Figure 5A

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(54) Title:  
CLL1-SPECIFIC MULTI-CHAIN CHIMERIC ANTIGEN RECEPTOR  

(57) Abstract:  
The present invention relates to a new generation of chimeric antigen receptors (CAR) referred to as multi-chain CARs, which are made specific to the antigen CLL1. Such CARs aim to redirect immune cell specificity and reactivity toward malignant cells expressing the tumor antigen CLL1. The alpha, beta, and gamma polypeptides composing these CARs are designed to assemble in juxtamembrane position, which forms flexible architecture closer to natural receptors, that confers optimal signal transduction. The invention encompasses the polynucleotides, vectors encoding said multi-chain CAR and the isolated cells expressing them at their surface, in particular for their use in immunotherapy. The invention opens the way to efficient adoptive immunotherapy strategies for treating cancer, especially leukemia.
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Field of the invention

The present invention relates to a new generation of chimeric antigen receptors (CAR) referred to as multi-chain CARs, which are made specific to the antigen CLL1. Such CARs aim to redirect immune cell specificity and reactivity toward malignant cells expressing the tumor antigen CLL1. The polypeptides composing these CARs are designed to assemble in juxtamembrane position, which forms flexible architecture closer to natural receptors, that confers tunable signal transduction. The invention encompasses the polynucleotides, vectors encoding said multi-chain CAR and the isolated cells resulting from their heterologous expression in immune cells, in particularly for their use in immunotherapy. The invention opens the way to efficient adoptive immunotherapy strategies for treating cancer, especially acute myeloid leukemia (AML).

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) Treating Cancer with Genetically Engineered T Cells. Trends Biotechnol. 29(11): 550-557) Transfer of viral antigen specific T cells is a well-established procedure used for the treatment of transplant associated viral infections and rare viral-related malignancies. Similarly, isolation and transfer of tumor specific T cells has been shown to be successful in treating melanoma.

Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs) (Jena, Dotti et al. (2010) Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 116(7): 1035-1044). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains to form a single-chain fusion molecule. However, this approach has so far proven efficiency only with respect to patients with acute lymphoblastic leukemia (ALL) by targeting malignant B cells bearing the antigen CD19 (Porter, D.L. et al. (2011) Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia. N. Engl. J. Med. 365:725-733).
Induction treatments for acute myeloid leukemia (AML) have remained largely unchanged for nearly 50 years and AML remains a disease of poor prognosis. Acute myeloid leukemia (AML) is a disease characterized by the rapid proliferation of immature myeloid cells in the bone marrow resulting in 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.

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 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.

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: Q5GGZ9 for human protein encoded by gene n°160364 in "Entrez Gene" database) that contains a 200 AA extracellular domain. CLL1 is also referred to in the literature and databases as M.1CL, 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 Rhenen et al, 2007). Meanwhile, CLL1 was revealed to be a diagnostic marker in AML (Larsen et al, 2012). Anti-CLL1 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.

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)

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 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 CLL1 using immune cells endowed with specific chimeric antigen receptors based on anti-CLL1 monoclonal antibodies, which redirect immune cell specificity towards CLL1 positive cells.

In the context of developing therapeutic grade engineered immune cells that can target malignant or infected cells, the inventors have sought for improved CAR architectures, which would be closer to natural ones and likely to behave accordingly using any extracellular mono or multi-specific ligand binding domains. In WO2014039523, they described a new generation of CARs involving separate polypeptide sub-units according to the present invention, referred to as "multi-chain CARs". According to this architecture, the signaling domains and co-stimulatory domains are located on different polypeptide chains. Such multi-chain CARs can be derived from FCERI (see Figure 1), by replacing the high affinity IgE binding domain of FCERI alpha chain by an extracellular ligand-binding domain such as scFv, whereas the N and/or C-termini tails of FCERI 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 the immune cell response. The fact that the different polypeptides derived from the alpha, beta and gamma polypeptides from FCERI 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.

The inventors have now designed multi-chain CAR bearing scFv extracellular domain binding CLL1, which are particularly suited to target malignant cells bearing CLL1 as a marker. This was
achieved, whereas very few antibodies had been so far described to act efficiently against CLLI positive cells for treating or preventing leukemia, in particular AML.

For the purposes of the invention, inventors have now provided T cells expressing anti-CLLI multi chain CARs. Due to the design and architecture of these new anti-CLLI CARs and to the properties of the present engineered immune cells, the kinetic of action and activity of engineered immune cells is unexpectedly modified so that less tumor cells may escape and long term effect is observed with reduced GVHD and side effects.

These original CARs specifically bind to and affect the survival of CLLI positive T cells, in particular to malignant CLLI positive cells developing during AML and selectively alter the viability of these malignant cells, with an expectation of displaying less toxic side effects including cytokine release. Moreover, 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 CLLI specific multi-chain (mcCAR) having different design and comprising different scFV derived from anti-CLLI specific antibodies. Said multi-chain CARs are preferably based on the alpha, beta and gamma polypeptides from FCERI as detailed herein.

In particular, The Inventors have developed anti-CLLI specific multi-chain CAR (mcCAR) 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 mcCARs constructions that bind to CLLI expressing cells and selectively destroy CLLI 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 mcCARs 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 (αβ - T-Cell receptors) to prevent Graft versus host reaction.
Immune-cells endowed with CLL1 specific CARs according to the invention may be further engineered to create T cells resistant to anti-cancer drugs, to be used in combination or sequentially 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 mcCARs 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.

According to a further aspect, the CLL1 specific CARs of the present invention comprises at least one epitope tagging sequence such as a CD20 mimotope, allowing a depletion of said immune cells by the use of antibodies against such epitope, to modulate the immune response of the CAR positive cells (i.e occurrence of adverse effect during their use in immunotherapy such as cytokine storm). Preferably, said at least one epitope is inserted in the extracellular ligand binding domain of the CAR, more preferably on the alpha chain of the multi-chain CAR exemplified herein.

The engineered immune cells of the present invention are particularly useful for therapeutic applications such as acute myeloma leukemia (AML) treatments.
Description of the Figures:

Figure 1: Schematic representation of the native FCERI from which derive the multi-chain CAR architecture according to the invention.

Figure 2: General structure of the polyclistron construct encoding the CLLI multi-chain CAR according to the invention.

Figure 3: Different architectures of the CLLI specific multi-chain CAR according to the invention. From left to right: polypeptide gamma (fused to ITAM of CD3zeta), polypeptide alpha (fused to ScFv), polypeptide beta (fused to co-stimulatory domain from either CD28 or 41BB). A and B: polypeptide beta is fused to co-stimulatory domain from 41BB, VL and VH fragments being in opposite orders. C and D: polypeptide beta is fused to co-stimulatory domain from CD28, VL and VH fragments being in opposite orders.

In Figure 3 C, and in Figure 4, VL and VH fragments are in opposite order as compared to construction in Figure 3D. In Figure 3C and in Figure 4, the VL fragment of the extracellular CLLI ligand binding domain is fused to a transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI), more precisely to a peptide comprising a CD8 fragment and a fragment of the alpha chain of high-affinity IgE receptor (FCERI).

Figure 4: Two architectures of the CLLI specific multi-chain CAR according to the invention (mcCLLI-41BB and mcCLLI-CD28) wherein the alpha fragment comprises a VL fragment is linked to a polypeptide comprising a CD8 fragment and to a VH fragment, and the beta chain comprises a co-stimulatory domain located in the C-terminus of the beta chain, said co-stimulatory domain is from 41BB (mcCLLI-41BB) or from CD28 (mcCLLI-CD28).

Figure 5A and 5B: Schematic representation of different strategies based mAb-epitope tagging using for instance the CD20 mimotope for T cell depletion designed to mitigate possible side effects associated with CAR positive cells injection: VI and v2 represents either VH or VL chain respectively, TM: transmembrane domain, L: linker.

(A) extracellular anti-CLLI ligand binding domain part of the multi-chain architecture according to the present invention, which does not include an epitope tagging sequence for sorting or depleting cells; VI: anti-CLLI monoclonal antibody VH; L: GS linker; V2: anti-CLLI monoclonal antibody VH; Hinge: preferably CD8 hinge; TM: preferably FcsRly-TM-IC.

(B) extracellular anti-CLLI domain of the multi-chain architectures according to the invention 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): both architectures presented here correspond to examples 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; the second epitope is inserted between the VH and VL chains, said 2nd epitope being also bordered by 2 at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 2nd epitope.

(D): both architectures presented here correspond to examples 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 also bordered by at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 1st epitope.

(E): one architecture is presented 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): both architectures presented here correspond to examples, 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 and the hinge. These two architectures differ by the linkers used bordering the 2nd epitope.

(G): extracellular anti-CLL1 domains of the multi-chain 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 can be considered where CD34 replaces any other previous CD20 epitopes.

(H): extracellular anti-CLL1 domains of the multi-chain architectures according to the invention, where at least two epitopes are inserted at the extremity of in the extracellular ligand binding domain.
Table 1: Exemplary sequences of the alpha polypeptide component of CLLI multi-chain CAR

<table>
<thead>
<tr>
<th>Functional domains</th>
<th>description</th>
<th>SEQ ID #</th>
<th>Raw amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcERly-SP</td>
<td>signal peptide</td>
<td>SEQ ID NO.1</td>
<td>MAPAMESPTLLCVALLFFAPDGVLA</td>
</tr>
<tr>
<td>CD8α hinge</td>
<td>hinge</td>
<td>SEQ ID NO.2</td>
<td>TTTPAARPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACD</td>
</tr>
<tr>
<td>VH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4SX3Linker</td>
<td>Linker VH-VL</td>
<td>SEQ ID NO.3</td>
<td>GGGSSTGGSAGGGGG</td>
</tr>
<tr>
<td>VL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcERly-TM-IC</td>
<td>Fc Receptor for IgE, alpha chain, transmembrane and intracellular domain</td>
<td>SEQ ID NO.4</td>
<td>FFIPLLVLVILFADVTGFLISTQQQVTFLKIKRTRKGFRLLNPHKPKNPN</td>
</tr>
</tbody>
</table>

Table 2: Exemplary sequences of the beta polypeptide component of CLLI multi-chain CAR

<table>
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<tr>
<th>Functional domains</th>
<th>description</th>
<th>SEQ ID #</th>
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<tbody>
<tr>
<td>FcERly-AITAM</td>
<td>Fc Receptor for IgE, beta chain, without ITAM</td>
<td>SEQ ID NO.5</td>
<td>MDTESN RRAN LALPQEPSSVPASFLEISPEVESSLRLLASSPPLHTWLTVKKEQEFGLVQLTAMICLCFGTVCVSLDISHIEGDFSSFKA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GYPFWGAI FFISGMLSISERRNATYLVRLSLGANTASSIAGGTGILL INLKKSLAYIHSCQKFFFFKF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ASFSTEIVVMLFLTILGLGSASVSLTICGAGEELKGKNKVPE</td>
</tr>
<tr>
<td>41BB-IC</td>
<td>41BB co-stimulatory domain</td>
<td>SEQ ID NO.6</td>
<td>KRGRKKLFLYFKQPFMRPVGTTQEDGCSRFPEEEEGGCEL</td>
</tr>
<tr>
<td>CD28-IC</td>
<td>CD28 co-stimulatory domain</td>
<td>SEQ ID NO.7</td>
<td>RSKRSGGHDYDMNMTPRRPGPTRKHYQPYAPRDFAAAYRS</td>
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### Table 3: Exemplary sequences of the gamma polypeptide component of CLL1 multi-chain CAR

<table>
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</thead>
<tbody>
<tr>
<td>FcεRI γ-SP</td>
<td>signal peptide</td>
<td>SEQ ID NO.8</td>
<td>MIPAVVLLLLLVEQAAA</td>
</tr>
<tr>
<td>FcεRIγ-ΔITAM</td>
<td>Fc Receptor for IgE, gamma chain, without ITAM</td>
<td>SEQ ID NO.9</td>
<td>LGEPQLCYILDAILFLYGLVLTLYCR LKIQRKAAITSEYK</td>
</tr>
<tr>
<td>CD3ζ-IC</td>
<td>CD3zeta intracellular domain comprising ITAM</td>
<td>SEQ ID NO.10</td>
<td>RVKFSRSDAPAYQGQNQNLYN ELNLGRREEYDVLKRRRGRDPGRKKPRRPQNPEGLYNELQDKDM AEAYSEIMGKERRRNGKHGDGLY QGLSTAKDTYDHALHMQALPR</td>
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### Table 4: Skip peptides linking the polypeptides forming the multi-subunit CAR

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<td>GSG-P2A</td>
<td>GSG-P2A ribosomal skip peptide</td>
<td>SEQ ID NO.11</td>
<td>GSGATNFSLLKQAGDVEENPGP</td>
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<tr>
<td>GSG-T2A</td>
<td>GSG-T2A ribosomal skip peptide</td>
<td>SEQ ID NO.12</td>
<td>GSGEGRGSLLTCGDVEENPGP</td>
</tr>
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</table>

### Table 5: Sequence of variable regions of exemplary anti-CLL1 VH and VL chains, and their respective CDRs

<table>
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<tr>
<th>ScFv sequences</th>
<th>SEQ ID #</th>
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<td>SC02-357 heavy chain variable region</td>
<td>SEQ ID NO. 13</td>
<td>QVQLQESGPGLVKPSETLSLTCVVSGLSISSSNNWWSWRQPPGKLE WIGEYHSQSPYNPSLKSRTISVDSKNSQFSLKSSVTADA7VYSSS GFFDYWGQGLTLVTSS</td>
</tr>
<tr>
<td>CDR1</td>
<td>SEQ ID NO.37</td>
<td>GSISSSNWWS</td>
</tr>
<tr>
<td>CDR2</td>
<td>SEQ ID NO.38</td>
<td>WIGEYHSQSPDY</td>
</tr>
<tr>
<td>CDR3</td>
<td>SEQ ID NO.39</td>
<td>KVSTGGFFDY</td>
</tr>
<tr>
<td>SC02-378 heavy chain variable region</td>
<td>SEQ ID NO.14</td>
<td>QVQLQESGPGLVKPSETLSLTCVVSGLSISSSNNWWSWRQPPGKLE WIGEYHSQSPYNPSLKSRTISVDSKNSQFSLKSSVTADA7VYSSS GFFDYWGQGLTLVTSS</td>
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<td>CDR1</td>
<td>SEQ ID NO.40</td>
<td>GSISSNNWWS</td>
</tr>
<tr>
<td>CDR2</td>
<td>SEQ ID NO.41</td>
<td>WIGEYHSQSPNY</td>
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<td>CDR3</td>
<td>SEQ ID NO.42</td>
<td>RSSSGFFFDY</td>
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<td>SC02-161 heavy chain variable region</td>
<td>SEQ ID NO. 15</td>
<td>QVQLQESGPGLVKPSETLSLTCVVSGLSISSSNNWWSWRQPPGKLE WIGEYHSQSPYNPSLKSRTISVDSKNSQFSLKSSVTADA7VYSSS GFFDYWGQGLTLVTSS</td>
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Unless specifically defined herein, all technical and scientific terms used have the same meaning as commonly understood by a skilled artisan in the fields of gene therapy, biochemistry, genetics, and molecular biology.

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


The present invention relates to:

1) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least:
   - a first transmembrane polypeptide comprising at least one extracellular ligand-binding domain, wherein the at least one extracellular ligand-binding domain binds to the cell surface CLL1 antigen; and;
- a second polypeptide comprising at least one signal-transducing domain;

wherein the signal transducing domain(s) of the multi-chain Chimeric Antigen Receptor is present on a polypeptide distinct from that carrying the extracellular ligand-binding domain(s).

2) The CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 1, wherein said signal-transducing domain containing polypeptide is a transmembrane polypeptide.

3) The CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 1 or embodiment 2, wherein at least one transmembrane polypeptide comprises a part of Fc receptor.

4) The CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 3, wherein said part of Fc receptor is selected from the group consisting of: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain.

5) The CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 3 or embodiment 4, wherein a transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) fused to an extracellular CLL1 ligand binding domain.

6) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) according to any one of embodiment 3 to embodiment 5 further comprising:
- said second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain;

7) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) according to any one of embodiment 3 to embodiment 6, further comprising:
- a third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain.

8) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 7, wherein said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy (V_H) and light (V_L) chains conferring specificity to CLL1.

9) A CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 8, wherein said V_H comprises a polypeptide sequence displaying at least 90% identity to one selected SEQ ID NO. 13, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35.
10) A CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 8, wherein said V_L comprises a polypeptide displaying at least 90 % identity to one selected from SEQ ID NO. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36.

11) A CLL1 specific multi-chain Chimeric Antigen Receptor of any one of embodiment 4 to embodiment 10, wherein said alpha chain of FCERI is fused to said extracellular ligand-binding domain by a hinge from CD8a, IgGl or FCRIIa proteins.

12) A CLL1 specific multi-chain Chimeric Antigen Receptor of embodiment 11, wherein said hinge comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO. 2.

13) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 3 to 12, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the TCR zeta chain, the FCERβ chain, the FcRIγ chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM).

14) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 13, wherein said signal transducing domain is from CD3zeta.

15) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 14, wherein said signal transducing domain comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO. 10.

16) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 15, wherein said second or third polypeptide comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule selected from CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, CD8, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

17) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 16, wherein said co-stimulatory domain is from 4-1BB and comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO. 6.

18) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 16, wherein said co-stimulatory domain is from CD28 and comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO. 7.
19) A CLL1 specific multi-chain Chimeric Antigen Receptor according to anyone of embodiments 1 to 18, wherein at least one epitope is inserted in at least one of the extracellular domain(s) of said CAR.

20) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 19, wherein said at least one epitope is inserted in one extracellular ligand binding domain of said CAR.

21) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 20, wherein said at least one epitope is inserted in the extracellular domain of said CAR that binds CLL1.

22) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 19 to 21, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

23) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 22, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.

24) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 23, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes.

25) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 23, wherein the extracellular binding domain comprises one of the following sequences:

Vi-L1-VHLK-Epitope - (L) x;

V1-L1-V2-(L) x - Epitope2-(L) x;

V1-L1-V2-(L) x - Epitope2-(L) x - Epitope3-(L) x;

(L) x - Epitope-iLK - Vi-L1-V2;

(L) x - Epitope-(L) x - Epitope2-(L) x - V1-L1-V2;

Epitope-(L) x - Epitope2-(L) x - Epitope3-(L) x - V1-L1-V2;

25) A CLL1 specific multi-chain Chimeric Antigen Receptor according to embodiment 23, wherein the extracellular binding domain comprises one of the following sequences:

(L) x - Epitope-(L) x - V1-L1-V2 - Epitope2-(L) x;

(L) x - Epitope-(L) x - V1-L1-V2 - Epitope3-(L) x;

(L) x - Epitope-(L) x - V1-L1-V2 - Epitope4-(L) x;

(L) x - Epitope2-(L) x - V1-L1-V2 - Epitope3-(L) x;
(L)_x·Epitopel-(L)_y·Epitope2-(L)_z·Epitope3-(L)_ω·Epitope4-(L)_η;

VHLK·Epitopel·(L)_ω·V_2;

V_1·(L)_x·Epitopel-(L)_y·V_2·(L)_z·Epitope2-(L)_η;

V_1·(L)_x·Epitopel-(L)_y·V_2·(L)_z·Epitope2-(L)_η·Epitope3-(L)_ω;

V_1·(L)_x·Epitopel-(L)_y·V_2·(L)_z·Epitope2-(L)_η·Epitope3-(L)_ω·Epitope4-(L)_η;

(L)_x·Epitopel-(L)_y·V_1·(L)_x·Epitope2-(L)_η·V_2·(L)_z·Epitope3-(L)_ω;

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 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.

26) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 22,

wherein the extracellular binding domain comprises the following sequence:

V_1·L_1·V_2·L·Epitope; V_1·L_1·V_2·L·Epitope-L; V_1·L_1·V_2·L·Epitope-L·Epitope2; V_1·L_1·V_2·L·

Epitope-L·Epitope2-L; V_1·L_1·V_2·L·Epitope-L·Epitope2-L·Epitope3; V_1·L_1·V_2·L·Epitope-L·

Epitope2-L·Epitope3-L; V_1·L_1·V_2·Epitope; V_1·L_1·V_2·Epitope-L; V_1·L_1·V_2·Epitope-L·

Epitope2; V_1·L_1·V_2·Epitope-L·Epitope2-L; V_1·L_1·V_2·Epitope-L·Epitope2-L·Epitope3; V_1·

L_1·V_2·Epitope-L·Epitope2-L·Epitope3-L; Epitope -V_1·L_1·V_2·L·Epitope-L·V_1·L_1·V_2·L·

Epitope2·V_1·L_1·V_2; L·Epitope-L·Epitope2·V_1·L_1·V_2; Epitope-L·Epitope2·V_1·L_1·V_2;

Epitope2·L·V_1·L_1·V_2·L·Epitope2·V_1·L_1·V_2; L·Epitope-L·Epitope2·L·V_1·L_1·V_2;

Epitope-L·Epitope2-L·Epitope3·V_1·L_1·V_2; Epitope-L·Epitope2-L·Epitope3·L·V_1·L_1·V_2;

Epitope-L·Epitope2-L·Epitope3·L·V_1·L_1·V_2; L·Epitope-L·Epitope2-L·Epitope3·L·V_1·L_1·V_2;

V_1·L·Epitope-L·V_2; L·Epitope-L·V_2·L·Epitope2·L·V_2; V_1·L·Epitope-L·V_2·L·Epitope2·L·V_1;
L-Epitope1-L-V2-L-Epitope2-L-Epitope3; V1-L-Epitope1-L-V2-L-Epitope2-Epitope3; VI-L-Epitope1-L-V2-L-Epitope2-L-Epitope3-epitope4; L-Epitope1-L-Vi-L-Epitope2-L-V2-L-Epitope3-L; Epi
tope1-L-Vi-L-Epitope2-L-V2-L-Epitope3-L; L-Epitope1-L-Vi-L-Epitope2-L-V2-L-Epitope3-L; L-Epitope1-L-Vi-L-Epitope2-L-V2-
L-Epitope3-L; L-Epitope1-L-Vi-Li-V2-L-Epitope2-L; L-Epitope1-L-Vi-Li-V2-L-Epitope2-L-
Epitope3-L; L-Epitope1-L-Vi-Li-V2-L-Epitope2-Epitope3, or Epitope1-L-Vi-Li-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_I;

L_i 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

differents.

27) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 25 or

26, wherein L_i is a linker comprising Glycine and/or Serine.

28) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 27,

wherein L_i 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.

29) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 27,

wherein L_i is a linker comprising the amino acid sequence (Gly4Ser)_4 or (Gly4Ser)_3.

30) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 27,

wherein L is a linker having an amino acid sequence selected from SGG, GGS, SGGS,

SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGG, SGGGG, SGGS, SGGGGGS;

31) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 30,

wherein L is a SGGGG, GGGGG or SGGGG.

32) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 22,

wherein said mAb-specific epitope(s) is(are) 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, panitumumab, QBEN D-10, alemtuzumab or ustekinumab.

33) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 22, wherein mAb-specific epitope is one comprising an amino acid sequence selected from SEQ ID NO 109, SEQ ID NO 110, SEQ ID NO 111, SEQ ID NO 112, SEQ ID NO 113, SEQ ID NO 114, SEQ ID NO 115 and SEQ ID NO 116.

34) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 25 or 26, wherein Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

35) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 25 or 26, wherein Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

36) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 25 or 26, wherein Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109 or SEQ ID NO 117 or SEQ ID NO 118.

37) A CLLI specific multi-chain Chimeric Antigen Receptor according to embodiment 25 or 26, wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

38) A CLLI specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 37, comprising a polypeptide sequence displaying at least 80 % identity to the full amino acid sequence of anti-CLLI SC02-357, anti-CLLI SC02-378, anti-CLLI SC02-161, anti-CLLI M26, anti-CLLI M31, anti-CLLI G4, anti-CLLI M22, anti-CLLI M29, anti-CLLI M2, anti-CLLI M5, anti-CLLI G12, anti-CLLI 21.26 and anti-CLLI 1075.7 as referred to in Table 6.

39) A polynucleotide comprising a nucleic acid sequence encoding a CLLI specific multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 38.

40) A vector comprising a polynucleotide of embodiment 39.

41) An engineered immune cell expressing at the cell surface membrane an anti-CLLI mcCAR according to any one of embodiments 1 to 38.
42) An engineered immune cell according to embodiment 41, derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

43) An engineered cell according to any one of embodiments 41 or 42 for use in therapy.

44) An engineered cell according to any one of embodiments 412 to 43 for use in therapy, wherein the patient is a human.

45) An engineered cell according to any one of embodiments 41 to 44 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by CLLI-expressing cells.

46) An engineered cell according to any one of embodiments 41 to 45 use in therapy, wherein the condition is a condition which is characterized by an overabundance of CLLI-expressing cells.

47) An engineered cell according to any one of embodiments 41 to 46 for use in therapy, wherein the condition is a hematological cancer condition.

48) An engineered cell according to any one of embodiments 41 to 47 for use in therapy, wherein the hematological cancer condition is leukemia.

49) An engineered cell according to any one of embodiments 41 to 48 for use in therapy, wherein the leukemia is acute myelogenous leukemia (AML).

50) An engineered cell according to any one of embodiments 41 to 49 wherein expression of TCR is suppressed in said immune cell.

51) An engineered cell according to any one of embodiments 41 to 50, wherein expression of at least one MHC protein, preferably β2m or HLA, is suppressed in said immune cell.

52) An engineered cell according to any one of embodiments 41 to 51, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.

53) A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to any one of embodiments 41 to 52 in an amount effective to cause impairment of said cancer cell.

54) A method of engineering an immune cell comprising:
(a) Providing an immune cell;
(b) Expressing at the surface of said cells at least one multi-chain Chimeric Antigen Receptor according to any one of the embodiments 1 to 38.

55) The method of engineering an immune cell of embodiment 54 comprising:

(a) Providing an immune cell;
(b) Introducing into said cell at least one polynucleotide encoding polypeptides composing at least one multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 38;
(c) Expressing said polynucleotides into said cell.

56) The method of engineering an immune cell of embodiment 36 comprising:

(a) Providing an immune cell;
(b) Expressing at the surface of said cell a population of multi-chain Chimeric Antigen Receptors according to any one of the embodiments 1 to 38 each one comprising different extracellular ligand-binding domains.

57) The method of engineering an immune cell of embodiment 56 comprising:

(a) Providing an immune cell;
(b) Introducing into said cell at least one polynucleotide encoding polypeptides composing a population of multi-chain Chimeric Antigen Receptors according to any one of embodiments 1 to 38 each one comprising different extracellular ligand binding domains.
(c) Expressing said polynucleotides into said cell.

58) An isolated immune cell obtainable from the method according to any one of embodiments 54 to 57.

59) An isolated immune cell comprising at least one multi-chain Chimeric Antigen Receptor according to any one of embodiments 1 to 38.

60) An isolated immune cell according to embodiment 58 or 59 for its use as a medicament.
61) An isolated cell according to any one of embodiments 58 to 60 derived from, NK cells, inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

62) A therapeutic composition comprising an isolated immune cell according to any one of embodiments 58 to 61.

63) A method for treating a patient in need thereof comprising:
   a) Providing a immune cell obtainable by a method according to any one of the embodiments 54 to 57;
   b) Administering said T-cells to said patient.

64) The method for treating a patient of embodiment 63, wherein said immune cells are recovered from donors.

   The method for treating a patient of embodiment 63, wherein said immune cells are recovered from patients.

Preliminary definitions

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. More preferably, said domain will be capable of interacting with a CLL1 cell surface molecule.

The term "derived from" means a polypeptide having an amino acid sequence which is equivalent to that an FcE receptor which include one or more amino acid modification(s) of the sequence of the FcE receptor. Such amino acid modification(s) may include amino acid substitution(s), deletion(s), addition(s) or a combination of any of those modifications, and may alter the biological activity of the Fc binding region relative to that of an Fc receptor. On the other hand, Fc binding regions derived from a particular Fc receptor may include one or more amino acid modification(s) which do not substantially alter the biological activity of the Fc binding region relative to that of an Fc receptor. Amino acid modification(s) of this kind will typically comprise conservative amino acid substitution(s).

"identity" refers to sequence identity between two nucleic acid molecules or polypeptides. Identity can be determined by comparing a position in each sequence which may
be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base, then the molecules are identical at that position. A degree of similarity or identity between nucleic acid or amino acid sequences is a function of the number of identical or matching nucleotides at positions shared by the nucleic acid sequences. Various alignment algorithms and/or programs may be used to calculate the identity between two sequences, including FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default setting. For example, polypeptides having at least 70%, 85%, 90%, 95%, 98% or 99% identity to specific polypeptides described herein and preferably exhibiting substantially the same functions, as well as polynucleotide encoding such polypeptides, are contemplated. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity of similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. The polynucleotide sequences of similar polypeptides are deduced using the genetic code and may be obtained by conventional means, in particular by reverse translating its amino acid sequence using the genetic code.

\textit{Anti-CLL1 multi-chain CARs of the invention}

The present invention relates to a multi-chain chimeric antigen receptor (CAR) particularly adapted to immune cells used in immunotherapy.

In particular, the present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least:

- a first transmembrane polypeptide comprising at least one extracellular ligand-binding domain, wherein the at least one extracellular ligand-binding domain binds to the cell surface CLL1 antigen; and;

- a second polypeptide comprising at least one signal-transducing domain;
wherein the signal transducing domain(s) of the multi-chain Chimeric Antigen Receptor is present on a polypeptide distinct from that carrying the extracellular ligand-binding domain(s).

By "a polypeptide distinct from that carrying the extracellular ligand-binding domain(s)" it is meant that there is no peptidic binding between the two polypeptides.

The present invention provides an anti-CLLI multi-chain chimeric antigen receptor (CAR) (CLLI mcCAR anti-CLLI mc) having a structure as illustrated in Figure 2, Figure 3, or Figure 4, and according to claim 1, 2 and/or 3 said structure comprising an extra cellular ligand binding-domain VH and VL from a monoclonal anti-CLLI antibody or CDR sequences.

In a preferred embodiment, said second polypeptide comprising at least one signal-transducing domain containing polypeptide is a transmembrane polypeptide.

In another preferred embodiment, said least one transmembrane polypeptide comprises a part of Fc receptor. More preferably, said part of Fc receptor is selected from the group consisting of: (a) FcERI alpha chain, (b) FcERI beta chain and (c) FcERI gamma chain.

According to an embodiment, said first transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FcERI) which is fused to an extracellular CLLI ligand binding domain.

According to an embodiment, the present invention provides a CLLI specific multi-chain Chimeric Antigen Receptor (mc CAR) as above further comprising:

- said second transmembrane polypeptide from the gamma or beta chain of FcERI which is fused to a signal transducing domain;

According to another embodiment, the present invention provides a CLLI specific multi-chain Chimeric Antigen Receptor (mc CAR) according as above, further comprising:

- A third transmembrane polypeptide from the gamma or beta chain of FcERI comprising a co-stimulatory domain.

The present invention preferably provides a CLLI specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising:

- a first transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FcERI) fused to an extracellular ligand binding domain specifically binding to CLLI comprising a single-chain variable fragment (scFv) comprising a heavy (VH) and a light (VL) chain conferring specificity to CLLI,
- a second transmembrane polypeptide from the gamma or beta chain of FCERI
fused to a signal transducing domain; and
- a third transmembrane polypeptide from the gamma or beta chain of FCERI
comprising a co-stimulatory domain.

In a more preferred embodiment said anti-CLL1 CARs are constructed with these
sequences and correspond to the constructions illustrated in figure 4.

In more particular embodiment, said multi-chain CAR can comprise a part of FCERI alpha
chain and a part of FCERI beta chain or variant thereof such that said FCERI chains spontaneously
dimerize together to form a dimeric Chimeric Antigen Receptor. In another embodiment, the
multi-chain Chimeric Antigen can comprise a part of FCERI alpha chain and a part of a FCERI
gamma chain or variant thereof such that said FCERI chains spontaneously trimerize together
to form a trimeric Chimeric Antigen Receptor, and in another embodiment the multi-chain
Chimeric Antigen Receptor can comprise a part of FCERI alpha chain, a part of FCERI beta chain
and a part of FCERI gamma chain or variants thereof such that said FCERI chains spontaneously
tetramerize together to form a tetrameric Chimeric Antigen Receptor.

As non-limiting example, different versions (architectures) of multi-chain CAR are
illustrated in Figure 3. In a preferred embodiment, two versions (architectures) of multi-chain
CAR are illustrated in Figure 4. In a more preferred embodiment, the multi-chain CARs of the
present invention comprises a polypeptide comprising amino acid sequences as set forth in
Table 6. In another preferred embodiment the multi-chain CAR comprise a polypeptide with
amino acid sequence that has at least 70%, prefera bly at least 80%, more prefera bly at least 90
%, 95 % 97 % or 99 % sequence identity with such amino amino acid sequences or with the
polynucleotide sequence encoding one two or three of the polypeptides constitutive of the
multi-chain polypeptide structure.

The present invention provides a polypeptide encoding a CLL1 specific multi-chain
Chimeric Antigen Receptor as above, comprising a polypeptide sequence displaying at least 80 %
identity to the full amino acid sequence of anti-CLL1 SC02-357, SC02-378, SC02-161, M26, M31,
G4, M22, M29, M2, M5, G12, 21.26 and 1075.7 as referred to in Table 6.

*Extracellular binding domains, hinges and transmembrane domains*
The distinguishing features of appropriate transmembrane polypeptides comprise the ability to be expressed at the surface of an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and to interact together for directing cellular response of immune cell against a predefined target cell. The different transmembrane polypeptides of the multi-chain CAR of the present invention comprising an extracellular ligand-binding domain and/or a signal transducing domain interact together to take part in signal transduction following the binding with a target ligand and induce an immune response. 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 δ, polypeptide constituting CD3 complex, IL2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Feγ receptor II or CD proteins. Alternatively the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above, wherein said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy (V_H) and light (V_L) chains conferring specificity to CLL1.

In a preferred embodiment, said extracellular ligand-binding domain is a single chain antibody fragment (scFv) comprising the light (V_L) and the heavy (V_H) variable fragment of a target antigen specific monoclonal antibody specific to CLL1 joined by a flexible linker. In a preferred embodiment, said scFv is an anti-CLL1 scFv, preferably provided in Table 5 as SEQ ID NO.13 to 36. Binding domain specific to CLL1 other than scFv can also be used for predefined targeting of lymphocytes, such as camelid or shark (VNAR) single-domain antibody fragments or receptor ligands like a vascular endothelial growth factor polypeptide, an integrin-binding peptide, heregulin or an IL-13 mutein, antibody binding domains, antibody hypervariable loops or CDRs as non-limiting examples.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said V_H comprises a polypeptide sequence having at least 80% to at least 90 % identity with one of the polypeptide sequences selected from SEQ ID NO. 13, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above wherein said V_L comprises a polypeptide having at least 80% to at least 90 % identity with
one of the polypeptide sequences selected from SEQ ID NO. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36.

In a more preferred embodiment, the present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor (mcAR) wherein said extra cellular ligand binding-domain comprises a VH from a monoclonal anti-CLL1 antibody containing at least one of the following CDR sequences: GSISSSNWWS (SEQ ID NO 37), WIGEIYHSGSPDY (SEQ ID NO 38), KVSTGGFFDY (SEQ ID NO 39), and GSISSSNWWS (SEQ ID NO 40), WIGEIYHSGSPNY (SEQ ID NO 41), RSSSGGFFDY (SEQ ID NO 42), and GSISSSNWWS (SEQ ID NO 43), WIGEIYHSGSPNY (SEQ ID NO 44), RQTTAGSFDY (SEQ ID NO 45), and GYTFTSYFIH (SEQ ID NO 49), WIGFIN PYNDGSKY (SEQ ID NO 50), TRDDGYYGYAMDY (SEQ ID NO 51), and GYTFTSYVMH (SEQ ID NO 55), WIGYIN PYNDGTKY (SEQ ID NO 56), ARPIYFDN DY (SEQ ID NO 57), and QQN NYDPW (SEQ ID NO 61), WIGPI NPYNDGTI (SEQ ID NO 62), ARTDDYDDYTMIDY (SEQ ID NO 63), and GYTFTRYWMH (SEQ ID NO 67), WIGIN IDPSDTETHY (SEQ ID NO 68), AIYYGN PSYYAMDY (SEQ ID NO 69), and GYIFTSYVMY (SEQ ID NO 73), WIGYI NPY (SEQ ID NO 74), ARYYDYDDYFDY (SEQ ID NO 75), and GYTFTSYFMH (SEQ ID NO 79), WIGFIN PYNDGTKY (SEQ ID NO 80), TRDDGYYDYAMDY (SEQ ID NO 81), and GFNIKDDYIH (SEQ ID NO 85), WIGWI DPEKGDYATYA (SEQ ID NO 86), TLTGRFDY (SEQ ID NO 87), and GYTFPSSNH (SEQ ID NO 91), WIGVIIYPNGDTSY (SEQ ID NO 92), AIYFVYNWHFDV (SEQ ID NO 93), and GYTFTRYWMH (SEQ ID NO 97), M IHPSSGSTSYNEKVK (SEQ ID NO 98), RDGDDYYGTYGDY (SEQ ID NO 99), and GYSITSAYYW (SEQ ID NO 103), YISYDGRNNYN PSLKN (SEQ ID NO 104) and

and comprises a VL from a monoclonal anti-CLL1 antibody containing at least one of the following CDR sequences: QSISSYLN (SEQ ID NO 46), LLIYAASSLQS (SEQ ID NO 47), QQSYSTPP (SEQ ID NO 48), and QELSGLS (SEQ ID NO 52), RLIYAASTLDS (SEQ ID NO 53), LQYAIYPP (SEQ ID NO 54), and ESVDSYGNSFMH (SEQ ID NO 58), LLIYLANLES (SEQ ID NO 59), QQN NYDPW (SEQ ID NO 60), HDISNYLN (SEQ ID NO 64), LLIYTSRLHS (SEQ ID NO 65), QQKTLKLW (SEQ ID NO 66), and QNLNLSNQQKKLYLN (SEQ ID NO 70), LLIYWASTRES (SEQ ID NO 71), QNDYSYPF (SEQ ID NO 72), and QDI NKYIA (SEQ ID NO 76), LLIHYSTLQP (SEQ ID NO 77), LQDYDLYW (SEQ ID NO 78), and QEISVYL (SEQ ID NO 82), RLIYAASTLDS (SEQ ID NO 83), LQYASYPF (SEQ ID NO 84), and QSLLYSSNQNKNLNA (SEQ ID NO 88), LLIYWASTRES (SEQ ID NO 89), QQYSSYR (SEQ ID NO 90), and ESVDGYDIFML (SEQ ID NO 94), LLIYFASNLES (SEQ ID NO 95), QQNN EDPY (SEQ ID NO 96), and RASSSYinMyH (SEQ ID NO 100), PWI FATSNLS (SEQ ID NO 101), QQWRSRALT (SEQ ID NO 102), and RASSNVISSYVH (SEQ ID NO 106), LWIYSTNLS (SEQ ID NO 107) and QQYSGYPLT (SEQ ID NO 108).
The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said alpha chain of FCERI is fused to said extracellular ligand-binding domain by a hinge from CD8a, IgGl or FcRIIa proteins.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said hinge comprises a polypeptide sequence displaying at least 90% identity with SEQ ID NO.2. In one preferred embodiment, said hinge comprises a polypeptide of SEQ ID NO.2.

In a preferred embodiment said first transmembrane polypeptide further comprises a stalk region between said extracellular ligand-binding domain and said transmembrane domain. The term “stalk region” used herein generally means any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, stalk region are used to provide more flexibility and accessibility for the extracellular ligand-binding domain. A stalk region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively the stalk region may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be an entirely synthetic stalk sequence. In a preferred embodiment said stalk region is a part of human CD8 alpha chain (e.g. NP_001139345.1) (SEQ ID NO: 2). Thus, the expression of multi-chain CAR in immune cells results in modified cells that selectively and eliminate defined targets, including but not limited to malignant cells carrying a respective tumor-associated surface antigen or virus infected cells carrying a virus-specific surface antigen, or target cells carrying a lineage-specific or tissue-specific surface antigen.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said alpha chain of FCERI is fused to said extracellular ligand-binding domain by a hinge from CD8a, IgGl or FcRIIa proteins.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said hinge comprises a polypeptide sequence displaying at least 90% identity to SEQ ID NO.2.

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 specific to target, the multi-chain CAR can comprise several 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 multi-chain CAR.

In another embodiment, the present invention relates to a population of multi-chain CARs comprising each one different extracellular ligand binding domains. In a particular one, 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 multi-chain 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 multi-chain CAR each one comprising different extracellular ligand binding domains. In a particular embodiment the method of engineering an immune cell comprises expressing at the surface of the cell at least a part of FCERI beta and/or gamma chain fused to a signal-transducing domain and several part of FCERI alpha chains fused to different extracellular ligand binding domains. In a more particular embodiment, said method comprises introducing into said cell at least one polynucleotide which encodes a part of FCERI beta and/or gamma chain fused to a signal-transducing domain and several FCERI alpha chains fused to different extracellular ligand binding domains. By population of multi-chain CARs, it is meant at least two, three, four, five, six or more multi-chain CARs 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.

Transduction signalling domains

The signal transducing domain or intracellular signaling domain of the multi-chain CAR of the invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the multi-chain CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines. Thus, the term “signal transducing domain” refers
to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the TCR zeta chain, the FCERP chain, the FcεRIγ chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM), preferably said signal transducing domain is from CD3ζ, more preferably comprising a polypeptide sequence of SEQ ID NO. 10.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the TCR zeta chain, the FCERP chain, the FcεRIγ chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM).

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the FCERP chain.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments, wherein said signal transducing domain fused to the gamma or beta chain of FCERI comprises an immunoreceptor tyrosine-based activation motif (ITAM).

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said signal transducing domain is from CD3ζ, preferably the present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said signal transducing domain comprises a polypeptide sequence displaying at least 90% identity to SEQ ID NO. 10. In one embodiment, said signal transducing domain comprises a polypeptide sequence of SEQ ID NO. 10.
Preferred examples of signal transducing domain for use in multi-chain CAR can be the cytoplasmic sequences of the Fc receptor or 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 as 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 multi-chain CAR can comprise the CD3zeta signaling domain, or the intracytoplasmic domain of the FCERI beta or gamma chains.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of the above, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the TCR zeta chain, the FCERB chain, the FCERY chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM).

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said signal transducing domain is from CD3zeta.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said signal transducing domain comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO.10.

In another particular embodiment, said signal transducing domain is a TNFR-associated Factor 2 (TRAF2) binding motifs, intracytoplasmic tail of costimulatory TNFR member family. Cytoplasmic tail of costimulatory TNFR family member contains TRAF2 binding motifs consisting of the major conserved motif (P/S/A)X(Q/E)E or the minor motif (PXQXXD), wherein X is any amino acid. TRAF proteins are recruited to the intracellular tails of many TNFRs in response to receptor trimerization.

In a preferred embodiment, the signal transduction domain of the multi-chain CAR of the present invention comprises a part of co-stimulatory signal molecule selected from the group consisting of 4-1BB (GenBank: AAA53133,) and CD28 (NP_006130.1).
Co-stimulatory domains

In particular embodiment, the signal transduction domain of the multi-chain CAR of the present invention comprises a co-stimulatory signal molecule. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient immune response.

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

The present invention is related to a CLL1 specific multi-chain Chimeric Antigen Receptor as any of the above embodiment, wherein said second or third polypeptide comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule selected from CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/1R6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

In a preferred embodiment, the present invention is related to a CLL1 specific multi-chain Chimeric Antigen Receptor as any of the above embodiment, wherein said second or third polypeptide comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule from CD28.

In a preferred embodiment, the present invention is related to a CLL1 specific multi-chain Chimeric Antigen Receptor as any of the above embodiment, wherein said second or third polypeptide comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule from 4-1BB.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as above, wherein said co-stimulatory domain is from 4-1BB and comprises a polypeptide
sequence displaying at least 90% identity with SEQ ID NO. 6. In one embodiment, said co-
stimulatory domain is from 4-1BB and comprises a polypeptide sequence of SEQ ID NO. 6.

The present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor as
above, wherein said co-stimulatory domain is from CD28 and comprises a polypeptide sequence
displaying at least 90% identity to SEQ ID NO. 7.

**Examply CARs**

In one embodiment, the present invention provides a CLL1 specific multi-chain Chimeric
Antigen Receptor (mc CAR) comprising:

- a transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) fused to an extracellular CLL1 ligand binding domain,
- a second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain; and
- a third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain.

wherein said extra cellular ligand binding-domain comprising a VH from a monoclonal
anti-CLL1 antibody containing at least one of the following CDR sequences: GSISSSNWWS (SEQ
ID NO 37), WIGEIYHSGPDDY (SEQ ID NO 38), KVSTGFFGDY (SEQ ID NO 39), and GSISSSNWWS
(SEQ ID NO 40), WIGEITGSGLSPNY (SEQ ID NO 41), RISSGGFDDY (SEQ ID NO 42), and
GSISSSNWWS (SEQ ID NO 43), WIGEIYHSGPSPNY (SEQ ID NO 44), RQTTAGSFDY (SEQ ID NO 45),
and GYFTSYFIH (SEQ ID NO 49), WIGFINPYNDSK (SEQ ID NO 50), TRDDGYYGAMYDY (SEQ ID
NO 51), and GYFTSYVMH (SEQ ID NO 55), WIGYINPYNDCNTKY (SEQ ID NO 56), ARPPYFDNY (SEQ
ID NO 57), and QQN YNPW (SEQ ID NO 61), WIGIPNYPNDGTI (SEQ ID NO 62), ARTDDYDDTMDY
(SEQ ID NO 63), and GYFTFRYWMH (SEQ ID NO 67), WIGN IDPSDTETHY (SEQ ID NO 68),
AIYYGNSYYAMDY (SEQ ID NO 69), and GYFTSYVMY (SEQ ID NO 73), WIGYIN PY (SEQ ID NO 74),
ARYYDYDFDY (SEQ ID NO 75), and GYFTSYFMMH (SEQ ID NO 79), WIGFINPYNDCNTKY (SEQ ID
NO 80), TRDDGYYDAMDY (SEQ ID NO 81), and GFIN KKKYDH (SEQ ID NO 85),
WIGWI DPEKGDTAYA (SEQ ID NO 86), TLTRFDY (SEQ ID NO 87), and GYTPPSNH (SEQ ID NO
91), WIGVYIPNNGDTSY (SEQ ID NO 92), AYFVYNHFDV (SEQ ID NO 93), and GYTFTRYWMH
(SEQ ID NO 97), M IHPSSGTSTSYEKVK (SEQ ID NO 98), RDGDYHYTGDY (SEQ ID NO 99), and
YSITSAAYWN (SEQ ID NO 103), YISYDGRNNYNPSLKN (SEQ ID NO 104) and
AKEGDDVDNGNYYAMDY (SEQ ID NO 105);
and comprising a VL from a monoclonal anti-CLL1 antibody containing at least one of the following CDR sequences: QSISYLN (SEQ ID NO 46), LLIYAASLSQ (SEQ ID NO 47), QQSYSTPP (SEQ ID NO 48), and QELSGYLS (SEQ ID NO 50), RLIYASTLDS (SEQ ID NO 53), LQAYAIYPY (SEQ ID NO 54), and ESVDSYGNSFMH (SEQ ID NO 58), LLIYLASNNES (SEQ ID NO 59), QQNYDPW (SEQ ID NO 60), HDISNYLN (SEQ ID NO 64), LLIYTSRLHS (SEQ ID NO 65), QQKTLLLW (SEQ ID NO 66), and QNLLNSGNQKKYL (SEQ ID NO 70), LLIYWASTRES (SEQ ID NO 71), QNDYSYPF (SEQ ID NO 72), and QDIKNYIA (SEQ ID NO 76), LLIYHTSTLQP (SEQ ID NO 77), LQYDYLW (SEQ ID NO 78), and QEQVYLS (SEQ ID NO 82), RLIYASTLDS (SEQ ID NO 83), LQYASYPY (SEQ ID NO 84), and QGLYSSNQNNLA (SEQ ID NO 88), LLIYWASTRES (SEQ ID NO 89), QQYYSYR (SEQ ID NO 90), and ESVDGYGDIFML (SEQ ID NO 94), LLIYFASNLAS (SEQ ID NO 95), QQQNEDPY (SEQ ID NO 96), and RASSIINYMH (SEQ ID NO 100), PWIFATSNLAS (SEQ ID NO 101), QQWRSNDALT (SEQ ID NO 102), and RASSNVISYVH (SEQ ID NO 106), LWIYSTSNLAS (SEQ ID NO 107) and QQYSGYPLT (SEQ ID NO 108).

In one embodiment, the present invention provides a CLL1 specific multi-chain Chimeric Antigen Receptor (CLL1 m c CAR) comprising:

- a first transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) fused to an extracellular CLL1 ligand binding domain,

- a second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain; and

- a third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain.

wherein said extra cellular ligand binding-domain comprising a VH from a monoclonal anti-CLL1 antibody containing at least one of the following CDR sequences: GSIISSNWWS (SEQ ID NO 37), WIGEIYHSGBPDY (SEQ ID NO 38), KVSTGGFFDY (SEQ ID NO 39), and GSIISSNWWS (SEQ ID NO 40), WIGEIYHSGBPNY (SEQ ID NO 41), RSSSGGFFDY (SEQ ID NO 42), and GSIISSNWWS (SEQ ID NO 43), WIGEIYHSGBPNY (SEQ ID NO 44), RQTTAGSFDY (SEQ ID NO 45), and GYFTTSYFHIH (SEQ ID NO 49), WIGFIN PYNDBGSKY (SEQ ID NO 50), TRDDGYGYAM DY (SEQ ID NO 51), and GYFTTSYVMH (SEQ ID NO 55), WIGIYNPDNTKY (SEQ ID NO 56), ARPIYFDNDY (SEQ ID NO 57), and QQNYDPW (SEQ ID NO 61), WIGPI NPYNNDGTI (SEQ ID NO 62), ARTDDYDDYTMDY (SEQ ID NO 63), and GYFTTRYWMH (SEQ ID NO 67), WIGN IDPSDTETHY (SEQ ID NO 68), AIYYGNPSYYAMDY (SEQ ID NO 69), and GYIFTSYVMY (SEQ ID NO 73), WIGYIN PY (SEQ ID NO 74), ARYYDYDDYFDY (SEQ ID NO 75), and GYFTTSYFMH (SEQ ID NO 79), WIGFIN PYNPDGTKY (SEQ ID NO 80).
NO 80), TRDDGYDYAM DY (SEQ ID NO 81), and GFNIKDDYIH (SEQ ID NO 85), WIGWI DPEKGDTAYA (SEQ ID NO 86), TLTGRFDY (SEQ ID NO 87), and GYTFPSN1H (SEQ ID NO 91), WIGVIYPGNGDTSY (SEQ ID NO 92), AIYFVNWHFDV (SEQ ID NO 93), and GYTFTRYWMH (SEQ ID NO 97), MHPSSGTSYNEKVK (SEQ ID NO 98), RGDYYGTGDY (SEQ ID NO 99), and GYSITSAYYWN (SEQ ID NO 103), YISYDGRN NYP SLKN (SEQ ID NO 104) and AKEGDYDVGNYAMD Y (SEQ ID NO 105):

and comprising a VL from a monoclonal anti-CLL antibody containing at least one of the following CDR sequences: QSISSYLN (SEQ ID NO 46), LLI YASSLOS (SEQ ID NO 47), QQYSTPPP (SEQ ID NO 48), and QELSGYLS (SEQ ID NO 52), RLIAA STLDS (SEQ ID NO 53), LQYAIYPY (SEQ ID NO 54), and ESVDSYGNSFMH (SEQ ID NO 58), LLIYLA SLES (SEQ ID NO 59), QQN YDPW (SEQ ID NO 60), HDISYLN (SEQ ID NO 64), LLIYYTSRLHS (SEQ ID NO 65), QQKTL LW (SEQ ID NO 66), and QNLLNSGNQKKYL N (SEQ ID NO 70), LLIYWASTRES (SEQ ID NO 71), QNDYSYPF (SEQ ID NO 72), and QDINKY IA (SEQ ID NO 76), LLHYTSTLP (SEQ ID NO 77), L OYDYLW (SEQ ID NO 78), and QEISYVLS (SEQ ID NO 82), RLIAA STLDS (SEQ ID NO 83), LQYASYPY (SEQ ID NO 84), and QSLLYSSNQKNNL A (SEQ ID NO 88), LLIYWASTRES (SEQ ID NO 89), QQYYSYR (SEQ ID NO 90), and ESVDGYDIFML (SEQ ID NO 94), LLIFASNL ES (SEQ ID NO 95), QQNN EDPY (SEQ ID NO 96), and RASSSINMYM (SEQ ID NO 100), PW I FATSNLAS (SEQ ID NO 101), QQWRS DRLT (SEQ ID NO 102), and RASSNVISSY VH (SEQ ID NO 106), LWIYSTSNLAS (SEQ ID NO 107) and QQGSGYPLT (SEQ ID NO 108).

and a hinge between VH and VL (alpha chain),

- wherein said signal transducing domain (or cytoplasmic transmembrane domain) comprises a CD3 zeta signaling domain (gamma chain), and
- wherein said co-stimulatory domain comprises a co-stimulatory transmembrane domain from 4-1BB or CD28 (beta chain).

The present invention provides CLL1 specific multi-chain Chimeric Antigen Receptors (CLL1 mc CARs) as any of the above embodiments, comprising the peptide sequences according to Table 6 as follows, and wherein the polypeptide sequences has at least 80% identity with the following peptide sequences:

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.14, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.19, SEQ ID NO.3, SEQ ID NO.20, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.19, SEQ ID NO.3, SEQ ID NO.20, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.21, SEQ ID NO.3, SEQ ID NO.22, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.21, SEQ ID NO.3, SEQ ID NO.22, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.23, SEQ ID NO.3, SEQ ID NO.24, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.25, SEQ ID NO.3, SEQ ID NO.26, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.27, SEQ ID NO.3, SEQ ID NO.28, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.28, SEQ ID NO.3, SEQ ID NO.29, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.I, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID NO.10, SEQ ID NO.II, SEQ ID N0.2, SEQ ID N0.31, SEQ ID N0.3, SEQ ID N0.32, SEQ ID N0.4, SEQ ID NO.12, SEQ ID N0.5, and SEQ ID N0.7 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID NO.10, SEQ ID NO.II, SEQ ID NO.I, SEQ ID N0.2, SEQ ID N0.33, SEQ ID N0.3, SEQ ID N0.34, SEQ ID N0.4, SEQ ID NO.12, SEQ ID N0.5, and SEQ ID N0.6 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID NO.10, SEQ ID NO.II, SEQ ID NO.I, SEQ ID N0.2, SEQ ID N0.33, SEQ ID N0.3, SEQ ID N0.36, SEQ ID N0.4, SEQ ID NO.12, SEQ ID N0.5, and SEQ ID N0.6 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID NO.10, SEQ ID NO.II, SEQ ID NO.I, SEQ ID N0.2, SEQ ID N0.35, SEQ ID N0.3, SEQ ID N0.36, SEQ ID N0.4, SEQ ID NO.12, SEQ ID N0.5, and SEQ ID N0.7.

In a preferred embodiment, the present invention provides CLLI specific multi-chain Chimeric Antigen Receptors (CLLI mc CARs), wherein the polypeptide sequences has at least 90% identity with the preceeding peptide sequences.

In a more preferred embodiment, the present invention provides CLLI specific multi-chain Chimeric Antigen Receptors (CLLI mc CARs), wherein the polypeptide sequences has at least 95% identity with the preceeding peptide sequences.

In the most preferred embodiment, the present invention provides CLLI specific multi-chain Chimeric Antigen Receptors (CLLI mc CARs), wherein the polypeptide sequences has at least 99% identity with the preceeding peptide sequences.

In all the above embodiments, said CLLI specific multi-chain Chimeric Antigen Receptor (CLLI mc CAR), retains, continuously or temporarily, their properties of binding to CLLI expressing cells and/or to affect the survival of said CLLI expressing cancer cells.

**Insertion of at least one epitope in the extracellular domain of the anti-CLLI multi-chain CAR**
An anti-CLL CAR of the invention may include at least the insertion of at least one epitope in one extracellular domain of said CAR, preferably the extracellular domain binding CLLI as illustrated in Figure. This is intended to deplete the immune cells endowed with the CAR in the event these later would cause adverse effects in vivo such as a cytokine storm. Moreover, such insertion of epitope or "epitope-tagging" may be useful to sort in vitro engineered immune cells for sake of purification. 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. 110), into the CAR polypeptide sequence. Different positions of the at least one CD20 mimotope are schematized in Figure 5.

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 VH chain.

In one embodiment, said at least one epitope is inserted between the VH and VL chains of the anti-CLL 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.

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.

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

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

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

According to another embodiment, two epitopes are inserted 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.
According to another embodiment, three epitopes are inserted 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.

Said linker may be the GS linker of SEQ ID NO. 10 or the like.

Said at least one epitope may be any antigenic peptide which is enough immunogenic to be bound by a specific antibody recognizing such peptide.

In a preferred embodiment, the epitope introduced within the chimeric scFv is the CD20 antigen (SEQ ID NO. 110) and the infused mAb which is being used to target it - for sorting and/or depletion purpose(s) is rixutimab.

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 (Riener et al., 2005), or a 24 aa for palivizumab (Arbiza e 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 7.
**Table 7**: Mimotopes and epitope with their corresponding mAb

<table>
<thead>
<tr>
<th></th>
<th>Mimotope</th>
<th>SEQ ID NO</th>
<th>Epitope C</th>
<th>SEQ ID NO</th>
<th>Epitope 1</th>
<th>SEQ ID NO</th>
<th>Epitope 2</th>
<th>SEQ ID NO</th>
<th>Epitope 3</th>
<th>SEQ ID NO</th>
<th>Epitope 4</th>
<th>SEQ ID NO</th>
<th>Rituximab</th>
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<td><strong>Rituximab</strong></td>
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<td></td>
<td>109</td>
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<tr>
<td>Mimotope</td>
<td>SEQ ID NO</td>
<td>109</td>
<td>CPYSNPSLC</td>
<td></td>
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<tr>
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<td>CQFDLSTRRLKC</td>
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<td>Mimotope 2</td>
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<td>112</td>
<td>CQYNLSRALKC</td>
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<td>CVWQRWOQSKYVC</td>
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<td><strong>Nivolumab</strong></td>
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<tr>
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</table>

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). The anti-CLL1 CAR of the present invention may thus 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 a preferred embodiment of the invention, the epitope introduced within the chimeric scFv is the CD20 mimotope of SEQ ID NO. 109 and the corresponding antibody used for depleting the CAR positive cells into the patient is rituximab.

In general, the term “linker” as used in the context of a scFv refers to a peptide 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 \((\text{Gly-Gly-Gly-Ser})_n\) or \((\text{Gly-Gly-Gly-Gly-Ser})_n\), 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 polypeptide linkers include, but are not limited to, \((\text{Gly-Ser})_n\) or \((\text{Gly-Ser})_3\). In another embodiment, the linkers include multiple repeats of \((\text{Gly-Ser})_n\), where \(x=1, 2, 3, 4, 5, 6, 7, 8, 9\) or 10, such as multiple repeats of \((\text{Gly-Ser}), (\text{Gly}_2\text{Ser})\) or \((\text{Gly}_5\text{Ser})\). Also included within the scope of the invention are linkers described in WO2012/138475, incorporated herein by reference.

According to one embodiment, the present invention relates to a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least:

- a first transmembrane polypeptide comprising at least one extracellular ligand-binding domain, wherein the at least one extracellular ligand-binding domain binds to the cell surface CLL1 antigen and comprises at least one epitope; and;

- a second polypeptide comprising at least one signal-transducing domain;

wherein the signal transducing domain(s) of the multi-chain Chimeric Antigen Receptor is present on a polypeptide distinct from that carrying the extracellular ligand-binding domain(s).

In a preferred embodiment, said anti-CLL1 mcCAR contains said signal-transducing domain containing polypeptide is a transmembrane polypeptide, and at least one transmembrane polypeptide comprises a part of Fc receptor.

In a preferred embodiment, the present invention relates to a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain,

the transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) is fused to an extracellular CLL1 ligand binding domain, and,

said extracellular CLL1 ligand binding domain contains at least one epitope.
In another preferred embodiment, the present invention relates to a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain,

the transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) is fused to an extracellular CLL1 ligand binding domain,

second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain, preferably CD3 ITAM;

third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain, preferably 4-1 BB costimulatory domain,

said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy ($V_H$) and light ($V_L$) chains conferring specificity to CLL1, and,

said extracellular CLL1 ligand binding domain contains at least one epitope.

In one embodiment, said previous CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprises said FCERI alpha chain in which one epitope is inserted between the 2 scFvs in its extracellular domain, said epitope being optionally bordered by one linker.

In another preferred embodiment, the present invention relates to a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain,

the transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) is fused to an extracellular CLL1 ligand binding domain,

second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain;

third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain,

said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy ($V_H$) and light ($V_L$) chains conferring specificity to CLL1, and,
said scFvs being linked to the transmembrane (TM) domain of said FCERI alpha chain by a hinge, preferably IgGl, CD8alpha or FCYRII a hinge, and

said extracellular CLL1 ligand binding domain contains two epitopes.

In one embodiment, said previous CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprises said FCERI alpha chain in which two epitopes are inserted in the extracellular domain of the CAR, one being inserted between the N-terminal end of the CAR and the VH chain, said epitope being optionally bordered by 2 linkers; the second epitope is inserted between the 2 scFvs, said 2nd epitope being optionally bordered by 2 linkers.

In another embodiment, said CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprises said FCERI alpha chain in which two epitopes are inserted in the extracellular domain of the CAR, one being inserted between the two scFvs; the other epitope being inserted between the VL chain and the hinge, each said epitope being optionally bordered by 2 linkers.

In another embodiment, said CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprises said FCERI alpha chain in which two epitopes are inserted in the extracellular domain of the CAR, one being inserted between the N-terminal end of the CAR and the VH chain, said epitope being optionally bordered by 2 linkers; the second epitope being inserted between the VL chain and the hinge, said 2nd epitope being optionally bordered by 2 linkers.

In another preferred embodiment, the present invention relates to a CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain,

the transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) is fused to an extracellular CLL1 ligand binding domain,

second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain;

third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain,
said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy (V_H) and light (V_L) chains conferring specificity to CLL1. and,

said extracellular CLL1 ligand binding domain contains three epitopes.

In another embodiment, said previous CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprises said FCERI alpha chain in which three epitopes are inserted in the extracellular domain of the CAR, the first one being inserted between the N-terminal end of the CAR and the VH chain, said epitope being optionally bordered by 2 linkers; the second epitope being inserted between the 2 scFvs, said epitope being optionally bordered by 2 linkers, and the third epitope being inserted between the VL chain and the hinge.

Said at least one epitope may be chosen preferably among those for which a corresponding monoclonal antibody exists and is approved by the National Health Organization (such as FDA). For instance, said epitope may be chosen among SEQ ID NO.109 to SEQ ID NO.116. Another epitope which may be selected is a CD34 epitope such as those of SEQ ID NO.117 or II8.

In a particular embodiment, said above anti-CLL1 mcCARs comprising at least an extracellular ligand binding-domain including VH and VL domains of monoclonal anti-CLL1 antibodies.

The present invention relates also to a method for depleting in a patient engineered lymphoid immune cell expressing a CLL1 specific mcCAR and at least one epitope such as disclosed in this application, by administering in said patient an antibody -preferably monoclonal- specific to said epitope in case of need, i.e. to avoid adverse effects such as cytokine storm.

In a preferred embodiment, the monoclonal antibody rituximab specific to the at least one CD20 antigen inserted in the extracellular domain of the CLL1 specific mcCAR is administered to the patient in order to deplete said engineered immune cells.

More specifically, the epitopes can be included into the extracellular domain of the CAR according to the present invention as follows:
In some embodiments, the extracellular binding domain comprises at least 1, 2, 3, 4, 5, 6, 7, 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.

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.

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):

\[ \text{VI} - L_1 - V_2 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ V_1 - L_1 - V_2 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
\[ (L)_x - \text{Epitope} - (L)_y - V_1 - L_1 - V_2 - V_3 - (L)_x - \text{Epitope} - (L)_y ; \]
V_1-(L)x-Epitope-(L)x-V_2;

V_1-(L)x-Epitope-(L)x-V_2-(L)x-Epitope2-(L)x;

V_i-L-Epitope-(L)x-V_i-Epitope2-(L)x;

Vi-i-L-Epitope-igx^-igx-Epitope2-igx-Epitope3-igx-Epitope^iLlx;

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(L)x-Epitope-(L)x-V_1-(L)x-Epitope2-(L)x-V_2;

(Llx-Epitope-igx-Vi-igx-Epitope2-igx-Vj-igx-Epitope3-ilLx;

Vi-Li-V_2-L-Epitope;

Vi-Li-V_2-L-Epitope-L;

Vi-Li-V_2-L-Epitope-L-Epitope2;

Vi-Li-V_2-L-Epitope-L-Epitope2-L;

Vi-Li-V_2-L-Epitope-L-Epitope2-L-Epitope3;

Vi-Li-V_2-L-Epitope-L-Epitope2-L-Epitope3-L;

Vi-Li-V_2-Epitope;

Vi-Li-V_2-Epitope-L;

Vi-Li-V_2-Epitope-L-Epitope2;

Vi-Li-V_2-Epitope-L-Epitope2-L;

Vi-Li-V_2-Epitope-L-Epitope2-L-Epitope3;

Vi-Li-V_2-Epitope-L-Epitope2-L-Epitope3-L;

Epitope-Vi-Li-V_2;

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Epitope-L-Vi-Li-V_2;

L-Epitope-Vi-Li-V_2;

L-Epitope-L-Vi-Li-V_2;

Epitope-L-Epitope2-Vi-Li-V_2;
wherein,

\( V_1 \) and \( V_2 \) are \( V_H \) and \( V_L \) of an ScFv (i.e., \( V_1 \) is \( V_L \) and \( V_2 \) is \( V_H \) or \( V_1 \) is \( V_H \) and \( V_2 \) is \( V_L \));

\( L_i \) 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,

\[ x = 0 \text{ or } 1 \text{ and each occurrence of } x \text{ is independently from the others}; \text{ and,} \]

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):

\[ \begin{align*}
V_H^1 & \cdot L \cdot V_L^1 \cdot \text{Epitope1} \cdot \text{Epitope2} \cdot L; \\
L \cdot \text{Epitope1} \cdot L \cdot V_H^2 \cdot L \cdot \text{Epitope2} \cdot L \cdot \text{Epitope3} \cdot L; \\
V_L^1 \cdot L^1 \cdot V_H^1 & \cdot L \cdot \text{Epitope1} \cdot \text{Epitope2} \cdot L; \text{ or,} \\
L \cdot \text{Epitope1} \cdot L \cdot V_L^1 \cdot L \cdot \text{Epitope2} \cdot L \cdot V_H^1 \cdot L \cdot \text{Epitope3} \cdot L
\end{align*} \]

wherein \( L, L_1, \) epitope 1, epitope 2 and epitope 3 are as defined above.

In some embodiments, \( L_1 \) is a linker comprising Glycine and/or Serine. In some embodiment, \( L_2 \) is a linker comprising the amino acid sequence \((\text{Gly-Gly-Ser})_n\) or \((\text{Gly-Gly-Gly-Gly-Ser})_n\), where \( n = 1, 2, 3, 4 \) or 5. In some embodiments \( L_3 \) is \((\text{Gly}_n\text{Ser})_4\) or \((\text{Gly}_n\text{Ser})_3\).

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, SGGGGS, GGGGGS, SGGGGG, SGGGGS, GGGGGG, SGGGGGG, or SGGGGGGS preferably SGG, SGGS, SSGGS, GGGG, SGGGGS, SGGGGG, GGGGGG, or SGGGGGGS GGGGGGS. In some embodiment, when the extracellular binding 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 SGGGGG. In some embodiments, the extracellular binding domain comprises several occurrences of \( L \) and all the \( L \)s are SGGGGG.
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 SEQ ID NO 109, SEQ ID NO 110, SEQ ID NO 111, SEQ ID NO 112, SEQ ID NO 113, SEQ ID NO 114, SEQ ID NO 115 or SEQ ID NO 116.

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-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.

In some embodiment, Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

In some embodiment, Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

In some embodiment, Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109.

In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 109 and Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 117.

In some embodiment, one of Epitope 1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID NO 117 or SEQ ID NO 118. In some embodiment, one of Epitope 1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID NO 117 or SEQ ID NO. 118 and the other mAb specific epitopes are CD20 mimotopes, preferably mimotope of SEQ ID NO 109.

Method for depleting CAR-expressing immune cells
The immune cells expressing the CLL1 specific 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).

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 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.

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 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 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 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.

Anti-CLLI CAR encoding polynucleotides and vectors.

The present invention also relates to polynucleotides, vectors encoding the above described multi-chain CAR according to the invention. The present invention provides polynucleotides, including DNA and RNA molecules that encode the transmembrane polypeptides disclosed herein that can be included in the multi-chain CAR. In particular, the invention relates to a polynucleotide comprising a nucleic acid sequence encoding at least one transmembrane polypeptide composing the multi-chain CAR as described above. More particularly the invention relates to a polynucleotide comprising two or more nucleic acid sequences encoding transmembrane polypeptides composing the multi-chain CAR as described above.

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

In a particular embodiment, the different nucleic acid sequences can be included in one polynucleotide or vector which comprises a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal “skip” from one codon to the next
without the formation of a peptide bond between the two amino acids encoded by the codons (see Donnelly 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. As a non-limiting example, in the present invention, 2A peptides have been used to express into the cell the different polypeptides of the multi-chain CAR.

To direct, transmembrane polypeptide such as FCER into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pro sequence) is provided in polynucleotide sequence or vector sequence. The secretory signal sequence may be that of FCER, or may be derived from another secreted protein (e.g., t-PA) or synthesized de novo. The secretory signal sequence is operably linked to the 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 residues 1 to 25 of the FCERI alpha chain (NP_001992.1) and has the amino acid sequence SEQ ID NO: 5.

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.
The present invention provides a polynucleotide comprising a nucleic acid sequence encoding a CLLl specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments.

The present invention provides a polynucleotide comprising a nucleic acid sequence encoding a CLLl specific multi-chain Chimeric Antigen Receptor comprising the following peptide sequences:

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.14, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.19, SEQ ID N0.3, SEQ ID N0.20, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.19, SEQ ID N0.3, SEQ ID N0.20, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.21, SEQ ID N0.3, SEQ ID N0.22, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.25, SEQ ID N0.3, SEQ ID N0.26, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.25, SEQ ID N0.3, SEQ ID N0.26, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.27, SEQ ID N0.3, SEQ ID N0.28, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.1, SEQ ID N0.2, SEQ ID N0.27, SEQ ID N0.3, SEQ ID N0.28, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;
The present invention provides a vector comprising a polynucleotide as above, preferably a vector encoding a CLL1 specific multi-chain Chimeric Antigen Receptor comprising the following peptide sequences:

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.34, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.36, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.35, SEQ ID NO.3, SEQ ID NO.36, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7.

The present invention provides a vector comprising a polynucleotide as above, preferably a vector encoding a CLL1 specific multi-chain Chimeric Antigen Receptor comprising the following peptide sequences:
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.13, SEQ ID NO.14, SEQ ID NO.15, SEQ ID NO.16, SEQ ID NO.17, SEQ ID NO.18, SEQ ID NO.19, and SEQ ID NO.20 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.13, SEQ ID NO.14, SEQ ID NO.15, SEQ ID NO.16, SEQ ID NO.17, SEQ ID NO.18, SEQ ID NO.19, SEQ ID NO.20, SEQ ID NO.21, SEQ ID NO.22, and SEQ ID NO.23 or.
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.21, SEQ ID N0.3, SEQ ID N0.22, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.25, SEQ ID N0.3, SEQ ID N0.26, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.25, SEQ ID N0.3, SEQ ID N0.26, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.27, SEQ ID N0.3, SEQ ID N0.28, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.27, SEQ ID N0.3, SEQ ID N0.28, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.29, SEQ ID N0.3, SEQ ID N0.30, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.29, SEQ ID N0.3, SEQ ID N0.30, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.31, SEQ ID N0.3, SEQ ID N0.32, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;
Methods for engineering immune cell

The present invention also provides with a method of engineering an immune cell as above comprising the following steps of:

(a) Providing an immune cell;

(b) Expressing at the surface of said cell a population of multi-chain Chimeric Antigen Receptors as above each one comprising different extracellular ligand-binding domains.

The present invention provides 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 polypeptides composing a population of multi-chain Chimeric Antigen Receptors as above each one comprising different extracellular ligand binding domains.

(c) Expressing said polynucleotides into said cell.
A method of engineering an immune cell endowing a CLLI specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments is part of the present invention, said method of engineering an immune cell is comprising the following steps:

(a) Providing an immune cell;

(b) Expressing at the surface of said cells at least one CLLI multi-chain Chimeric Antigen Receptor according to any one of the above embodiments.

In one embodiment, the present invention provides method of engineering an immune cell endowing a CLLI specific multi-chain Chimeric Antigen Receptor according to any one of the above embodiments comprising:

(a) Providing an immune cell;

(b) Introducing into said cell at least one polynucleotide encoding polypeptides composing a CLLI multi-chain Chimeric Antigen Receptor according to any one of the above; preferably encoding the following peptide sequences:

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.13, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.14, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.17, SEQ ID NO.3, SEQ ID NO.18, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.19, SEQ ID NO.3, SEQ ID NO.20, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.19, SEQ ID NO.3, SEQ ID NO.21, SEQ ID NO.22, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.19, SEQ ID NO.3, SEQ ID NO.21, SEQ ID NO.3, SEQ ID NO.22, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.23, SEQ ID NO.3, SEQ ID NO.24, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.23, SEQ ID NO.3, SEQ ID NO.24, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.25, SEQ ID NO.3, SEQ ID NO.26, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.25, SEQ ID NO.3, SEQ ID NO.26, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.27, SEQ ID NO.3, SEQ ID NO.28, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.27, SEQ ID NO.3, SEQ ID NO.28, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.34, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.34, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.36, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.35, SEQ ID NO.3, SEQ ID NO.36, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7.

(c) Expressing said polynucleotides into said cell.
In a preferred embodiment, said method of engineering an immune cell is comprising:

(a) Providing an immune cell;

(b) Expressing at the surface of said cell a population of CLL1 multi-chain Chimeric Antigen Receptors according to any one of the above embodiments each one comprising different extracellular ligand-binding domains.

In a preferred embodiment, the method of engineering an immune cell is further comprising:

(a) Providing an immune cell;

(b) Introducing into said cell at least one polynucleotide encoding polypeptides composing a population of CLL1 multi-chain Chimeric Antigen Receptors according to any one of the above embodiments each one comprising different extracellular ligand binding domains.

(c) Expressing said polynucleotides into said cell.

**Isolated immune cells**

According to another aspect, the present invention provides an isolated immune cell obtainable from the method according to any one of the above embodiments, preferably an isolated immune cell expressing a CLL1 multi-chain Chimeric Antigen Receptors comprising the following peptide sequences:

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.13, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.14, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;

- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.2, SEQ ID NO.15, SEQ ID NO.3, SEQ ID NO.16, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.15, SEQ ID N0.3, SEQ ID N0.16, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.15, SEQ ID N0.3, SEQ ID N0.16, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.17, SEQ ID N0.3, SEQ ID N0.18, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.19, SEQ ID N0.3, SEQ ID N0.20, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.19, SEQ ID N0.3, SEQ ID N0.20, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.21, SEQ ID N0.3, SEQ ID N0.22, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.21, SEQ ID N0.3, SEQ ID N0.22 SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.6 or;

- SEQ ID N0.8, SEQ ID N0.9, SEQ ID N0.10, SEQ ID N0.11, SEQ ID N0.2, SEQ ID N0.23, SEQ ID N0.3, SEQ ID N0.24, SEQ ID N0.4, SEQ ID N0.12, SEQ ID N0.5, and SEQ ID N0.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.12, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.25, SEQ ID NO.3, SEQ ID NO.26, SEQ ID NO.4, and SEQ ID NO.5 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.25, SEQ ID NO.3, SEQ ID NO.26, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.27, SEQ ID NO.3, SEQ ID NO.28, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.29, SEQ ID NO.3, SEQ ID NO.30, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.31, SEQ ID NO.3, SEQ ID NO.32, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.34, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.6 or;
- SEQ ID NO.8, SEQ ID NO.9, SEQ ID NO.10, SEQ ID NO.11, SEQ ID NO.1, SEQ ID NO.2, SEQ ID NO.33, SEQ ID NO.3, SEQ ID NO.34, SEQ ID NO.4, SEQ ID NO.12, SEQ ID NO.5, and SEQ ID NO.7 or;
The present invention provides an isolated cell said isolated cell is selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes, said isolated cell further comprises at least one anti-CLL multi-chain (CAR) of the invention.

In a preferred embodiment, said isolated cell provided in the present invention is an isolated immune T cell and said isolated immune T cell expresses at least one anti-CLL multi-chain (CAR) of the invention.

In another preferred embodiment, said isolated immune cell is an isolated immune T cell and said isolated immune T cell expresses at least one anti-CLL multi-chain (CAR) of the invention.

In one embodiment, said isolated immune cell is further engineered and is an engineered primary isolated immune cell comprising at least one anti-CLL multi-chain (CAR) of the invention.

In a preferred embodiment, said engineered primary isolated immune cell comprises at least one anti-CLL multi-chain (CAR) of the invention.

In another preferred embodiment, said engineered primary isolated immune cell comprises at least one anti-CLL multi-chain (CAR) comprising at least one anti-CLL multi-chain (CAR) of the invention.

**Pharmaceutical composition**

In one aspect, the present invention provides a pharmaceutical composition as described above.

The present invention provides pharmaceutical composition comprising at least one pharmaceutically acceptable vehicle and at least one primary cell endowed with at least one anti-CLL multi-chain (CAR).
In one embodiment said pharmaceutical composition comprises at least one pharmaceutically acceptable vehicle and at least one primary cell endowed with at least one anti-CLL1 multi-chain (CAR) of the invention.

Methods of engineering an immune cell

In encompassed particular embodiment, the invention relates to a method of preparing immune cells for immunotherapy comprising introducing into said immune cells the polypeptides composing said multi-chain CAR and expanding said cells. In particular embodiment, the invention relates to a method of engineering an immune cell comprising providing a cell and expressing at the surface of said cell at least one multi-chain CAR as described above. In particular embodiment, the method comprises transforming the cell with at least one polynucleotide encoding polypeptides composing at least one multi-chain CAR as described above, and expressing said polynucleotides into said cell.

In another embodiment, the present invention relates to a method of preparing cells for immunotherapy comprising introducing into said cells the different polypeptides composing said multi-chain CAR and expanding said cells.

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

The invention relates to a method of preparing primary immune cells for immunotherapy comprising introducing into said primary immune cells the polypeptides composing at least one anti-CLL1 multi-chain (CAR) of the invention.

In another embodiment, the present invention provides a method of preparing primary immune cells for immunotherapy comprising introducing into said immune cells a polynucleotide encoding at least one anti-CLL1 multi-chain (CAR) of the invention.
Delivery methods

The different methods described above involve introducing multi-chain CAR, pTalpha or functional variants thereof, rare cutting endonuclease, TALE-nuclease, CAR optionally with DNA-end processing enzyme or exogenous nucleic acid into a cell.

As non-limiting example, said multi-chain CAR can be introduced as transgenes encoded by one or as different plasmidic vectors. Different transgenes can be included in one 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 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. As non-limiting example, in the present invention, 2A peptides have been used to express into the cell the rare-cutting endonuclease and a DNA end-processing enzyme or the different polypeptides of the multi-chain CAR.

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 animal 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.

Electroporation

In particular 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 (Cellectis property) 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. In one aspect of the present invention, the inventor describe the steps that led to achievement of >95% transfection efficiency of mRNA in T cells, and the use of the electroporation protocol to transiently express different kind of proteins in T cells. In particular the invention relates to a method of transforming T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence consisting of:

(a) one electrical pulse with a voltage range from 2250 to 3000 V per centimeter, a pulse width of 0.1 ms and a pulse interval of 0.2 to 10 ms between the electrical pulses of step (a) and (b);

(b) one electrical pulse with a voltage range from 2250 to 3000 V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c) ; and

(c) 4 electrical pulses with a voltage of 325 V with a pulse width of 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses.

In particular embodiment, the method of transforming T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence consisting of:
(a) one electrical pulse with a voltage of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V per centimeter, a pulse width of 0.1 ms and a pulse interval of 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ms between the electrical pulses of step (a) and (b);

(b) one electrical pulse with a voltage range from 2250, of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V with a pulse width of 100 ms and a pulse interval of 100 ms between the electrical pulse of step (b) and the first electrical pulse of step (c); and

(c) 4 electrical pulses with a voltage of 325 V with a pulse width of 0.2 ms and a pulse interval of 2 ms between each of 4 electrical pulses.

Any values included in the value range described above are disclosed in the present application. Electroporation medium can be any suitable medium known in the art. Preferably, the electroporation medium has conductivity in a range spanning 0.01 to 1.0 milliSiemens.

In particular embodiments, as non-limiting examples, said RNA encodes a rare-cutting endonuclease, one monomer of the rare-cutting endonuclease such as Half-TALE-nuclease, a Chimeric Antigen Receptor, at least one component of the multi-chain chimeric antigen receptor, a pTalpha or functional variant thereof, an exogenous nucleic acid, one additional catalytic domain.

**Engineered immune cells**

The present invention also relates to isolated cells or cell lines susceptible to be obtained by said method to engineer cells. In particular, said isolated cell comprises at least one multi-chain CAR as described above. In another embodiment, said isolated cell comprises a population of multi-chain CARs each one comprising different extracellular ligand binding domains. In particular, said isolated cell comprises exogenous polynucleotide sequences encoding polypeptides composing at least one multi-chain CAR.

In the scope of the present invention is also encompassed an isolated immune cell, preferably a T-cell obtained according to any one of the methods previously described.

Said immune cell refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response. Said immune cell according to the present invention can be derived from a stem cell. The stem cells can be adult
stem cells, 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.

In a preferred embodiment, said isolated cell is an isolated stem CD34+ cell, said isolated stem CD34+ cell comprises at least one anti-CLL multi-chain (CAR) of the present invention.

In another preferred embodiment, said isolated cell is an isolated stem CD34+ cell, said isolated stem CD34+ cell comprises at least one anti-CLL multi-chain (CAR) comprising comprises at least one anti-CLL multi-chain (CAR) of the present invention.

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 mentioned previously, such cells can be also genetically engineered to inactivate one or several genes selected, for instance, from the group consisting of CD52, GR, TCR alpha, TCR beta, HLA gene, immune check point genes such as PD1 and CTLA-4, or can express a pTalpha transgene.

In another embodiment, TCR is rendered not functional in the cells according to the invention by inactivating TCR alpha gene and/or TCR beta gene(s). The above strategies are used more particularly to avoid GvHD. In a particular aspect of the present invention is a method to
obtain modified cells derived from an individual, wherein said cells can proliferate independently of the Major Histocompatibility Complex signaling pathway. Said method comprises the following steps:

(a) Recovering cells from said individual;

(b) Genetically modifying said cells ex-vivo by inactivating TCR alpha and/or TCR beta genes;

(c) Cultivating genetically modified T-cells in vitro in appropriate conditions to amplify said cells.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention.

Modified cells, which can proliferate independently of the Major Histocompatibility Complex signaling pathway, susceptible to be obtained by this method are encompassed in the scope of the present invention. Said modified cells can be used in a particular aspect of the invention for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified cells comprising inactivated TCR alpha and/or TCR beta genes.

In a more preferred embodiment, said method comprises:

(a) Providing a T-cell, preferably from a cell culture or from a blood sample;

(b) Transforming said T-cell with nucleic acid encoding a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break at least one gene encoding a component of the T-cell receptor (TCR);

(c) Expressing said rare-cutting endonucleases into said T-cells;

(d) Sorting the transformed T-cells, which do not express TCR on their cell surface;

(e) Expanding said cells.

In another embodiment, said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease or a TALE-nuclease. In a preferred embodiment, said rare-cutting endonuclease
is a TALE-nuclease. Preferred methods and relevant TALE-nucleases have been described in WO2013176915.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention inducing from 50% to 100% less Host versus Graft (HvG) rejection than primary non engineered T cell.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention inducing from 50% to 100% less Host versus Graft (HvG) rejection than primary non engineered T cell.

**Anti-CLL1 Immune cells made resistant to chemotherapy**

According to a preferred embodiment of the invention, the immune cells endowed with an anti CLL1 multi-chain CAR are engineered to be resistant to chemotherapy drugs, in particular to purine nucleotide analogues (PNAs), making them suitable for cancer treatments in order to combine adoptive immunotherapy and chemotherapy. Purine nucleotide analogues enter chemotherapy compositions for many cancer treatments, especially leukemia. It is particularly used as a standard of care in AML. The most widely used PNAs are clofarabine, fludarabine, cytarabine and decitabine (Dacogen), alone or in combination. PNAs are metabolized by enzymes having deoxycytidine kinase (dCK) activity [EC 2.7.1.74] into mono, -di and tri-phosphate PNA. Their tri-phosphate forms and particularly clorofarabine triphosphate compete with ATP for DNA synthesis, acts as pro-apotptotic agent and are potent inhibitors of ribonucleotide reductase (RNR), which is involved in trinucleotide production.

The present invention thus includes a method of producing ex-vivo immune cells, preferably T-cells, which are resistant to a purine analogue drug and that can target CLL1 positive malignant cells. Said method comprises one or several of the following steps of:

(a) Providing an immune cell from a patient (autologous treatment) or from a donor;

(b) transfecting said immune cell with a nucleic acid sequence encoding a rare-cutting endonuclease specifically targeting a gene expressing an enzyme having deoxycytidine kinase activity (dCK - EC 2.7.1.74), in particular the human deoxycytidine kinase gene (NCBI Gene ID: 1633).
(c) expressing said endonuclease into said immune cells to obtain targeted inactivation of said dck gene;

(d) Expanding the engineered immune cells obtained in step c), optionally in the presence of said purine analogue drug; and

(e) Introducing into said immune cell an anti-CLL1 multi-chain CAR as previously described.

The present inventors have successfully created anti-CLL1 T-cells resistant to purine nucleotide analogues, more particularly clorofarabine and/or fludarabine, by mediating the inactivation of dck gene expression into said cells particularly by using TAL-nucleases. Transfection of the T-cells using mRNA encoding specific TAL-nuclease directed against dck genes, preferably by using electroporation as described in WO2013176915, induced a significant resistance to the drugs, while maintaining T-cells cytotoxic activity towards CLL1 bearing cells.

The present application thus provides with anti-CLL1 T-cells, which expression of deoxycytidine kinase has been repressed or inactivated for the treatment of leukemia.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention, in which expression of deoxycytidine kinase has been repressed or inactivated for the treatment of leukemia, preferably AML.

The present invention provides primary engineered T cell comprising at least one anti-CLL1 multi-chain (CAR) of the present invention, in which expression of deoxycytidine kinase has been repressed or inactivated for the treatment of leukemia, preferably AML.

Activation and expansion of T cells

Whether prior to or after genetic modification of the T cells, the T cells can be 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. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody. For example, the agents providing each signal may be in solution or coupled to a surface. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. In further embodiments of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. 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-mercaptoethanoli. 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 administrating said cell into the subject.

**Medicament**

The pharmaceutical composition of the invention for use as a medicament to prevent or treat AML comprises engineered primary immune cells, preferably primary immune T cells, comprising at least one anti-CLLI multi-chain (CAR) of the invention, with at least one pharmaceutically acceptable vehicle.

The present invention provides an isolated immune cell according to above embodiments for its use as a medicament, preferably an isolated immune T cell endowed with a CLL1 mc CAR of the invention for its use as a medicament.

In one embodiment the present application provides an isolated immune T cells endowed with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat refractory /relapse AML.

The present invention provides an isolated inflammatory T-lymphocyte with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat AML.

The present invention provides an isolated cytotoxic T-lymphocyte endowed with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat AML.

The present invention provides an isolated regulatory T-lymphocyte endowed with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat AML.

The present invention provides an isolated helper T-lymphocyte endowed with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat AML.

The present invention provides an isolated immune NK cell endowed with at least one anti-CLLI multi-chain (CAR) of the invention for its use as a medicament to prevent or treat AML.
In another aspect the present invention provides a pharmaceutical composition for use as a medicament for the prevention or treatment of a pathological condition such as cancer, in particular a cancer of hematopoietic cells, more particularly AML.

**Therapeutic indications**

Preferably, the present invention provides a method for treating a patient in need thereof comprising:

a) Providing an isolated immune T cell obtainable by a method according to any one of the above embodiments;

b) Administering said T-cells to said patient, wherein said patients is suffering from a cancer selected from AML, more preferably refractory/relapse AML.

The present invention provides a method for treating a patient as above wherein said immune cells are recovered from donors.

The present invention provides a method for treating a patient as above wherein said immune cells are recovered from a patient, preferably from the patient itself, the patient to be treated by said method.

In the present application a patient or a subject means non-human primates or humans.

A donor means a healthy individual or an individual suffering from a disease.

**Leukemia/AML**

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 leukemia, such as acute myeloid leukemia (AML).

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

The term "relapsed" refers to a situation where a subject 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. T cells comprising an anti CLL1 multi-chain CAR of the invention are provided as a treatment in patients diagnosed with 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 and in particular acute myelogenous leukemia (AML).

AML subtypes / markers

AML or AML subtypes that may be treated using the anti CLL1 multi-chain CAR-expressing cells of the present invention may be in particular, acute myeloblasts leukemia, minimally differentiated acute myeloblasts leukemia, acute myeloblasts leukemia without maturation, acute myeloblasts 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 leukemia, acute panmyelosis with myelofibrosis, whether involving CLL1-positive malignant cells.

Subtypes of AML also include, hairy cell leukemia, Philadelphia chromosome-positive acute lymphoblastic leukemia.

AML or AML subtypes that may be treated using the anti CLL1 multi-chain CAR-expressing cells of the present invention may be AML with specific genetic abnormalities. Classification is based on the ability of karyotype to predict response to induction therapy, relapse risk, survival.

Accordingly, AML that may be treated using the anti CLL1 multi-chain 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 (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 multi-chain CAR-expressing cells 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)\textsuperscript{16-19} and for the treatment of patients with t(8;21)(q22;q22) or inv(16)(pl3q22)/t(16; 16)(pl3;q22) identified using repetitive doses of high-dose cytarabine.

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

In another embodiment, isolated cell obtained by the different methods or cell line derived from said isolated cell as previously described can be used as a medicament.

In another embodiment, said medicament can be used for treating cancer or infections in a patient diagnosed with a pathology linked to CLL1 positive cells. 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, especially AML.

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) Administering said transformed immune cells to said patient,

On one embodiment, said T cells of the invention can undergo robust \textit{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.

The invention is particularly suited for allogenic immunotherapy, insofar as it enables the transformation of T-cells, typically obtained from donors, into non-alloreactive cells. This may be done under standard protocols and reproduced as many times as needed. The resulted modified T cells may be pooled and administrated to one or several patients, being made available as an "off the shelf" therapeutic product.
Cells that can be used with the disclosed methods are described in the previous section. Said treatment can be used to treat patients diagnosed with cancer, viral infection, autoimmune disorders or Graft versus Host Disease (GvHD). Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise nonsolid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the multi-chain CARs of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

**Administration: routes/posology**

According to a preferred embodiment of the invention, said treatment can be administrated into patients undergoing an immunosuppressive treatment. Indeed, the present invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T-cells according to the invention within the patient. The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administrated to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

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

The administration of the cells or population of cells can consist of the administration of \(10^5\) to \(10^9\) cells per kg body weight, preferably \(10^5\) to \(10^8\) cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as
more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administrated 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.

Combination with other(s) treatment(s)

It can be a treatment 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.

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 efalizimab 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 CAM PATH, anti-CD3 antibodies or other antibody therapies, cytokin, fludaribine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 11; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Cittr. Opin. mm n. 5:763-773, 93). 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 CAM PATH, 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. Said modified cells obtained by any one of the methods described here can be used in a particular aspect of the invention for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified (engineered) cells comprising inactivated TCR alpha and/or TCR beta genes.

EXEMPLES

All methods disclosed in document PCT/EP2015/055848 are incorporated herein by references.

Example 1: Design of multi-chain CARs

Multi-chain CARs targeting the CLL1 antigen were designed based on the high affinity receptor for IgE (FCERI) such as depicted in Figure 2 to Figure 4. The FCERI expressed on mast cells and basophiles triggers allergic reactions. It is a tetrameric complex composed of a single a subunit, a single β subunit and two disulfide-linked γ subunits. The a subunit contains the IgE-binding domain. The β and γ subunits contain ITAMs that mediate signal transduction. In every multi-chain CAR, the extracellular domain of the FcRa chain was deleted and replaced by the respective scFv referred to Table 5 respectively and the CD8α hinge (SEQ ID NO: 2) and the ITAM of the FcRβ chain and/or the FcRγ chain was deleted. The resulting constructions had the structure detailed in table 6.

Example 2: Expression of Anti-CLL1 mcCARs in human T cells

Primary T-cell cultures

T cells were purified from Buffy coat samples provided by EFS (Etablissement Frangais 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 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).

Models of AML and clorofarabine, fludarabine or cytarabine resistant AML

Originally, an AML-positive cell line, such as MOLM13 cell line, has been established from the peripheral blood of a 20-year-old man with acute myeloid leukemia AML FAB M5a at relapse in 1995 after initial myelodysplastic syndromes (MDS, refractory anemia with excess of blasts, RAEB).

To establish the MOLM13-Luc cell line and dck Knock out MOLM13-Luc cell line (clorofarabine, fludarabine or cytarabine resistant MOLM13-Luc cell line), MOLM13 cells (DSMZ ACC 554) were transfected with a nucleic acid sequence encoding a rare-cutting endonuclease specifically targeting a gene expressing an enzyme having deoxycytidine kinase activity (dcK - EC 2.7.1.74), namely the human deoxycytidine kinase gene (NCBI Gene ID: 1633), and with a lentivirus encoding the GFP and the firefly luciferase (amsbio LVP438-PBS).

The GFP-positive cells have been selected with Neomycin (ref 10131-027, Gibco, Life Technologies, Saint-Aubin, France). Resistance to clorofarabine, fludarabine or cytarabine of cdk KO MOLM13-Luc cells was tested in the presence of clorofarabine, fludarabine or cytarabine.

Transiently expression in T cells

The live T cells engineered using polycistronic mRNAs expressed the multi-chain CARs on their surface. Multi-chain CARs can be expressed in human T cells after electroporation of polycistronic mRNA. T cells were electroporated with capped and polyadenylated polycistronic mRNA that were produced using the mMMESSAGE mMACH IN E kit and linearized plasmids as template. The plasmids used as template contained the T7 RNA polymerase promoter followed by a polycistronic DNA sequence encoding the different CAR variants.

The electroporation of the polycistronic mRNAs into the human T cells was done using the CytoLVT-S device (Cellectis), according to the following protocol: 5X10⁶ T cells preactivated
several days (3-5) with anti CD3/CD28 coated beads and IL2 were resuspended in cytoporation buffer T, and electroporated in 0.4cm cuvettes with 45µg of mRNA.

24 hours post electroporation, human T cells engineered using polycistronic mRNAs encoding the multi-chain CARs were labeled with a fixable viability dye eFluor-780 and a PE-conjugated goat anti mouse IgG F(ab')2 fragment specific, and analysed by flow cytometry.

The human T cells engineered using polycistronic mRNAs encoding the multi-chain CARs were co-cultured with target (Daudi) or AML cell line control cells for 24 hours. The supernatants were then harvested and analysed using the TH1/TH2 cytokine cytometric bead array kit to quantify the cytokines produced by the T cells. The assay aims to show that the human T cells expressing the multi-chain CARs produce IFNγ, IL8 and IL5 in coculture with CLL1 expressing target cells but not in coculture with control cells.

T-cell transduction and CAR detection

Transduction of T-cells with recombinant lentiviral vectors along the expression of mcCAR was carried out three days after T-cell purification/activation. Lentiviral vectors were produced by Vectalis SA (Toulouse, France) by transfection of genomic and helper plasmids in HEK-293 cells. Transductions were carried out at a multiplicity of infection of 5, using 10⁵ cells per transduction. CAR 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 IgGl Fc fragment (produced by LakePharma). Binding of this protein to the CAR molecule was detected with a PE-conjugated secondary antibody (Jackson Immunoresearch) targeting the mouse Fc portion of the protein, and analyzed by flow cytometry.

Example 3: Degranulation of T cells transiently expressing the anti-CLL1 mcCARs following coculture with target cells

24 hours post electroporation, human T cells engineered using polycistronic mRNAs encoding the multi-chain CARs were co-cultured with target (Daudi) or AML cell line control cells for 6 hours. The CD8+ T cells were then analyzed by flow cytometry to detect the expression of the degranulation marker CD107a at their surface. This experiment aims to check that the human CD8+ T cells expressing the CLL1 multi-chain CARs degranulate in coculture with CLL1 expressing target cells but not in coculture with control cells.

Degranulation assay (CD107a mobilization)
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 8g/ml of anti-CD49d (BD Pharmingen), 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 8-10 days after T-cell transduction with mcCAR.

Example 4: Lyse of target cells by T cells transiently expressing the anti-CLL1 mcCARs

24 hours post electroporation, human T cells engineered using polycistronic mRNAs encoding the multi-chain CARs were co-cultured with target (Daudi) or AML cell line control cells for 4 hours. The target cells were then analysed by flow cytometry to analyse their viability. This assay aims to show that the different cells expressing the CLL1 multi-chain CARs lyse the CLL1 expressing target cells but not the control cells.

Cytotoxicity assay

T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing various levels of 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 CAR+ T-cells (mcCAR+ T-cells or mcCAR+ 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 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.

Example 5: Anti-tumor mouse model

Animal housing and experimental procedures were carried out by Oncodesign (Dijon, France; http://www.oncodesign.com/), according to the French and European Regulations and...
NRC Guide for the Care and Use of Laboratory Animals. Immunodeficient female NOG (NOG) mice (NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac) mice (NOD stands for non-obese diabetic), 6-8 weeks old, were obtained from Taconic (Ry, Danemark). In one arm of the experiment, mice received chlorofarabine or fludarabine. Mice were intravenously (iv) injected with MOLM13-Luciferase cells or with chlorofarabine resistant MOLM13-Luciferase cells as an AML and an chlorofarabine resistant AML mouse model, respectively. Mice were then iv injected (7 days after injection of the tumor cell line) with different doses of mccAR+ T-cells (from $10^4$ to $5 \times 10^5$), or with T-cells that were not transduced with any CAR lentiviral vector.

Bioluminescent signals were determined the day before T-cell injection (D-1) and at D7 and 14 after T-cell injection, in order to follow tumoral progression on the different animals.

**Example 6  Proliferation of TCRalpha inactivated cells expressing a CLLI-mcCAR**

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 8.

<table>
<thead>
<tr>
<th>Target</th>
<th>Target sequence</th>
<th>Repeat sequence</th>
<th>Half TALE-nuclease</th>
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<tr>
<td>TRAC_T01</td>
<td>TTGTCCCCCACAGATATCC Agaacctgtacccctg CCGTGTACCAGCTGAGA (SEQ ID NO: 119)</td>
<td>Repeat TRAC_T01-L (SEQ ID NO: 120)</td>
<td>TRAC_T01-L TALEN (SEQ ID NO: 122)</td>
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<tr>
<td></td>
<td></td>
<td>Repeat TRAC_T01-R (SEQ ID NO: 121)</td>
<td>TRAC_T01-R TALEN (SEQ ID NO: 123)</td>
</tr>
</tbody>
</table>

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-CLLI mcCAR previously described (SEQ ID NO: 18 to 37). 2 days post-transduction, CD3\textsubscript{n0} cells were purified using anti-CD3 magnetic beads and 5 days post-transduction cells were reactivated with soluble anti-CD28 (5 µg/ml).
1. 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 CLLI mcCARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

To investigate whether the human T cells expressing the CLLI-mcCAR 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 CLLI mcCAR 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.
CLAIMS

1) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) comprising at least:
   - a first transmembrane polypeptide comprising at least one extracellular ligand-binding domain, wherein the at least one extracellular ligand-binding domain binds to the cell surface CLL1 antigen; and;
   - a second polypeptide comprising at least one signal-transducing domain;
   wherein the signal transducing domain(s) of the multi-chain Chimeric Antigen Receptor is present on a polypeptide distinct from that carrying the extracellular ligand-binding domain(s).

2) The CLL1 specific multi-chain Chimeric Antigen Receptor of claim 1, wherein said signal-transducing domain containing polypeptide is a transmembrane polypeptide.

3) The CLL1 specific multi-chain Chimeric Antigen Receptor of claim 1 or claim 2, wherein at least one transmembrane polypeptide comprises a part of Fc receptor.

4) The CLL1 specific multi-chain Chimeric Antigen Receptor of claim 3, wherein said part of Fc receptor is selected from the group consisting of: (a) FCERI alpha chain, (b) FCERI beta chain and (c) FCERI gamma chain.

5) The CLL1 specific multi-chain Chimeric Antigen Receptor of claim 3 or claim 4, wherein a transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FCERI) is fused to an extracellular CLL1 ligand binding domain.

6) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) according to any one of claim 3 to claim 5 further comprising:
   - said second transmembrane polypeptide from the gamma or beta chain of FCERI fused to a signal transducing domain;

7) A CLL1 specific multi-chain Chimeric Antigen Receptor (mc CAR) according to any one of claim 3 to claim 6, further comprising:
   - a third transmembrane polypeptide from the gamma or beta chain of FCERI comprising a co-stimulatory domain.
8) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of claims 1 to 7, wherein said CLL1 ligand binding domain fused to said alpha chain of FCERI is a single-chain variable fragment (scFv) comprising heavy (V\text{H}) and light (V\text{L}) chains conferring specificity to CLL1.

9) A CLL1 specific multi-chain Chimeric Antigen Receptor of claim 8, wherein said V\text{H} comprises a polypeptide sequence displaying at least 90 % identity to one selected SEQ ID NO. 13, 14, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35.

10) A CLL1 specific multi-chain Chimeric Antigen Receptor of claim 8, wherein said V\text{L} comprises a polypeptide displaying at least 90 % identity to one selected from SEQ ID NO. 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and 36.

11) A CLL1 specific multi-chain Chimeric Antigen Receptor of any one of claim 4 to claim 10, wherein said alpha chain of FCERI is fused to said extracellular ligand-binding domain by a hinge from CD8a, IgGl or FCRIIa proteins.

12) A CLL1 specific multi-chain Chimeric Antigen Receptor of claim 11, wherein said hinge comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO.2.

13) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of claims 3 to 12, wherein said signal transducing domain fused to the gamma or beta chain of FCERI is from the TCR zeta chain, the FCER\beta chain, the FCER\gamma chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM).

14) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 13, wherein said signal transducing domain is from CD3zeta.

15) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 14, wherein said signal transducing domain comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO.10.

16) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of claims 1 to 15, wherein said second or third polypeptide comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule selected from CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, CD8, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.
17) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 16, wherein said co-stimulatory domain is from 4-1BB and comprises a polypeptide sequence displaying at least 90% identity to SEQ ID NO.6.

18) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 16, wherein said co-stimulatory domain is from CD28 and comprises a polypeptide sequence displaying at least 90% identity to SEQ ID NO.7.

19) A CLL1 specific multi-chain Chimeric Antigen Receptor according to anyone of claim 1 to 18, wherein at least one epitope is inserted in at least one one of the extracellular domain(s) of said CAR.

20) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 19, wherein said at least one epitope is inserted in one extracellular ligand binding domain of said CAR.

21) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 20, wherein said at least one epitope is inserted in the extracellular domain of said CAR that binds CLL1.

22) A CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of claims 19 to 21, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

23) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 22, wherein the extracellular binding domain comprises 1, 2, 3 or 4 mAb-specific epitopes.

24) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 23, wherein the extracellular binding domain comprises 2, 3 or 4 mAb-specific epitopes.

25) A CLL1 specific multi-chain Chimeric Antigen Receptor according to claim 23, wherein the extracellular binding domain comprises one of the following sequences:

$$V_1^\alpha \cdot L_1^\beta \cdot V_2^\gamma \cdot (L)_x^\delta \cdot \text{Epitope}_1^\epsilon \cdot (L)_y^\zeta \cdot \text{Epitope}_2^\eta \cdot (L)_\zeta \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\text{Epitope}_2^\eta \cdot (L)_\zeta \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\cdot (L)_\iota \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\cdot (L)_\iota \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\cdot (L)_\iota \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$

$$\cdot (L)_\iota \cdot \text{Epitope}_3^\iota \cdot (L)_\iota \cdot \text{Epitope}_4^\kappa$$
Epitope1-(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-V_{1,1}^-L_{1,1}^-V_{2}^-;

(L)_x-Epitope1-(L)_x-V_{1,1}^-L_{1,1}^-V_{2}^-.(L)_x-Epitope2-(L)_x;

(Ljx-Epitope1-Ljx-Vl-Ujx-Vz-Ljx-Epitope2-Ljx-Epitope3-Ljx-;

(L)_x-Epitope1-(L)_x-V_{1,1}^-L_{1,1}^-V_{2}^-.(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

(Ljx-Epitope1-Ljx-Epitope2-(Ljx-Vl-Ljx-Vz-Ljx-Epitope3-Ljx-;

(L)_x-Epitope1-(L)_x-V_{1,1}^-L_{1,1}^-V_{2}^-.(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

VHLK-Epitope-Ljx-Vl-Ljx-Epitope2-Ljx-Epitope3-Ljx-;

V_{1,1}^-V_{1,1}^-L_{1,1}^-V_{2}^-.(L)_x-V_{1,1}^-L_{1,1}^-V_{2}^-.(L)_x-Epitope2-(L)_x-Epitope3-(L)_x-Epitope4-(L)_x;

wherein,

V_{1,1} is V_L and V_{2} is V_H or V_{1,1} 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.

26) A CLLI specific multi-chain Chimeric Antigen Receptor according to claim 22, wherein the extracellular binding domain comprises the following sequence:

V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope1; V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope1-L; V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope1-L-Epitope2; V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope2-L; V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope2-L-Epitope3; V_{1,1}^-L_{1,1}^-V_{2,1}^-L-Epitope1-L-Epitope2-L-Epitope3;
wherein

\[ V_1 \text{ is } V_L \text{ and } V_2 \text{ is } V_H \text{ or } V_1 \text{ is } V_H \text{ and } V_2 \text{ is } V_L; \]

\[ L_i \text{ is any linker suitable to link the } V_H \text{ chain to the } V_L \text{ chain;} \]

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

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

27) A CLLI specific multi-chain Chimeric Antigen Receptor according to claim 25 or 26, wherein \( L_i \) is a linker comprising Glycine and/or Serine.

28) A CLLI specific multi-chain Chimeric Antigen Receptor according to claim 27, wherein \( L_i \) 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.

29) A CLLI specific multi-chain Chimeric Antigen Receptor according to claim 27, wherein \( L_i \) is a linker comprising the amino acid sequence \((\text{Gly}_3\text{Ser})_4\) or \((\text{Gly}_3\text{Ser})_3\).
30) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 27, wherein
L is a linker having an amino acid sequence selected from SGG, GGS, SGGS, SGSGS,
GSG, SGSGG, GGGS, SGSGGGS, GSGGGS, SGSGGG, SGSGGGG, GSGGGGG,
SGSGGGGG, SGSGGGGGS, SGSGGGGGG, or SGSGGGGGGG.

31) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 30, wherein
L is a SGGG, GGGGS or SGSGG.

32) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 22, wherein
said mAb-specific epitope(s) is(are) specifically recognized by ibritumomab, tiuxetan,
umromonab-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, QBEN D-10, alemtuzumab or ustekinumab.

33) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 22, wherein
mAb-specific epitope is one comprising an amino acid sequence selected from SEQ ID
NO 109, SEQ ID NO 110, SEQ ID NO 111, SEQ ID NO 112, SEQ ID NO 113, SEQ ID NO
114, SEQ ID NO 115 and SEQ ID NO 116.

34) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 25 or 26,
wherein Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ
ID NO 109.

35) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 25 or 26,
wherein Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ
ID NO 109.

36) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 25 or 26,
wherein Epitope 3 is an mAb-specific epitope having an amino acid sequence of SEQ
ID NO 109 or SEQ ID NO 117 or SEQ ID NO 118.

37) A CLLl specific multi-chain Chimeric Antigen Receptor according to claim 25 or 26,
wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ
ID NO 109.

38) A CLLl specific multi-chain Chimeric Antigen Receptor according to any one of
claims 1 to 37, comprising a polypeptide sequence displaying at least 80 % identity to the full
amino acid sequence of anti-CLL1 SC02-357, anti-CLL1 SC02-378, anti-CLL1 SC02-161, anti-CLL1 M26, anti-CLL1 M31, anti-CLL1 G4, anti-CLL1 M22, anti-CLL1 M29, anti-CLL1 M2, anti-CLL1 M5, anti-CLL1 G12, anti-CLL1 21.26 and anti-CLL1 1075.7 as referred to in Table 6.

39) A polynucleotide comprising a nucleic acid sequence encoding a CLL1 specific multi-chain Chimeric Antigen Receptor according to any one of claims 1 to 38.

40) A vector comprising a polynucleotide of claim 39.

41) An engineered immune cell expressing at the cell surface membrane an anti-CLL1 mccAR according to any one of claims 1 to 38.

42) An engineered immune cell according to claim 41, derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

43) An engineered cell according to any one of claims 41 or 42 for use in therapy.

44) An engineered cell according to any one of claims 412 to 43 for use in therapy, wherein the patient is a human.

45) An engineered cell according to any one of claims 41 to 44 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by CLL1-expressing cells.

46) An engineered cell according to any one of claims 41 to 45 use in therapy, wherein the condition is a condition which is characterized by an overabundance of CLL1-expressing cells.

47) An engineered cell according to any one of claims 41 to 46 for use in therapy, wherein the condition is a hematological cancer condition.

48) An engineered cell according to any one of claims 41 to 47 for use in therapy, wherein the hematological cancer condition is leukemia.

49) An engineered cell according to any one of claims 41 to 48 for use in therapy, wherein the leukemia is acute myelogenous leukemia (AML).

50) An engineered cell according to any one of claims 41 to 49 wherein expression of TCR is suppressed in said immune cell.

51) An engineered cell according to any one of claims 41 to 50, wherein expression of at least one MHC protein, preferably β2m or HLA, is suppressed in said immune cell.
52) An engineered cell according to any one of claims 41 to 51, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.

53) A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to any one of claims 41 to 52 in an amount effective to cause impairment of said cancer cell.

54) A method of engineering an immune cell comprising:
   (c) Providing an immune cell;
   (d) Expressing at the surface of said cells at least one multi-chain Chimeric Antigen Receptor according to any one of the claims 1 to 38.

55) The method of engineering an immune cell of claim 54 comprising:
   (d) Providing an immune cell;
   (e) Introducing into said cell at least one polynucleotide encoding polypeptides composing at least one multi-chain Chimeric Antigen Receptor according to any one of claims 1 to 38;
   (f) Expressing said polynucleotides into said cell.

56) The method of engineering an immune cell of claim 36 comprising:
   (c) Providing an immune cell;
   (d) Expressing at the surface of said cell a population of multi-chain Chimeric Antigen Receptors according to any one of the claims 1 to 38 each one comprising different extracellular ligand-binding domains.

57) The method of engineering an immune cell of claim 56 comprising:
   (d) Providing an immune cell;
   (e) Introducing into said cell at least one polynucleotide encoding polypeptides composing a population of multi-chain Chimeric Antigen Receptors according to any one of claims 1 to 38 each one comprising different extracellular ligand binding domains.
   (f) Expressing said polynucleotides into said cell.

58) An isolated immune cell obtainable from the method according to any one of claims 54 to 57.
59) A n isolated immune cell comprising at least one multi-chain Chimeric Antigen Receptor according to any one of claims 1 to 38.

60) An isolated immune cell according to claim 58 or 59 for its use as a medicament.

61) An isolated cell according to any one of claims 58 to 60 derived from, NK cells, inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

62) A therapeutic composition comprising an isolated immune cell according to any one of claims 58 to 61.

63) A method for treating a patient in need thereof comprising:

  c) Providing a immune cell obtainable by a method according to any one of the claims 54 to 57;
  d) Administering said T-cells to said patient,

64) The method for treating a patient of claim 63, wherein said immune cells are recovered from donors.

65) The method for treating a patient of claim 63, wherein said immune cells are recovered from patients.
Native FcεRI

Figure 1
Structure of the polycistronic mCCAR construct
Figure 3
Anti-CLL1 mc CAR with 4.1BB as costimulatory domain

Anti-CLL1 mc CAR with CD28 as costimulatory domain

Figure 4
Figure 5A
Figure 5B
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. forming part of the international application as filed:
      - in the form of an Annex C/ST.25 text file.
      - on paper or in the form of an image file.
   b. furnished together with the international application under PCT Rule 13ter.1 (a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. furnished subsequent to the international filing date for the purposes of international search only:
      - in the form of an Annex C/ST.25 text file (Rule 13ter.1 (a)).
      - on paper or in the form of an image file (Rule 13ter.1 (b) and Administrative Instructions, Section 713).

2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C07K16/28

**ADD.**

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>X</td>
<td>wo 2014/191128 AI (CELLECTIS [FR]) 4 December 2014 (2014-12-04) page 31, line 31 - page 37, line 23</td>
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<tr>
<td>X</td>
<td>wo 2014/145252 A2 (MI LONE MICHAEL C [US]; WANG ENXIU [US]) 18 September 2014 (2014-09-18) page 78, line 20 - page 88, line 8 page 131, line 22 - page 133, line 15 figure 1</td>
<td>1-65</td>
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**X** Further documents are listed in the continuation of Box C. **X** See patent family annex.

* Special categories of cited documents:
  - “X” document defining the general state of the art which is not considered to be of particular relevance
  - “E” earlier application or patent but published on or after the international filing date
  - “L” document which may throw doubts on priority claim(s) or which cited to establish the publication date of another citation or other special reason (as specified)
  - “O” document referring to an oral disclosure, use, exhibition or other means
  - “P” document published prior to the international filing date but later than the priority date claimed
  - “T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  - “X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  - “Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  - “Z” document member of the same patent family

**Date of the actual completion of the international search**

24 March 2016

**Date of mailing of the international search report**

25/04/2016

**Name and mailing address of the ISA/European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016**

Authorized officer

Ulbrecht, Matthias

Form PCT/ISA/210 (second sheet) (April 2000)
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<td>WO 2014/039523 A1 (CELLECTIS [FR]) cited in the application on page 14, paragraph 2 - page 21, last paragraph page 40, paragraph 2 - page 41, paragraph 3 figures 4A-4C</td>
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<td>A</td>
<td>MARC CARTELLIERI ET AL: &quot;A Novel Ex Vivo Isolation and Expansion Procedure for Chimeric Anti gen Receptor Engrafted Human T Cells&quot;., PL0S ONE, vol. 9, no. 4, 3 April 1 2014 (2014-04-03), page e93745, XP055153686, DOI: 10.1371/journal.pone.0093745 abstract page 9, left-hand column, paragraph 4 - page 10, right-hand column, paragraph 3 figures 1-5</td>
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