-161 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.51)

10 MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 15 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT
 QEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

20 **sc SC02-161 CAR-v6** (SEQ ID NO.1 + SEQ ID NO.52)

MALPVTALLLPLALLLHAARPQVQLQESGPGLVKPSSETLSLTCVVSGGSISSSNWWSWVRQPPGKGLEWIGEYHS
 GSPNYPNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYCARQTTAGSFDYWGQGTLVTVSSGGGGSGGGGSGG
 GGS
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTIS
 SLQPEDFATYYCQQSYSTPPTFGQGTKVEIKEPKSPDKHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 25 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQT
 TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

30

sc M26 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.53)

MALPVTALLLPLALLLHAARP
 EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSSGGGGSGGGGS
 GGGGS
 DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASTLDGVPKRFSGNRSGLDYS

TISSLESEDFADYYCLQYAIYPYTFGGGTKLEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLLSLVITLYCKRGRKKL
 LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
 DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

5 **sc M26 CAR-v2** (SEQ ID NO.1 + SEQ ID NO.54)

MALPVTALLLPLALLLHAARP EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSSGGGGSGGGGS
 GGGGS DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASLTDSGVPKRFSGNRS GSDYSL
 TISSLESEDFADYYCLQYAIYPYTFGGGTKLEIKRGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTRFSVVKRGRKK
 10 LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M26 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.55)

MALPVTALLLPLALLLHAARP EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 15 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSSGGGGSGGGGS
 GGGGS DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASLTDSGVPKRFSGNRS GSDYSL
 TISSLESEDFADYYCLQYAIYPYTFGGGTKLEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
 IYIWAPLAGTCGVLLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAY
 QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGH
 20 DGLYQGLSTATKDTYDALHMQALPPR

sc M26 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.56)

MALPVTALLLPLALLLHAARP EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSSGGGGSGGGGS
 25 GGGGS DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASLTDSGVPKRFSGNRS GSDYSL
 TISSLESEDFADYYCLQYAIYPYTFGGGTKLEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
 IISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPA
 YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGG
 HDGLYQGLSTATKDTYDALHMQALPPR

30

sc M26 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.57)

MALPVTALLLPLALLLHAARP EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSSGGGGSGGGGS

GGGGS[DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASLTDSGVPKRFSGNRS GSDYSL
 TISSESEDFADYYCLQYAIYPYTFGGG TKLEIKR]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTC
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
 KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDK
 5 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
 TTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
 PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M26 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.58)

10 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYFIHWVKQKPGQGLEWIGFINPYN
 DGSKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCTRDDGYGYAMDYWGQGTSTVTVSS]GGGGSGGGGS
 GGGGS[DIQMTQSPSSLSASLGERVSLTCRATQELSGYLSWLQQKPDGTIKRLIYAASLTDSGVPKRFSGNRS GSDYSL
 TISSESEDFADYYCLQYAIYPYTFGGG TKLEIKR]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTC
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
 15 KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPV
 QTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

20 **sc M31 CAR-v1** (SEQ ID NO.1 + SEQ ID NO.59)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
 NDGTKYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLKVSS]GGGGSGGGGS
 GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR
 TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGG TKLEIK]GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYC
 25 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
 LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQ
 ALPPR

sc M31 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.60)

30 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
 NDGTKYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLKVSS]GGGGSGGGGS
 GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR
 TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGG TKLEIK]GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSV
 VKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYD

VLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M31 CAR-v3 (SEQ ID NO.1 + SEQ ID NO. 61)

5 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
[NDGTYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLVSS]GGGGSGGGGS
GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR]
[TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR
10 SADAPAYQQGQNQLYNELNLGRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGE
RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M31 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.62)

15 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
[NDGTYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLVSS]GGGGSGGGGS
GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR]
[TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
20 ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M31 CAR-v5 (SEQ ID NO.1 + SEQ ID NO. 63)

25 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
[NDGTYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGQGTTLVSS]GGGGSGGGGS
GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR]
[TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIA
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLY
SKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP
FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLKRRGRDPEMG
30 GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M31 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.64)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
 NDGTKYNEKFKGKATLTSDTSSSTAYMELNSLTSEDSAVYFCARPIYFDNDYFDYWGGQGTTLKVSSGGGGSGGGGS
 GGGGS[TIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYLASNLESGVPARFSGSGSR
 TDFTLTIDPVEADDAATYYCQQNNYDPWTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIA
 5 RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLY
 SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQ
 PFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
 GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

10

sc G4 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.65)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDAVYYCARTDDYDDYTMDYWGGQGTSTVTVSSGGGGSGGGG
 SGGGGS[EIQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 15 LTISNLEQEDIATYFCQQGKTLLWTFGGGKLEIK]GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
 LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc G4 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.66)

20 MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDAVYYCARTDDYDDYTMDYWGGQGTSTVTVSSGGGGSGGGG
 SGGGGS[EIQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 LTISNLEQEDIATYFCQQGKTLLWTFGGGKLEIK]GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVKRGRK
 KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
 25 GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc G4 CAR-v3 (SEQ ID NO.1 + SEQ ID NO. 67)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDAVYYCARTDDYDDYTMDYWGGQGTSTVTVSSGGGGSGGGG
 30 SGGGGS[EIQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 LTISNLEQEDIATYFCQQGKTLLWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 CDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAP
 AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
 GHDGLYQGLSTATKDTYDALHMQALPPR

sc G4 CAR-v4 (SEQ ID NO.1 + SEQ ID NO. 68)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDPVYYCARTDDYDDYTMDYWGQGTSVTVSS]GGGGSGGGG
 5 SGGGGS[EQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 LTISNLEQEDIATYFCQQGKTLLWTFGGGGTKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 CDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA
 PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
 KGHGGLYQGLSTATKDTYDALHMQALPPR

10

sc G4 CAR-v5 (SEQ ID NO.1 + SEQ ID NO. 69)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDPVYYCARTDDYDDYTMDYWGQGTSVTVSS]GGGGSGGGG
 SGGGGS[EQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 15 LTISNLEQEDIATYFCQQGKTLLWTFGGGGTKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPV
 QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
 20 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc G4 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.70)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHEKNLEWIGPINPYN
 DGTIYNPNFKGKATLTVDKASSTAYMELLSLTSDDPVYYCARTDDYDDYTMDYWGQGTSVTVSS]GGGGSGGGG
 25 SGGGGS[EQMTQTPSSLSASLGDRVTISCRASHDISNYLNWYQQKPDGTLKLLIYYTSRLHSGVPSRFSGSGSGTDYS
 LTISNLEQEDIATYFCQQGKTLLWTFGGGGTKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRP
 30 VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M22 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.71)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGNIDP
 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 GSGGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIYWASTRESGVPD

 RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLS
 5 LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGR
 REEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYD
 ALHMQALPPR

sc M22 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.72)

10 MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGNIDP
 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 GSGGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIYWASTRESGVPD

 RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELKGLAVSTISSFFPPGYQIISFFLALTSTALLFLFF
 LTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLG
 15 RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTY
 DALHMQALPPR

sc M22 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.73)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGNIDP
 20 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 GSGGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIYWASTRESGVPD

 RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
 GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
 25 GMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M22 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.74)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGNIDP
 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 30 GSGGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIYWASTRESGVPD

 RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG
 GAVHTRGLDFACDIISFFLALTSTALLFLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGC
 ELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYS
 EIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M22 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.75)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPQGQGLEWIGNIDP
 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 5 GGSGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIWASTRESGVDP

RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELK

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPK
 DTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
 VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
 DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLLSLVITLYCKRGRKKL
 10 LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR
 DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M22 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.76)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGASVKLSCKASGYTFTRYWMHWVKQRPQGQGLEWIGNIDP
 15 SDTETHYNQQFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAIYYGNPSYYAMDYWGQGTSVTVSSGGGGSGG
 GGSGGGGS

DIVMTQSPSSLTVTAGEKVTMSCKSSQNLLNSGNQKKYLNWYQQKPGQPPKLLIWASTRESGVDP

RFTGSGSGTDFTLTISVQAEDLAVYFCQNDYSYPFTFGAGTKLELK

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPK
 DTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
 VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
 20 DGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKK
 LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
 RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M29 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.77)

MALPVTALLLPLALLLHAARP

EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGQGLEWIGYINPY

 NDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGQGTTTLTVSSGGGGSGGGGSG
 GGS

DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDISFSI

SNLEPEDIATYYCLQYDYLWTFGGGKLEIK

GLAVSTISSFFPPGYQIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF
 KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE
 30 MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M29 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.78)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEWIGYINPY]
 [NDGTTYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGGQGTTLTVSS]GGGGSGGGGSG
 GGG[DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDYSFSI]
 [SNLEPEDIATYYCLQYDYLWTFGGGKLEIK][GLAVSTISSFFPPGYQIISSFLALTSTALLFLLFFLTLRFVVKRGRKKLLY]
 5 IFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
 PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHGGLYQGLSTATKDTYDALHMQALPPR

sc M29 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.79)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEWIGYINPY]
 10 [NDGTTYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGGQGTTLTVSS]GGGGSGGGGSG
 GGG[DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDYSFSI]
 [SNLEPEDIATYYCLQYDYLWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYI
 WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQ
 GQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHG
 15 LYQGLSTATKDTYDALHMQALPPR

sc M29 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.80)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEWIGYINPY]
 [NDGTTYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGGQGTTLTVSS]GGGGSGGGGSG
 20 GGG[DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDYSFSI]
 [SNLEPEDIATYYCLQYDYLWTFGGGKLEIK]TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIIS
 FFLALTSTALLFLLFFLTLRFVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQ
 QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHG
 GLYQGLSTATKDTYDALHMQALPPR

25

sc M29 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.81)

MALPVTALLLPLALLLHAARP[EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEWIGYINPY]
 [NDGTTYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGGQGTTLTVSS]GGGGSGGGGSG
 GGG[DIQMTQSPSSLSASLGGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDYSFSI]
 30 [SNLEPEDIATYYCLQYDYLWTFGGGKLEIK]EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
 VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTT

QEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M29 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.82)

5 MALPVTALLLPLALLLHAARP EVQLQQSGPELVKPGASVKMSCKASGYIFTSYVMYWVKQKPGQGLEWIGYINPY
NDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARYYDYDYFDYWGGQGTTLTVSSGGGGSGGGGSG
GGGS DIQMTQSPSSLSASLGKVTITCKASQDINKYIAWYQHKGPGKGPRLLIHYTSTLQPGIPSRFSGSGSGRDISFSI
SNLEPEDIATYYCLQYDYLWTFGGGKLEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
10 KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFLLTLRFSVVKRGRKKLLYIFKQPFMRPVQT
TQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP
QEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

15 **sc M2 CAR-v1** (SEQ ID NO.1 + SEQ ID NO.83)

MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSTVTVSSGGGGSGGG
GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
SLTISSESEDFADYYCLQYASYPYTFGGGKLEIKR GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLSLVITLYCKRGRK
20 KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M2 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.84)

MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
25 NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSTVTVSSGGGGSGGG
GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
SLTISSESEDFADYYCLQYASYPYTFGGGKLEIKR GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFLLTLRFSVVKRGR
KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKR
RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPP
30 R

sc M2 CAR-v3 (SEQ ID NO.1 + SEQ ID NO. 85)

MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
 NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSVTVSSGGGGSGGG
 GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
 SLTISSLESEDFADYYCLQYASYPYTFGGGKLEIKR TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 5 CDIIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAP
 AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
 GHDGLYQGLSTATKDTYDALHMQALPPR

sc M2 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.86)

10 MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
 NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSVTVSSGGGGSGGG
 GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
 SLTISSLESEDFADYYCLQYASYPYTFGGGKLEIKR TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFA
 CDIIFFLALTSTALLFLLFFLT LRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA
 15 PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
 KGHGGLYQGLSTATKDTYDALHMQALPPR

sc M2 CAR-v5 (SEQ ID NO.1 + SEQ ID NO. 87)

MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
 20 NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSVTVSSGGGGSGGG
 GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
 SLTISSLESEDFADYYCLQYASYPYTFGGGKLEIKR EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEV
 TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
 TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTV
 25 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRP
 VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHGGLYQGLSTATKDTYDALHMQALPPR

sc M2 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.88)

30 MALPVTALLLPLALLLHAARP EVQLRQSGPELVKPGASVKMSCKASGYTFTSYFMHWVKQKPGQGLEWIGFINPY
 NDGTKYNEKFKGKATLTSDKSSSTAYMELNSLTSEDSAVYYCTRDDGYDYAMDYWGQGTSVTVSSGGGGSGGG
 GSGGGGS DIQMTQSPSSLSASLGERVSLTCRASQEISVYLSWLQKQKPDGTIKRLIYAASLTDSGVPERFSGSRSGSDY
 SLTISSLESEDFADYYCLQYASYPYTFGGGKLEIKR EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAARTPEV
 TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK

TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQPFMR
PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPR
RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

5

sc M5 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.89)

MALPVTALLPLALLHAARP[EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK]
GDTAYASKFQDKATITSDTSSNTAYLQLSSLTSEDTAIVYYCTLTGRFDYWGGTTTLTVSSGGGGSGGGGSGGGGSD
IVMSQSPSSLAVSVGEKVTMSCKSSQSLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
10 TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG
RDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M5 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.90)

15 MALPVTALLPLALLHAARP[EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK]
GDTAYASKFQDKATITSDTSSNTAYLQLSSLTSEDTAIVYYCTLTGRFDYWGGTTTLTVSSGGGGSGGGGSGGGGSD
IVMSQSPSSLAVSVGEKVTMSCKSSQSLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTRFSVVKRGRK
KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
20 GRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc M5 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.91)

MALPVTALLPLALLHAARP[EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK]
GDTAYASKFQDKATITSDTSSNTAYLQLSSLTSEDTAIVYYCTLTGRFDYWGGTTTLTVSSGGGGSGGGGSGGGGSD
25 IVMSQSPSSLAVSVGEKVTMSCKSSQSLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIKTTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
HDGLYQGLSTATKDTYDALHMQALPPR

30

sc M5 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.92)

MALPVTALLPLALLHAARP[EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK]
GDTAYASKFQDKATITSDTSSNTAYLQLSSLTSEDTAIVYYCTLTGRFDYWGGTTTLTVSSGGGGSGGGGSGGGGSD

IVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
 TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
 DIISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAP
 AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK
 5 GHDGLYQGLSTATKDTYDALHMQALPPR

sc M5 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.93)

MALPVTALLLPLALLLHAARP EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK
 GDTAYASKFQDKATITSNTAYLQLSSLTSEDTA VYYCTLTGRFDYWGQGTTLVSSGGGGSGGGGSGGGGSD
 10 IVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
 TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPV
 15 QTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
 NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc M5 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.94)

MALPVTALLLPLALLLHAARP EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYIHWVKQRPEQGLEWIGWIDPEK
 20 GDTAYASKFQDKATITSNTAYLQLSSLTSEDTA VYYCTLTGRFDYWGQGTTLVSSGGGGSGGGGSGGGGSD
 IVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNNLAWYQQKPGQSPKLLIWASTRESGVPDRFTGSGSGTDF
 TLTISSVQAEDLAVYYCQQYYSYRTFGGGTKLEIK EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVT
 CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
 ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 25 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQPFMRP
 VQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
 RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc G12 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.95)

MALPVTALLLPLALLLHAARP QVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG
 30 NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSSGGGGSGGGGSG
 GGGSNIVLTQSPASLAVSLGQRATISCRASESDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT
 DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGTKEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCK
 RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL

DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQA
LPPR

sc G12 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.96)

5 MALPVTALLPLALLHAARPQVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG
NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSSGGGGSGGGGSG
GGGSNIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT
DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGKLEIKRGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTRFSV
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV
10 LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQ
ALPPR

sc G12 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.97)

15 MALPVTALLPLALLHAARPQVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG
NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSSGGGGSGGGGSG
GGGSNIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT
DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGKLEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIYWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
20 RRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc G12 CAR-v4 (SEQ ID NO.1 + SEQ ID NO.98)

25 MALPVTALLPLALLHAARPQVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG
NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSSGGGGSGGGGSG
GGGSNIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT
DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGKLEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
GLDFACDIISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLDDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

30

sc G12 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.99)

MALPVTALLPLALLHAARPQVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG
NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTVTVSSGGGGSGGGGSG

GGGS[NIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT]
 DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGKLEIKR[EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIA
 RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLY
 5 SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP
 FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
 GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc G12 CAR-v6 (SEQ ID NO.1 + SEQ ID NO.100)

10 MALPVTALLPLALLHAARP[QVQLQQPGAELVKPGASMKMSCKASGYTFPSSNIHWLKQTPGQGLEWIGVIYPG]
 NGDTSYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAIYFCARVYNWHFDVWGAGTTTVTVSSGGGGSGGGGSG
 GGGS[NIVLTQSPASLAVSLGQRATISCRASESVDGYGDIFMLWYQQKPGQPPKLLIYFASNLESGVPARFSGSGSRT]
 DFTLTIDPVEADDAATYYCQQNNEDPYTFGGGKLEIKR[EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIA
 RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 15 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFLY
 SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTRFSVVKRGRKKLLYIFKQ
 PFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEM
 GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

20 **sc 21.26 CAR-v1** (SEQ ID NO.1 + SEQ ID NO.101)

MALPVTALLPLALLHAARP[QVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGMIHP]
 SSGTSYNEKVKNKATLTVDRSSTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
 GSGGGGS[QIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS]
 YSLTISRVEAEDAATYYCQQWRSDRALTFGAGTKLEL[GLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRG
 25 RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDK
 RRRGRDPEMGKGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
 PR

sc 21.26 CAR-v2 (SEQ ID NO.1 + SEQ ID NO.102)

30 MALPVTALLPLALLHAARP[QVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWWKQRPQGQGLEWIGMIHP]
 SSGTSYNEKVKNKATLTVDRSSTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
 GSGGGGS[QIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS]
 YSLTISRVEAEDAATYYCQQWRSDRALTFGAGTKLEL[GLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTRFSVVKR
 GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD

KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQAL
PPR

sc 21.26 CAR-v3 (SEQ ID NO.1 + SEQ ID NO.103)

5 MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIGMIHP
SSGSTSYNEKVKNKATLTVDRSSTTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
GSGGGGSQIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS
YSLTISRVEAEDAATYYCQQWRSDRALTFGAGTKLELTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSA
10 DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc 21.26 CAR-v4 (SEQ ID NO.1 + SEQ ID NO. 104)

15 MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIGMIHP
SSGSTSYNEKVKNKATLTVDRSSTTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
GSGGGGSQIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS
YSLTISRVEAEDAATYYCQQWRSDRALTFGAGTKLELTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
DFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
20 RRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc 21.26 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.105)

25 MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWVKQRPGQGLEWIGMIHP
SSGSTSYNEKVKNKATLTVDRSSTTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
GSGGGGSQIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS
YSLTISRVEAEDAATYYCQQWRSDRALTFGAGTKLELEPKSPDKTHTCPAPVAGPSVFLFPPKPKDTLMIARTP
EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM
30 RPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP
RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

sc 21.26 CAR-v6 (SEQ ID NO.1 + SEQ ID NO. 106)

MALPVTALLLPLALLLHAARPQVQLQQPGAELVKPGTSVKLSCKASGYTFTRYWMHWWVKQRPQGQGLEWIGMIHP
 SSGSTSYNEKVKNKATLTVDRSSTTAYMQLSSLTSEDSAVYYCARDGDYYYGTGDYWGQGTTTLTVSSGGGGSGGG
 GSGGGGSQIVLSQSPAILSASPGEKVTMTCRASSSINYMHWYQQKPGSSPKPWIFATSNLASGVPSRFSGSGSGTS
 YSLTISRVEAEDAATYYCQWRSDRALTFGAGTKLELEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTP
 5 EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
 EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL
 TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLFFLTLRFSVVKRGRKKLLYIFKQPF
 MRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
 KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

10

sc 1075.7 CAR-v1 (SEQ ID NO.1 + SEQ ID NO.107)

MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
 RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGTSTVTVSSGGGGSGGG
 GSGGGGSENVLTQSPAISASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
 15 GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKR
 GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD
 KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL
 PPR

20 **sc 1075.7 CAR-v2** (SEQ ID NO.1 + SEQ ID NO.108)

MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
 RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGTSTVTVSSGGGGSGGG
 GSGGGGSENVLTQSPAISASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
 GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELGLAVSTISSFFPPGYQIISFFLALTSTALLFLFFLTLRFSVVK
 25 RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL
 DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
 LPPR

30 **sc 1075.7 CAR-v3** (SEQ ID NO.1 + SEQ ID NO.109)

MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
 RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGTSTVTVSSGGGGSGGG
 GSGGGGSENVLTQSPAISASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
 GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL

DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR

5 **sc 1075.7 CAR-v4** (SEQ ID NO.1 + SEQ ID NO.110)

MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGSTVTVSSGGGGSGGG
GSGGGGSENVLTQSPAIMSASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL
10 DFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
RRGKGHDGLYQGLSTATKDTYDALHMQALPPR

sc 1075.7 CAR-v5 (SEQ ID NO.1 + SEQ ID NO.111)

15 MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGSTVTVSSGGGGSGGG
GSGGGGSENVLTQSPAIMSASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
20 PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM
RPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP
RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

25 **sc 1075.7 CAR-v6** (SEQ ID NO.1 + SEQ ID NO.112)

MALPVTALLLPLALLLHAARPDIQLQESGPGLVKPSQSLSLTCSVTGYSITSAYYWNWIRQFPGNKLEWMGYISYDG
RNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCAKEGDYDVGNYYAMDYWGQGSTVTVSSGGGGSGGG
GSGGGGSENVLTQSPAIMSASPGEKVTMTCRASSNVISSYVHWYQQRSGASPKLWIYSTSNLASGVPARFSGSGS
GTSYSLTISSVEAEDAATYYCQQYSGYPLTFGAGTKLELEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART
30 PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK
LTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

REFERENCES:

- Arbiza J., Taylor G., López J.A., Furze J., Wyld S., Whyte P., Stott E.J., Wertz G., Sullender W., Trudel M. , et al. (1992), Characterization of two antigenic sites recognized by neutralizing monoclonal antibodies directed against the fusion glycoprotein of human respiratory syncytial virus. *J Gen Virol.*;73 (9):2225-34).
- 5 Arimondo, P. B., C. J. Thomas, et al. (2006). "Exploring the cellular activity of camptothecin-triple-helix-forming oligonucleotide conjugates." *Mol Cell Biol* **26**(1): 324-33.
- Atkins, J. F., N. M. Wills, et al. (2007). "A case for "StopGo": reprogramming translation to augment codon meaning of GGN by promoting unconventional termination (Stop) after addition of glycine and then allowing continued translation (Go)." *Rna* **13**(6): 803-10.
- 10 Bakker AB, Van den Oudenrijn S, Bakker AQ, Feller N, van Meijer M, Bia JA, Jongeneelen MA, Visser TJ, Bijl N, Geuijen CA, Marissen WE, Radosevic K, Throsby M, Schuurhuis GJ, Ossenkoppele GJ, de Kruif J, Goudsmit J, Kruisbeek AM, (2004) "C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia", *Cancer Res.* 64(22):8443-50).
- 15 Bardenheuer, W., K. Lehmberg, et al. (2005). "Resistance to cytarabine and gemcitabine and in vitro selection of transduced cells after retroviral expression of cytidine deaminase in human hematopoietic progenitor cells." *Leukemia* **19**(12): 2281-8.
- Betts, M. R., J. M. Brenchley, et al. (2003). "Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation." *J Immunol Methods* **281**(1-2): 65-78.
- 20 Bierer, B. E., G. Hollander, et al. (1993). "Cyclosporin A and FK506: molecular mechanisms of immunosuppression and probes for transplantation biology." *Curr Opin Immunol* **5**(5): 763-73.
- Boch, J., H. Scholze, et al. (2009). "Breaking the code of DNA binding specificity of TAL-type III effectors." *Science* **326**(5959): 1509-12.
- 25 Brewin, J., C. Mancao, et al. (2009). "Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of posttransplantation lymphoproliferative disease." *Blood* **114**(23): 4792-803.
- Choulika, A., A. Perrin, et al. (1995). "Induction of homologous recombination in mammalian chromosomes by using the I-SceI system of *Saccharomyces cerevisiae*." *Mol Cell Biol* **15**(4): 1968-73.

Christian, M., T. Cermak, et al. (2010). "Targeting DNA double-strand breaks with TAL effector nucleases." Genetics **186**(2): 757-61.

Cong, L., F. A. Ran, et al. (2013). "Multiplex genome engineering using CRISPR/Cas systems." Science **339**(6121): 819-23.

5 Critchlow, S. E. and S. P. Jackson (1998). "DNA end-joining: from yeast to man." Trends Biochem Sci **23**(10): 394-8.

Dasgupta, A., D. McCarty, et al. (2011). "Engineered drug-resistant immunocompetent cells enhance tumor cell killing during a chemotherapy challenge." Biochem Biophys Res Commun **391**(1): 170-5.

10 Deltcheva, E., K. Chylinski, et al. (2011). "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Nature **471**(7340): 602-7.

Deng, D., C. Yan, et al. (2012). "Structural basis for sequence-specific recognition of DNA by TAL effectors." Science **335**(6069): 720-3.

15 Donnelly, M. and G. Elliott (2001). "Nuclear localization and shuttling of herpes simplex virus tegument protein VP13/14." J Virol **75**(6): 2566-74.

Doronina, V. A., C. Wu, et al. (2008). "Site-specific release of nascent chains from ribosomes at a sense codon." Mol Cell Biol **28**(13): 4227-39.

Eisenschmidt, K., T. Lanio, et al. (2005). "Developing a programmed restriction endonuclease for highly specific DNA cleavage." Nucleic Acids Res **33**(22): 7039-47.

20 Garneau, J. E., M. E. Dupuis, et al. (2010). "The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA." Nature **468**(7320): 67-71.

Gasiunas, G., R. Barrangou, et al. (2012). "Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria." Proc Natl Acad Sci U S A **109**(39): E2579-86.

25 Geissler, R., H. Scholze, et al. (2011). "Transcriptional activators of human genes with programmable DNA-specificity." PLoS One **6**(5): e19509.

Hacke, K., J. A. Treger, et al. (2013). "Genetic modification of mouse bone marrow by lentiviral vector-mediated delivery of hypoxanthine-Guanine phosphoribosyltransferase short hairpin RNA confers chemoprotection against 6-thioguanine cytotoxicity." Transplant Proc **45**(5): 2040-4.

Henderson, D. J., I. Naya, et al. (1991). "Comparison of the effects of FK-506, cyclosporin A and rapamycin on IL-2 production." Immunology **73**(3): 316-21.

Huang, P., A. Xiao, et al. (2011). "Heritable gene targeting in zebrafish using customized TALENs." Nat Biotechnol **29**(8): 699-700.

5 Jena, B., G. Dotti, et al. (2010). "Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor." Blood **116**(7): 1035-44.

Jinek, M., K. Chylinski, et al. (2012). "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Science **337**(6096): 816-21.

Jonnalagadda, M., C. E. Brown, et al. (2013). "Engineering human T cells for resistance to
10 methotrexate and mycophenolate mofetil as an in vivo cell selection strategy." PLoS One **8**(6): e65519.

Kalish, J. M. and P. M. Glazer (2005). "Targeted genome modification via triple helix formation." Ann N Y Acad Sci **1058**: 151-61.

Kushman, M. E., S. L. Kabler, et al. (2007). "Expression of human glutathione S-transferase P1
15 confers resistance to benzo[a]pyrene or benzo[a]pyrene-7,8-dihydrodiol mutagenesis, macromolecular alkylation and formation of stable N2-Gua-BPDE adducts in stably transfected V79MZ cells co-expressing hCYP1A1." Carcinogenesis **28**(1): 207-14.

Larsen HØ, Roug AS, Just T, Brown GD, Hokland P (2012), "Expression of the hMICL in acute myeloid leukemia-a highly reliable disease marker at diagnosis and during follow-up". Cytometry B Clin Cytom. **82**(1):3-8).
20

Li, L., M. J. Piatek, et al. (2012). "Rapid and highly efficient construction of TALE-based transcriptional regulators and nucleases for genome modification." Plant Mol Biol **78**(4-5): 407-16.

Li, T., S. Huang, et al. (2011). "TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain." Nucleic Acids Res **39**(1): 359-72.

25 Li, T., S. Huang, et al. (2011). "Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes." Nucleic Acids Res **39**(14): 6315-25.

Liu, J., M. W. Albers, et al. (1992). "Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity." Biochemistry **31**(16): 3896-901.

Ma, J. L., E. M. Kim, et al. (2003). "Yeast Mre11 and Rad1 proteins define a Ku-independent mechanism to repair double-strand breaks lacking overlapping end sequences." Mol Cell Biol **23**(23): 8820-8.

5 Mahfouz, M. M., L. Li, et al. (2012). "Targeted transcriptional repression using a chimeric TALE-SRDX repressor protein." Plant Mol Biol **78**(3): 311-21.

Mahfouz, M. M., L. Li, et al. (2011). "De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks." Proc Natl Acad Sci U S A **108**(6): 2623-8.

10 Mak, A. N., P. Bradley, et al. (2012). "The crystal structure of TAL effector PthXo1 bound to its DNA target." Science **335**(6069): 716-9.

Mali, P., L. Yang, et al. (2013). "RNA-guided human genome engineering via Cas9." Science **339**(6121): 823-6.

15 McLaughlin P, et al. (1998) Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol. 16(8):2825-33

Miller, J. C., S. Tan, et al. (2011). "A TALE nuclease architecture for efficient genome editing." Nat Biotechnol **29**(2): 143-8.

20 Morbitzer, R., P. Romer, et al. (2011). "Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors." Proc Natl Acad Sci U S A **107**(50): 21617-22.

Moscou, M. J. and A. J. Bogdanove (2009). "A simple cipher governs DNA recognition by TAL effectors." Science **326**(5959): 1501.

Mussolino, C., R. Morbitzer, et al. (2011). "A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity." Nucleic Acids Res **39**(21): 9283-93.

25 Nivens, M. C., T. Felder, et al. (2004). "Engineered resistance to camptothecin and antifolates by retroviral coexpression of tyrosyl DNA phosphodiesterase-I and thymidylate synthase." Cancer Chemother Pharmacol **53**(2): 107-15.

Paques, F. and P. Duchateau (2007). "Meganucleases and DNA double-strand break-induced recombination: perspectives for gene therapy." Curr Gene Ther **7**(1): 49-66.

Park, T. S., S. A. Rosenberg, et al. (2011). "Treating cancer with genetically engineered T cells." Trends Biotechnol **29**(11): 550-7.

5 Peipp, M., D. Saul, et al. (2004). "Efficient eukaryotic expression of fluorescent scFv fusion proteins directed against CD antigens for FACS applications." J Immunol Methods **285**(2): 265-80.

Perrin, A., M. Buckle, et al. (1993). "Asymmetrical recognition and activity of the I-SceI endonuclease on its site and on intron-exon junctions." Embo J **12**(7): 2939-47.

Pingoud, A. and G. H. Silva (2007). "Precision genome surgery." Nat Biotechnol **25**(7): 743-4.

10 Porteus, M. H. and D. Carroll (2005). "Gene targeting using zinc finger nucleases." Nat Biotechnol **23**(8): 967-73.

Ravetch, J.V., Perussia, B., (1989). "Alternative membrane forms of Fc gamma RIII(CD16) on human natural killer cells and neutrophils. Cell type-specific expression of two genes that differ in single nucleotide substitutions". J. Exp. Med. 170, 481-497.

15 Riemer A.B., Kurz H., Klinger, M., Scheiner, O., Zielinski, C., and Jensen-Jarolim, E. (2005), Vaccination with cetuximab mimotopes and biological properties of induced anti-epidermal growth factor receptor antibodies, J Natl Cancer Inst.;97(22):1663-70

)Rouet, P., F. Smih, et al. (1994). "Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease." Mol Cell Biol **14**(12): 8096-106.

20 Sander, J. D., L. Cade, et al. (2011). "Targeted gene disruption in somatic zebrafish cells using engineered TALENs." Nat Biotechnol **29**(8): 697-8.

Sangiolo, D., M. Lesnikova, et al. (2007). "Lentiviral vector conferring resistance to mycophenolate mofetil and sensitivity to ganciclovir for in vivo T-cell selection." Gene Ther **14**(21): 1549-54.

25 Schweitzer, B. I., A. P. Dicker, et al. (1990). "Dihydrofolate reductase as a therapeutic target." Faseb J **4**(8): 2441-52.

Sorek, R., C. M. Lawrence, et al. (2013). "CRISPR-mediated Adaptive Immune Systems in Bacteria and Archaea." Annu Rev Biochem.

Stoddard, B. L. (2005). "Homing endonuclease structure and function." Q Rev Biophys **38**(1): 49-95.

Sugimoto, Y., S. Tsukahara, et al. (2003). "Drug-selected co-expression of P-glycoprotein and gp91 in vivo from an MDR1-bicistronic retrovirus vector Ha-MDR-IRES-gp91." J Gene Med **5**(5): 366-76.

Takebe, N., S. C. Zhao, et al. (2001). "Generation of dual resistance to 4-hydroperoxycyclophosphamide and methotrexate by retroviral transfer of the human aldehyde dehydrogenase class 1 gene and a mutated dihydrofolate reductase gene." Mol Ther **3**(1): 88-96.

Tesson, L., C. Usal, et al. (2011). "Knockout rats generated by embryo microinjection of TALENs." Nat Biotechnol **29**(8): 695-6.

Van Rhenen A, van Dongen GA, Kelder A, Rombouts EJ, Feller N, Moshaver B, Stigter-van Walsum M, Zweegman S, Ossenkoppele GJ, Jan Schuurhuis G Blood. (2007) "The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood, 110(7):2659-66.

Weber, E., R. Gruetzner, et al. (2011). "Assembly of designer TAL effectors by Golden Gate cloning." PLoS One **6**(5): e19722.

Yam, P., M. Jensen, et al. (2006). "Ex vivo selection and expansion of cells based on expression of a mutated inosine monophosphate dehydrogenase 2 after HIV vector transduction: effects on lymphocytes, monocytes, and CD34+ stem cells." Mol Ther **14**(2): 236-44.

Zhang Hongyong , Luo Juntao, Li Yuanpei, Henderson Paul T, Wachsmann-Hogiu Sebastian, Lam Kit S. , and Pan Chong-xian (2011) « Characterization of high-affinity peptides and their feasibility for use in nanotherapeutics targeting leukemia stem cells" Nanomedicine; 8(7): 1116–1124.

Zhang, F., L. Cong, et al. (2011). "Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription." Nat Biotechnol **29**(2): 149-53.

Zielske, S. P., J. S. Reese, et al. (2003). "In vivo selection of MGMT(P140K) lentivirus-transduced human NOD/SCID repopulating cells without pretransplant irradiation conditioning. "J Clin Invest 112(10)1561-70.

CLAIMS

1. A CLL1 specific chimeric antigen receptor (anti-CLL1 CAR) comprising at least:
 - an extra cellular ligand binding-domain anti-CLL1,
 - a transmembrane domain, and
 - a cytoplasmic signaling domain.
2. A CLL1 specific chimeric antigen receptor according to claim 1, further comprising a co-stimulatory domain.
3. A CLL1 specific chimeric antigen receptor according to claim 1, further comprising a CD28 or a 4-1BB co-stimulatory domain.
4. A CLL1 specific chimeric antigen receptor according to any one of claims 1 to 3, wherein said transmembrane domain is a CD8 α transmembrane domain.
5. A CLL1 specific chimeric antigen receptor according to any one of claims 1 to 4, further comprising a hinge.
6. A CLL1 specific chimeric antigen receptor according to any one of claims 1 to 5, wherein said cytoplasmic signaling domain is a T-cell activating domain.
7. A CLL1 specific chimeric antigen receptor according to any one of claims 1 to 6, wherein said chimeric antigen receptor is expressed under the form of a single polypeptide.
8. A CLL1 specific chimeric antigen receptor according to any one of claims 1 to 7, wherein said extra cellular ligand binding-domain is from a monoclonal anti-CLL1 antibody.
9. A CLL1 specific chimeric antigen receptor according to claim 8, wherein extra cellular ligand binding-domain comprises CDRs from VH and VL domains of monoclonal anti-CLL1 antibody(ies).

10. A CLL1 specific chimeric antigen receptor according to claim 9, wherein said CDRs are selected from SEQ ID NO. 109 to 190.
11. A CLL1 specific scCAR according to any one of claims 1 to 10 having one of the polypeptide structure selected from V1, V3 or V5, as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CLL1 antibody, a hinge, a CD8 α transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain.
12. A CLL1 specific scCAR according to any one of claims 1 to 11, wherein said structure V1 comprises a Fc γ RIII α hinge and CD8 α transmembrane domain.
13. A CLL1 specific scCAR according to any one of claims 1 to 11, wherein said structure V3 comprises a CD8 α hinge and a CD8 α transmembrane domain.
14. A CLL1 specific scCAR according any one of claims 1 to 11, wherein said structure V5 comprises an IgG1 hinge and a CD8 α transmembrane domain.
15. A CLL1 specific scCAR according to any one of claims 1 to 14, wherein said VH and VL have at least 80 % identity with a polypeptide sequence selected from SEQ ID NO. 11 to 34.
16. A CLL1 specific scCAR according to any one of claims 1 to 15, wherein co-stimulatory domain from 4-1BB has at least 80 % identity with SEQ ID NO.8.
17. A CLL1 specific scCAR according to any one of claims 1 to 16, wherein said CD3 zeta signaling domain has at least 80 % identity with SEQ ID NO. 9.
18. A CLL1 specific scCAR according to any one of claims 1 to 12 or 15 to 17, wherein said Fc γ RIII α hinge has at least 80 % identity with SEQ ID NO.3.
19. A CLL1 specific scCAR according to any one of claims 1 to 11, 13 or 15 to 17, wherein said CD8 α hinge has at least 80 % identity with SEQ ID NO.4.

20. A CLL1 specific scCAR according to any one of claims 1 to 11 or 14 to 19, wherein said IgG1 hinge has at least 80 % identity with SEQ ID NO.5.
21. A CLL1 specific scCAR according to any one of claims 1 to 20, wherein said CD8 α transmembrane domain has at least 80 % identity with SEQ ID NO.6.
22. A CLL1 specific scCAR according to any one of claims 1 to 21 further comprising another extracellular ligand binding domain which is not specific for CLL1.
23. A CLL1 specific scCAR of structure V1 according to any one of claims 1 to 12, 15 to 17 or 21 to 22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101 or 107.
24. A CLL1 specific scCAR of structure V3 according to any one of claims 1 to 11, 13, 15 to 17 or 21 to 22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103 or 109.
25. A CLL1 specific scCAR of structure V5 according to any one of claims 1 to 11, 14 to 19 or 21 to 22 which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO.39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105 or 111.
26. A CLL1 specific scCAR according to any one of claims 1 to 25 further comprising a signal peptide.
27. A CLL1 specific scCAR according to claim 26, wherein said signal peptide has at least 80 % sequence identity with SEQ ID NO.1 or SEQ ID NO.2.
28. The polypeptide according to any one of claims 1 to 27, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.
29. The polypeptide according to any one of claims 1 to 28, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.
30. The polypeptide according to any one of claims 1 to 29, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes

31. The polypeptide according to any one of claims 1 to 30, wherein the extracellular binding domain comprises the following sequence

$V_1-L_1-V_2-(L)_x$ -Epitope1- $(L)_x$;

$V_1-L_1-V_2-(L)_x$ -Epitope1- $(L)_x$ -Epitope2- $(L)_x$;

$V_1-L_1-V_2-(L)_x$ -Epitope1- $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ - $V_1-L_1-V_2$;

$(L)_x$ -Epitope1- $(L)_x$ -Epitope2- $(L)_x$ - $V_1-L_1-V_2$;

Epitope1- $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$ - $V_1-L_1-V_2$;

$(L)_x$ -Epitope1- $(L)_x$ - $V_1-L_1-V_2$ - $(L)_x$ -Epitope2- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ - $V_1-L_1-V_2$ - $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ - $V_1-L_1-V_2$ - $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$ -Epitope4- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ -Epitope2- $(L)_x$ - $V_1-L_1-V_2$ - $(L)_x$ -Epitope3- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ -Epitope2- $(L)_x$ - $V_1-L_1-V_2$ - $(L)_x$ -Epitope3- $(L)_x$ -Epitope4- $(L)_x$;

V_1 - $(L)_x$ -Epitope1- $(L)_x$ - V_2 ;

V_1 - $(L)_x$ -Epitope1- $(L)_x$ - V_2 - $(L)_x$ -Epitope2- $(L)_x$;

V_1 - $(L)_x$ -Epitope1- $(L)_x$ - V_2 - $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$;

V_1 - $(L)_x$ -Epitope1- $(L)_x$ - V_2 - $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$ -Epitope4- $(L)_x$;

$(L)_x$ -Epitope1- $(L)_x$ - V_1 - $(L)_x$ -Epitope2- $(L)_x$ - V_2 ; or,

$(L)_x$ -Epitope1- $(L)_x$ - V_1 - $(L)_x$ -Epitope2- $(L)_x$ - V_2 - $(L)_x$ -Epitope3- $(L)_x$;

wherein,

V_1 is V_L and V_2 is V_H or V_1 is V_H and V_2 is V_L ;

L_1 is a linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

x is 0 or 1 and each occurrence of x is selected independently from the others; and,

Epitope 1, Epitope 2 and Epitope 3 are mAb-specific epitopes and can be identical or different.

32. The polypeptide according to any one of claim 31, wherein the extracellular binding domain comprises the following sequence

V₁-L₁-V₂-L-Epitope1; V₁-L₁-V₂-L-Epitope1-L; V₁-L₁-V₂-L-Epitope1-L-Epitope2; V₁-L₁-V₂-L-Epitope1-L-Epitope2-L; V₁-L₁-V₂-L-Epitope1-L-Epitope2-L-Epitope3; V₁-L₁-V₂-L-Epitope1-L-Epitope2-L-Epitope3-L; V₁-L₁-V₂-Epitope1; V₁-L₁-V₂-Epitope1-L; V₁-L₁-V₂-Epitope1-L-Epitope2; V₁-L₁-V₂-Epitope1-L-Epitope2-L; V₁-L₁-V₂-Epitope1-L-Epitope2-L-Epitope3; V₁-L₁-V₂-Epitope1-L-Epitope2-L-Epitope3-L; Epitope1-V₁-L₁-V₂; Epitope1-L-V₁-L₁-V₂; L-Epitope1-V₁-L₁-V₂; L-Epitope1-L-V₁-L₁-V₂; Epitope1-L-Epitope2-V₁-L₁-V₂; Epitope1-L-Epitope2-L-V₁-L₁-V₂; L-Epitope1-L-Epitope2-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-V₁-L₁-V₂; Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂; Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-Epitope3-V₁-L₁-V₂; L-Epitope1-L-Epitope2-L-Epitope3-L-V₁-L₁-V₂; V₁-L-Epitope1-L-V₂; L-Epitope1-L-V₁-L-Epitope2-L-V₂; V₁-L-Epitope1-L-V₂-L-Epitope2-L; V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3; V₁-L-Epitope1-L-V₂-L-Epitope2-Epitope3; V₁-L-Epitope1-L-V₂-L-Epitope2-L-Epitope3-Epitope4; L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L; Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3-L; L-Epitope1-L-V₁-L-Epitope2-L-V₂-L-Epitope3; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3; L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-Epitope3, or Epitope1-L-V₁-L₁-V₂-L-Epitope2-L-Epitope3-Epitope4

wherein

V₁ is V_L and V₂ is V_H or V₁ is V_H and V₂ is V_L;

L₁ is any linker suitable to link the V_H chain to the V_L chain;

L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

epitope 1, epitope 2 and epitope 3 are mAb-specific epitopes and can be identical or different.

33. The polypeptide according to claim 31 or 32, wherein L_1 is a linker comprising Glycine and/or Serine.
34. The polypeptide according to claim 33, wherein L_1 is a linker comprising the amino acid sequence $(\text{Gly-Gly-Gly-Ser})_n$ or $(\text{Gly-Gly-Gly-Gly-Ser})_n$, where n is 1, 2, 3, 4 or 5.
35. The polypeptide according to claim 34, wherein L_1 is a linker comprising the amino acid sequence $(\text{Gly}_4\text{Ser})_4$ or $(\text{Gly}_4\text{Ser})_3$.
36. The polypeptide according to any one of claims 31 to 35 wherein L is a linker comprising Glycine and/or Serine.
37. The polypeptide according to claim 36, wherein L is a linker having an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGGS, SGGGGS, SGGGGG, GSGGGGS, GGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGGS SGGGGS.
38. The polypeptide according to claim 37, wherein L is a SGGGG, GGGGS or SGGGGS.
39. The polypeptide according to any one of claims 31 to 38 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.
40. The polypeptide according to any one of claims 31 to 38 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes having an amino acid sequence of SEQ ID NO 113, SEQ ID NO 191, , SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 195, SEQ ID NO 195, SEQ ID NO 196, SEQ ID NO 197, SEQ ID NO 198 or SEQ ID NO 199.
41. The polypeptide according to any one of claims 31 to 40 wherein Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.

42. The polypeptide according to any one of claims 31 to 41 wherein Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.
43. The polypeptide according to any one of claims 31 to 42 wherein Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113, SEQ ID NO 198 or SEQ ID NO 199.
44. The polypeptide according to any one of claims 31 to 43 wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 113.
45. A polynucleotide encoding a chimeric antigen receptor according to any one of claims 1 to 44.
46. An expression vector comprising a nucleic acid of claim 39.
47. An engineered lymphoid immune cell expressing at the cell surface membrane an anti-CLL1 CAR according to any one of claims 1 to 44.
48. An engineered lymphoid immune cell according to claim 41 derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.
49. An engineered cell according to claim 47 or 48 for use in therapy.
50. An engineered cell according to any one of claims 41 to 43 for use in therapy, wherein the patient is a human.
51. An engineered cell according to any one of claims 47 to 50 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by CLL1-expressing cells.
52. An engineered cell according to any one of claims 47 to 51 for use in therapy, wherein the condition is a condition which is characterized by an overabundance of CLL1-expressing cells.
53. An engineered cell according to any one of claims 51 to 52 for use in therapy, wherein the condition is a hematological cancer condition.

54. An engineered cell according to claim 53 for use in therapy, wherein the hematological cancer condition is leukemia.
55. An engineered cell according to claim 54 for use in therapy, wherein the leukemia is acute myelogenous leukemia (AML).
56. An engineered cell according to any one of claims 47 to 55, wherein expression of TCR is suppressed in said immune cell.
57. An engineered cell according to any one of claims 47 to 55, wherein expression of at least one MHC protein, preferably $\beta 2m$ or HLA, is repressed or suppressed in said immune cell.
58. An engineered cell according to any one of claims 47 to 57, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.
59. A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to any one of claims 47 to 58 in an amount effective to cause impairment of said cancer cell.
60. A method of engineering an immune cell comprising:
- (c) Providing an immune cell,
 - (d) Expressing at the surface of said cell at least one CLL1 single-chain specific chimeric antigen receptor according to any one of claims 1 to 44.
61. The method of engineering an immune cell of claim 60 comprising:
- (d) Providing an immune cell,
 - (e) Introducing into said cell at least one polynucleotide encoding said CLL1 single-chain specific chimeric antigen receptor ,
 - (f) Expressing said polynucleotide into said cell.
62. The method of engineering an immune cell of claim 61 comprising:
- (d) Providing an immune cell,
 - (e) Introducing into said cell at least one polynucleotide encoding said anti-CLL1 single-chain specific chimeric antigen receptor ,

- (f) Introducing at least one other chimeric antigen receptor which is not specific for CLL1.

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

- (c) Providing an immune cell expressing at the surface an anti-CLL1 single-chain specific chimeric antigen receptor according to any one of claims 1 to 44;
- (d) Administering said immune cells to said patient.

64. A method according to claim 63, wherein said immune cell is provided from a donor.

65. A method according to claim 63, wherein said immune cell is provided from the patient himself.

1/4

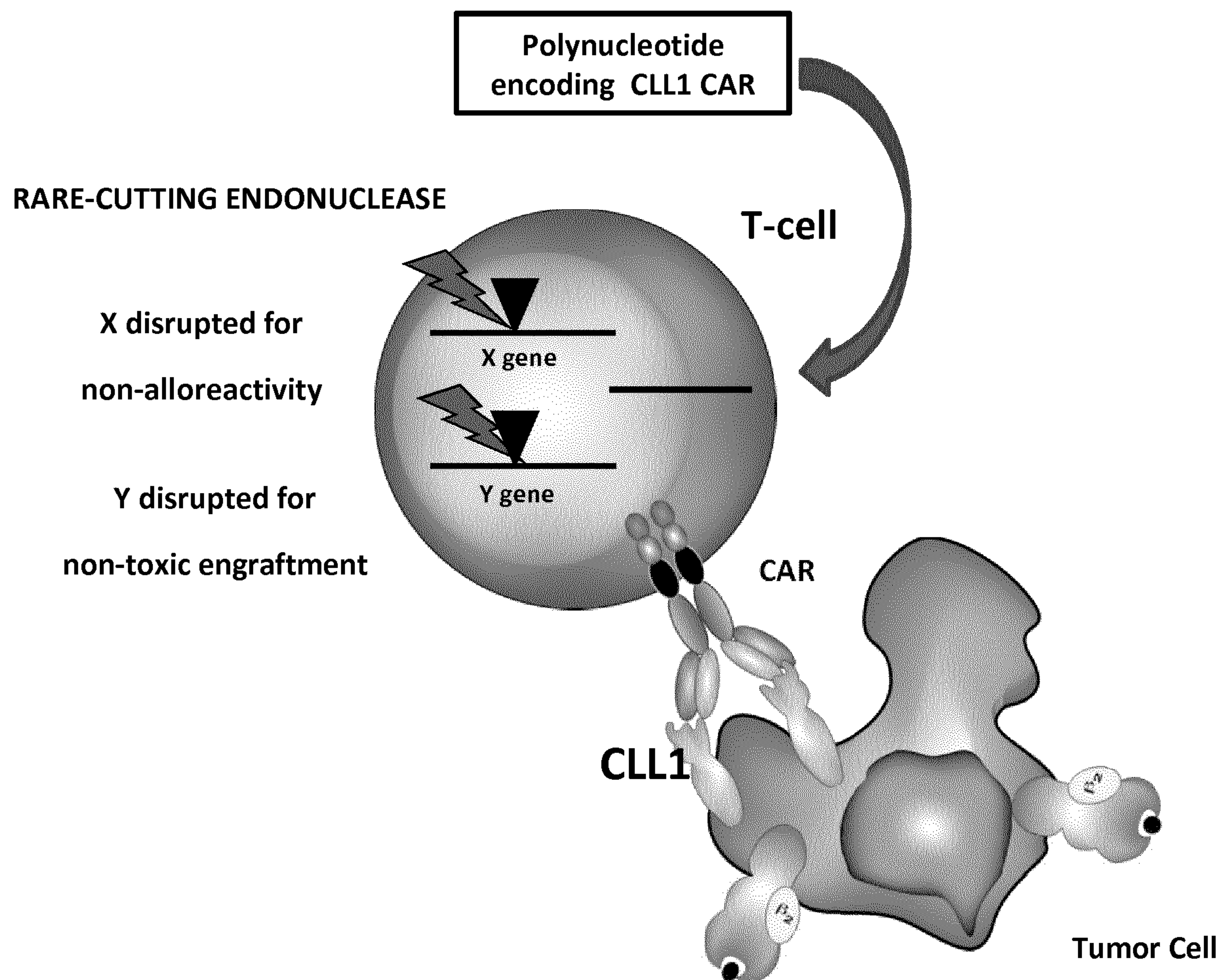


Figure 1

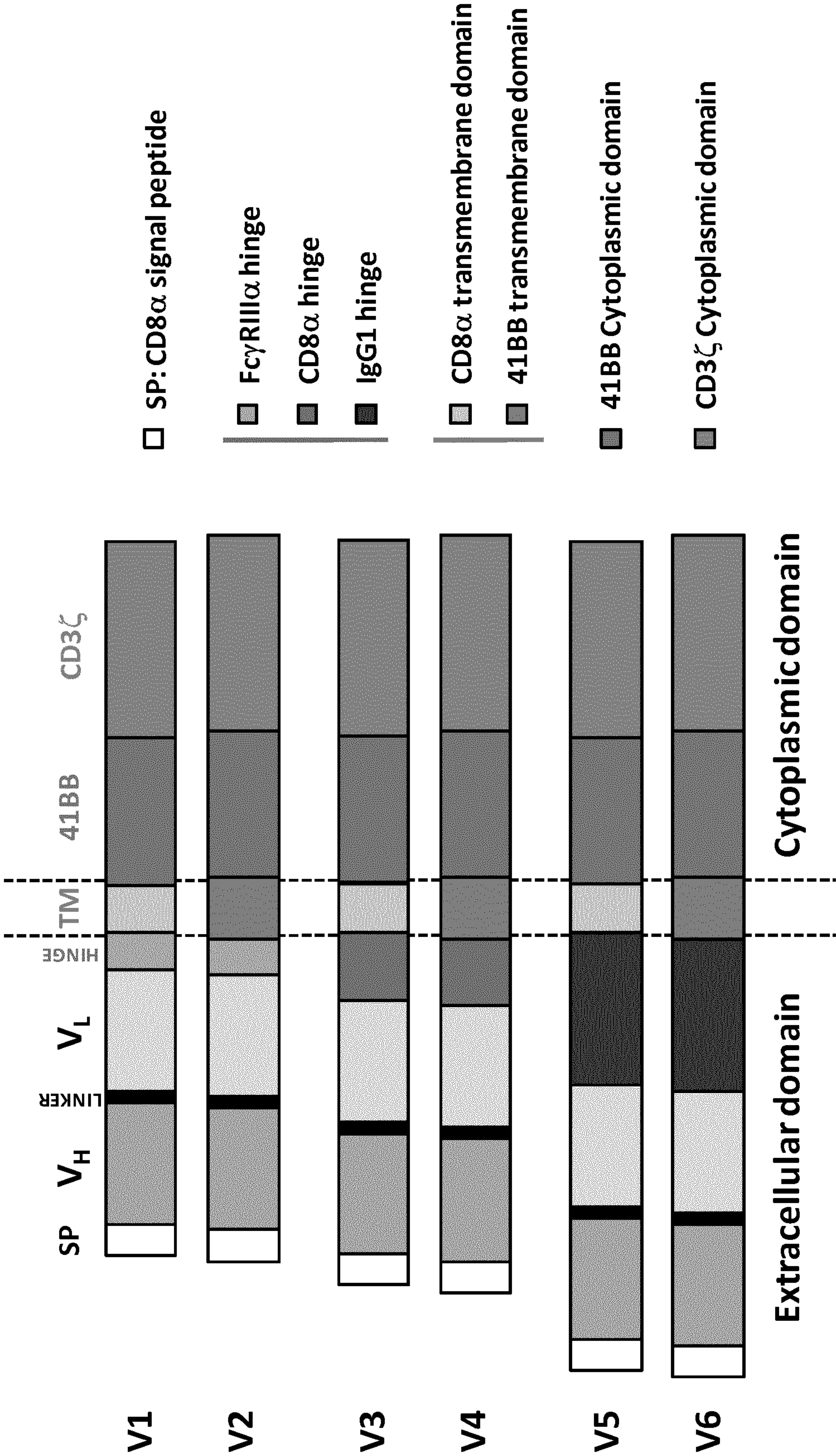


Figure 2

3/4

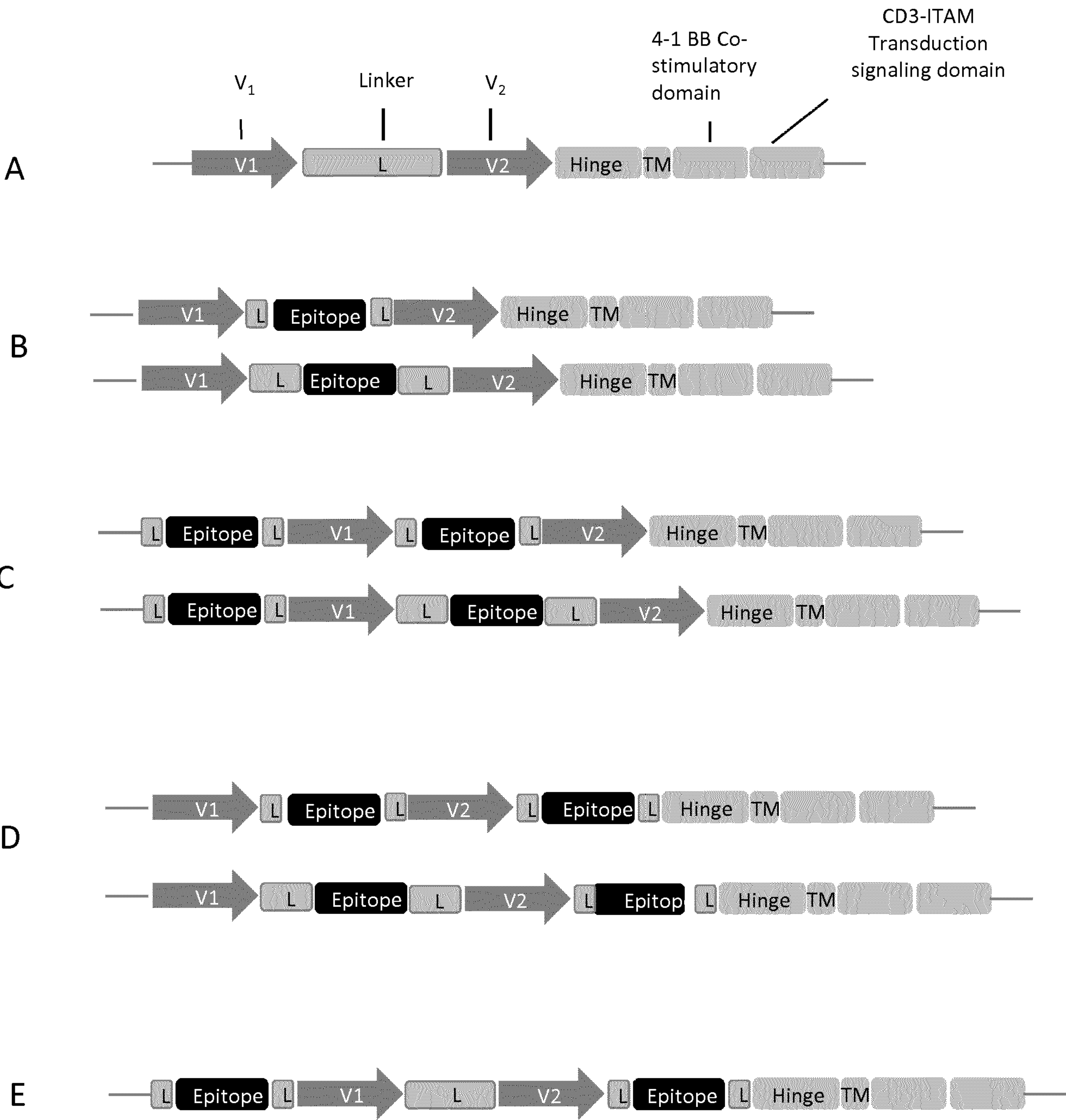


Figure 3A

4/4

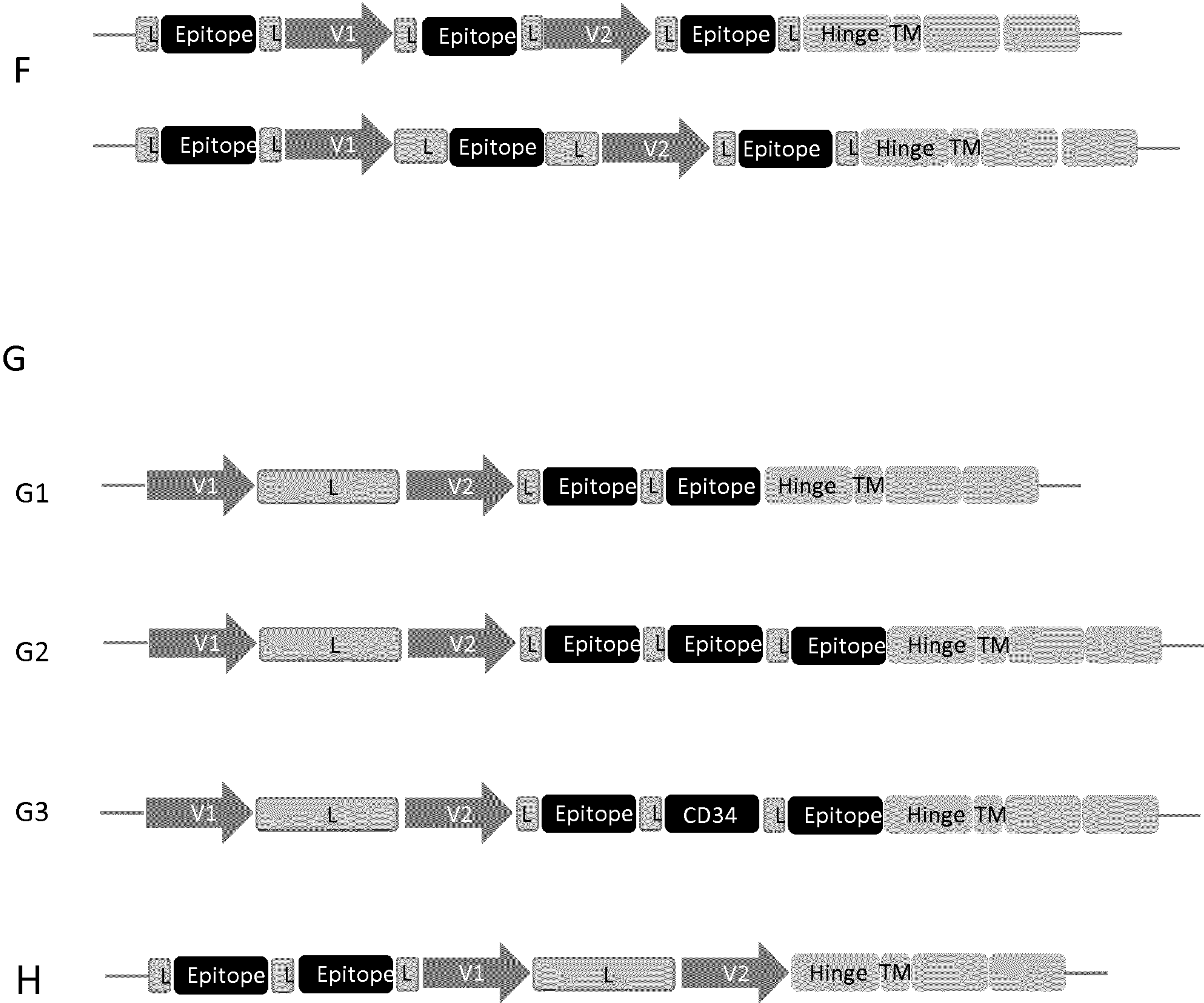


Figure 3B

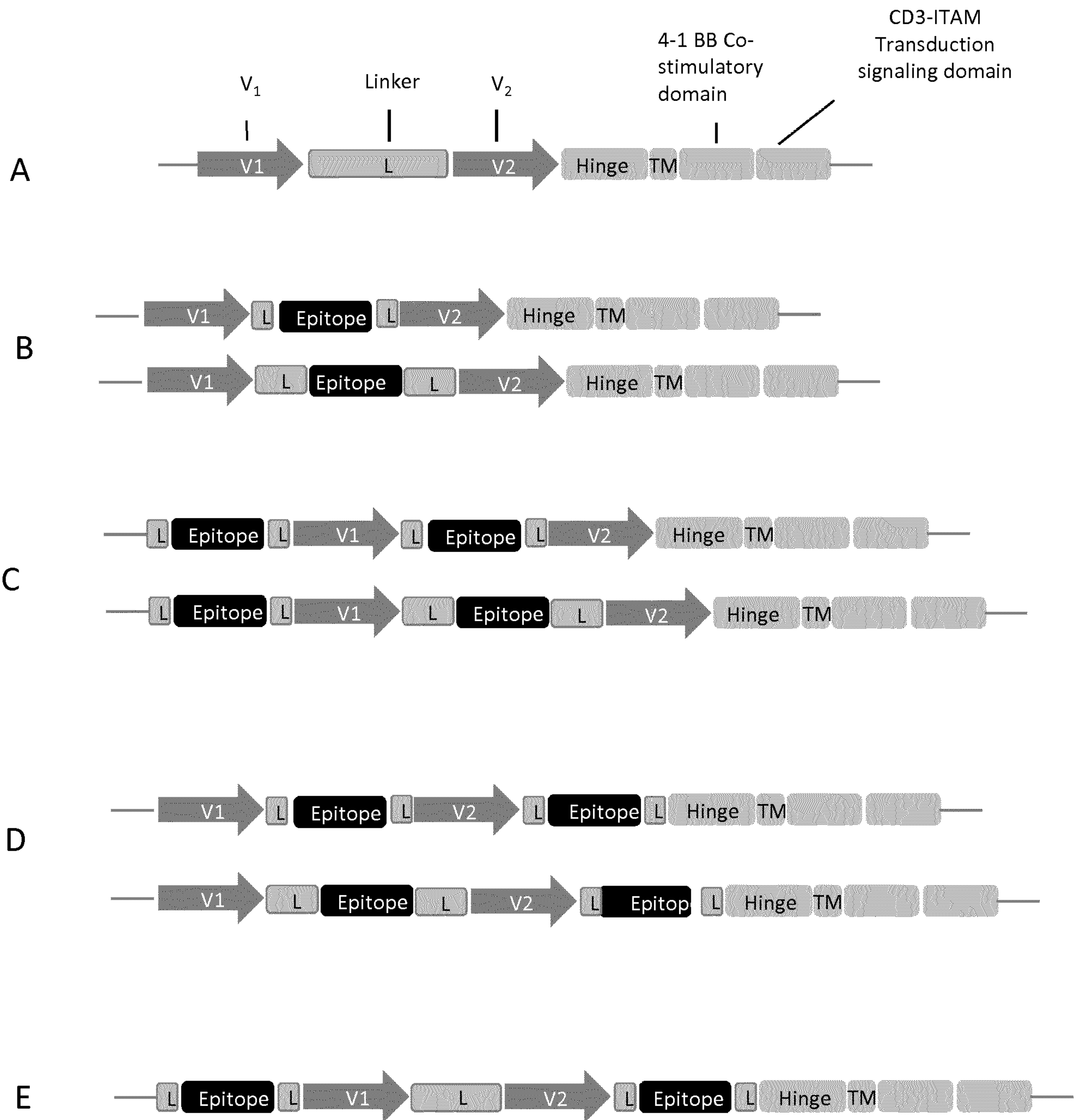


Figure 3A