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(54) **T CELL RECEPTOR KNOCK OUT ENGINEERED IMMUNE CELLS, ENDOWED WITH CHIMERIC ANTIGEN RECEPTORS BINDING TO CD123 FOR THE TREATMENT OF RELAPSED/REFRACTORY ACUTE MYELOID LYMPHOMA OR BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM**

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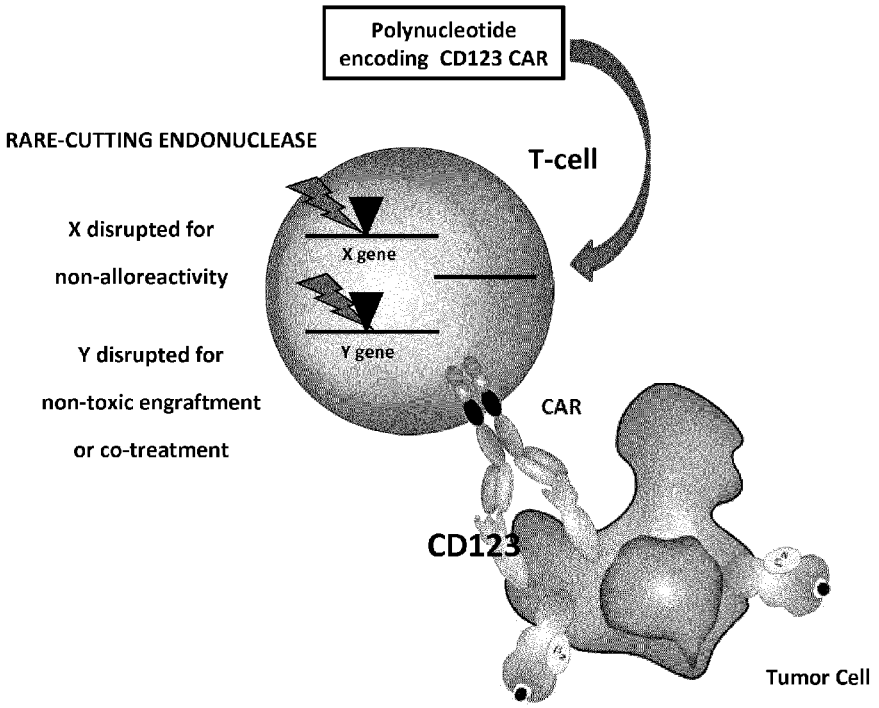
(57) **ABSTRACT**

The present invention relates to a TCR KO—or TCR KO and dCK KO—engineered immune cells expressing a Chimeric Antigen Receptors (CAR) specific for CD123 that is a recombinant chimeric protein able to redirect immune cell specificity and reactivity toward CD123-expressing cells, and more particularly in which extracellular ligand binding is a scFV derived from a CD123 monoclonal antibody, conferring specific immunity against CD123 positive cells. The engineered immune cells endowed with such CD123 CARs are particularly suited for treating relapse refractory AML and blastic plasmacytoid dendritic cell neoplasm and for use as a treatment before bone marrow transplantation.



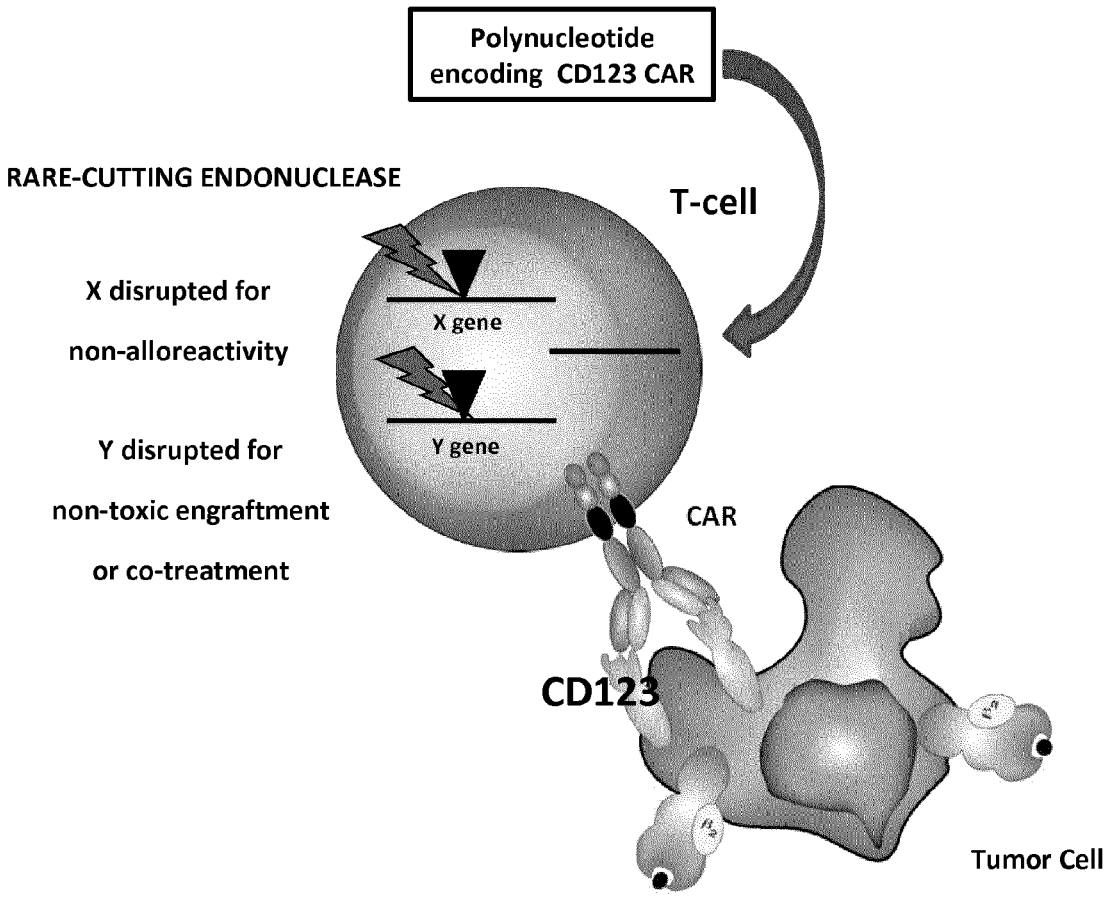

Polynucleotide
encoding CD123 CAR
RARE-CUTTING ENDONUCLEASE
T-cell
X disrupted for
non-alloreactivity
X gene
Y gene
Y disrupted for
non-toxic engraftment
or co-treatment
CAR
CD123
Tumor Cell

Figure 1

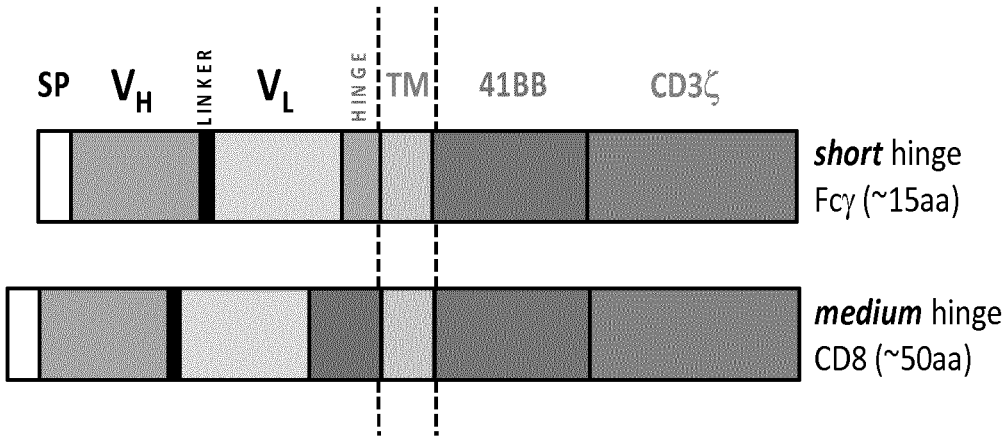

SP
V_H
LINKER
V_L
HINGE
TM
41BB
CD3ζ
short hinge
Fcγ (~15aa)
medium hinge
CD8 (~50aa)

Figure 2

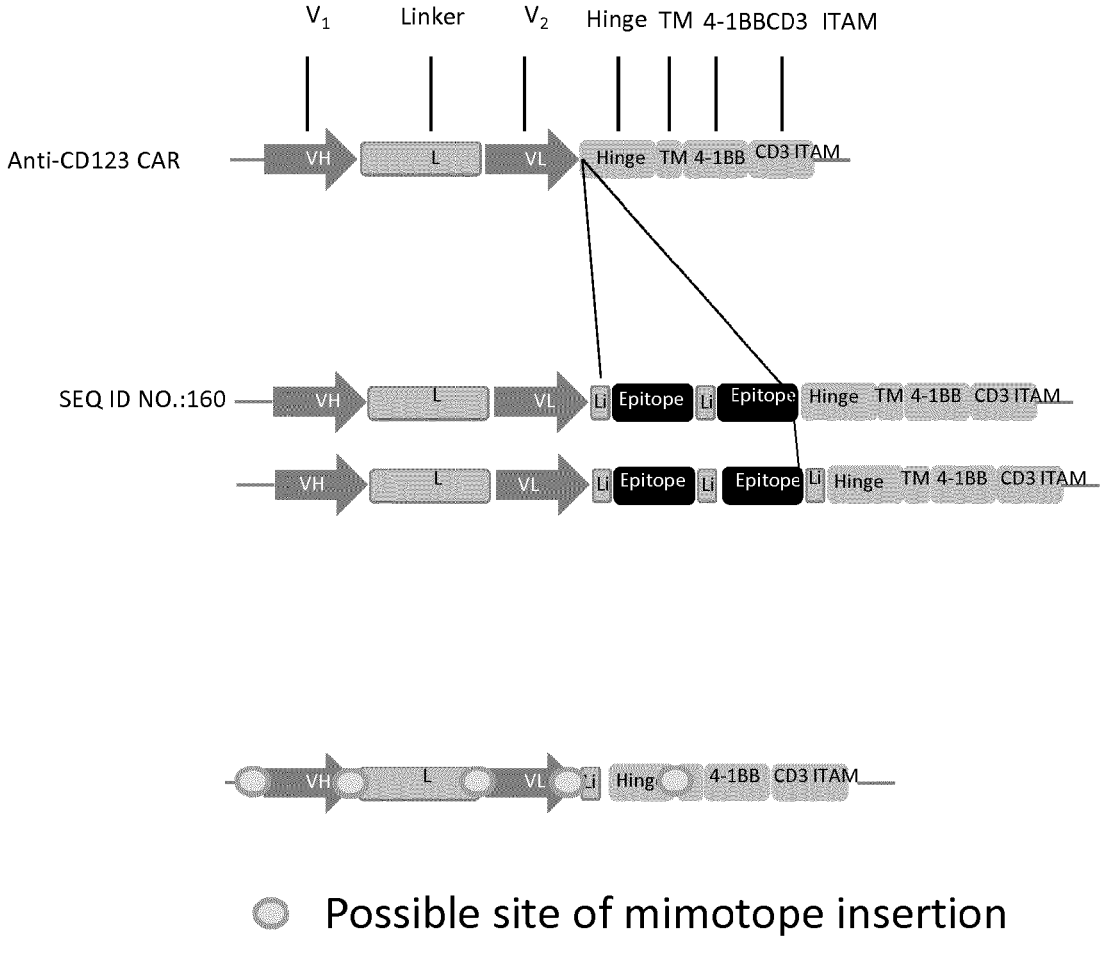

V1
Linker
V2
Hinge TM 4-1BBCD3 ITAM
Anti-CD123 CAR
VH
L
VL
Hinge TM 4-1BB CD3 ITAM
SEQ ID NO.:160
VH
L
VL
Li
Epitope
Li
Epitope
Hinge TM 4-1BB CD3 ITAM
VH
L
VL
Li
Epitope
Li
Epitope
Li
Hinge TM 4-1BB CD3 ITAM
VH
L
VL
Li
Hing
4-1BB CD3 ITAM
Possible site of mimotope insertion

Figure 3

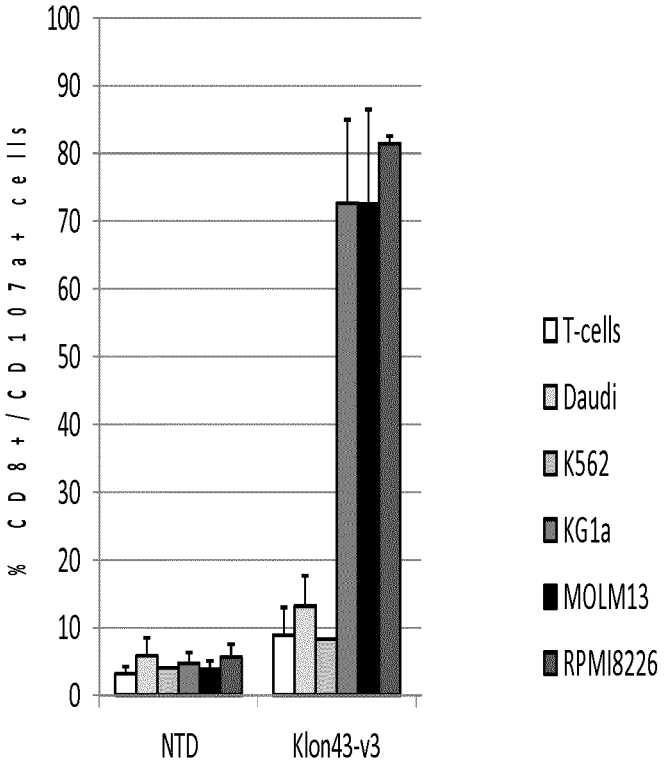

100
90
80
70
60
50
40
30
20
10
0
% CD8+/CD107a+ cells
NTD
Klon43-v3
T-cells
Daudi
K562
KG1a
MOLM13
RPMI8226

Figure 4

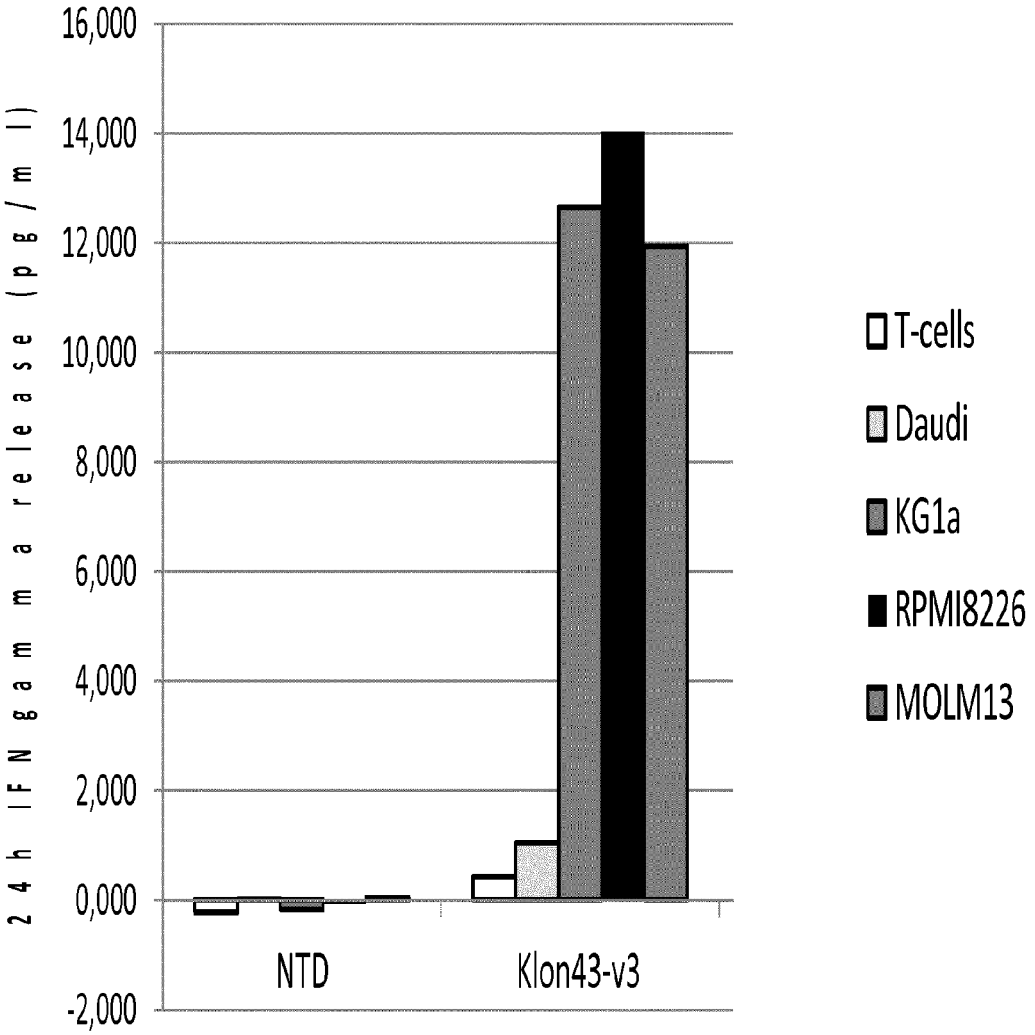

16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0,000
-2,000
24h IFNgamma release (pg/ml)
NTD
Klon43-v3
T-cells
Daudi
KG1a
RPMI8226
MOLM13

Figure 5

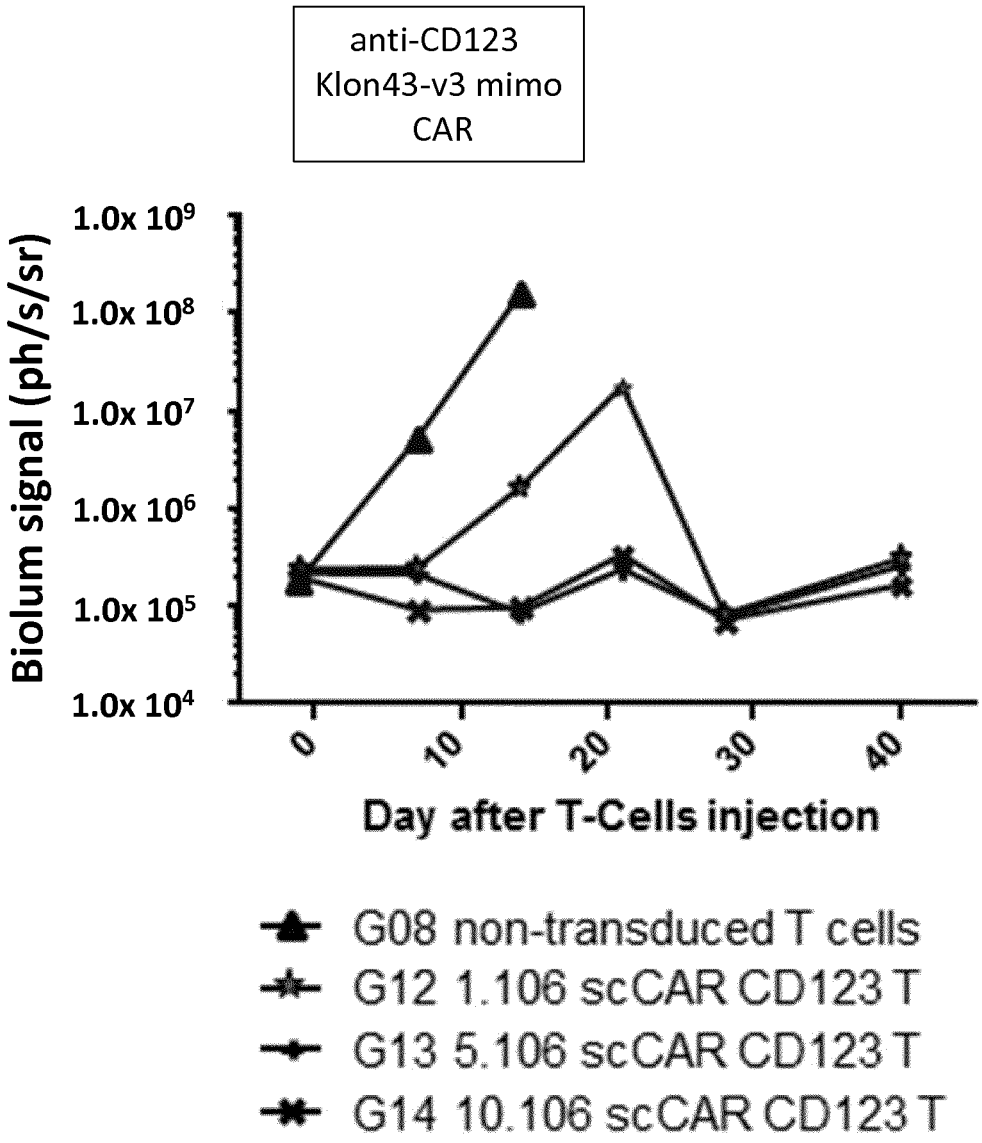

anti-CD123
Klon43-v3 mimo
CAR
Biolum signal (ph/s/sr)
1.0x 10^9
1.0x 10^8
1.0x 10^7
1.0x 10^6
1.0x 10^5
1.0x 10^4
0
10
20
30
40
Day after T-Cells injection
G08 non-transduced T cells
G12 1.106 scCAR CD123 T
G13 5.106 scCAR CD123 T
G14 10.106 scCAR CD123 T

Figure 6

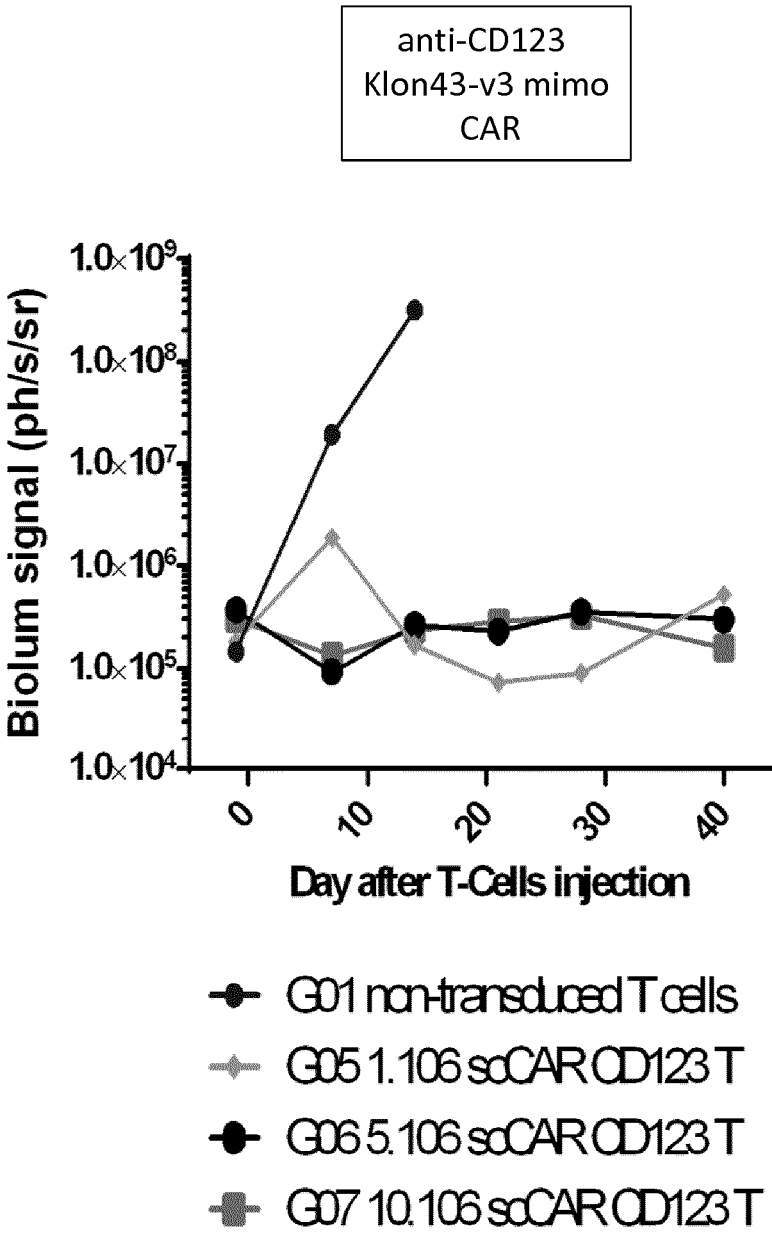

anti-CD123
Klon43-v3 mimo
CAR
Biolum signal (ph/s/sr)
1.0×10⁹
1.0×10⁸
1.0×10⁷
1.0×10⁶
1.0×10⁵
1.0×10⁴
0
10
20
30
40
Day after T-Cells injection
G01 non-transduced T cells
G05 1.106 scCAR CD123 T
G06 5.106 scCAR CD123 T
G07 10.106 scCAR CD123 T

Figure 7

T CELL RECEPTOR KNOCK OUT ENGINEERED IMMUNE CELLS, ENDOWED WITH CHIMERIC ANTIGEN RECEPTORS BINDING TO CD123 FOR THE TREATMENT OF RELAPSED/REFRACTORY ACUTE MYELOID LYMPHOMA OR BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

FIELD OF THE INVENTION

[0001] The present invention relates generally to a TCR gene Knock out T cell engineered to express a Chimeric Antigen Receptor (CAR) specific for interleukin 3 receptor alpha chain (IL-3Ra, cluster of differentiation 123 CD123) and their use, e.g., for the treatment of a disease or a condition associated with expression of IL-3Ra, CD123, namely acute myeloid leukemia (AML) and Blastic plasmacytoid dendritic cell neoplasm (BPDCN).

[0002] The present invention relates to a T cell receptor (TCRalpha or beta gene) and/or human deoxycytidine kinase (dCK gene or dck gene) Knock out (KO) immune T cells engineered to express:

[0003] a Chimeric Antigen Receptor (CAR) specific for the cluster of differentiation 123 (CD123) comprising at least one extracellular ligand binding domain from Klon43, optionally humanized, a hinge, an intracellular domain and a costimulatory domain,

[0004] a suicide domain, optionally inserted into a said hinge,

said CD123 CAR being specific for human CD123 and conferring a specific immunity against CD123 positive cells, in one embodiment; this object for its used for the treatment of acute myeloid leukemia AML, or of a complication of AML is provided,

in one embodiment; this object is provided for the treatment of Blastic plasmacytoid dendritic cell neoplasm (BPDCN). in one embodiment; this object is provided as a treatment before bone marrow transplant as a bridge of transplant.

[0005] The engineered immune cells endowed with the CD123 CARs according to the invention show high efficiency in view of treating lymphomas and leukemias as compared to previous CD123 CAR, and can be used in the presence of purine analogue with less side effects than previous treatments in patients.

BACKGROUND OF THE INVENTION

[0006] Induction treatments for lymphoproliferative diseases such as leukemia and in particular for acute myeloid leukemia (AML) have remained largely unchanged for nearly 50 years. Such standard induction chemotherapy can induce complete remissions, but many patients eventually relapse and succumb to the disease, calling for the development of novel therapeutics for AML, in particular for relapsed refractory AML.

[0007] Similar observations were reported for aggressive lymphoproliferative diseases such as BPDCN. These diseases remain of poor prognosis. Immunophenotyping of these cancerous cells have revealed that the interleukin 3 receptor alpha chain (IL-3Rα; CD123—NCBI reference: NP_001254642) is a potential immunotherapeutic target since it is over-expressed on these tumor cells compared to normal cells. Additionally, two phase I trials for CD123-specific therapeutics have been completed with both drugs displaying good safety profiles (ClinicalTrials.gov ID: NCT00401739 and NCT00397579). Unfortunately, these CD123 targeting drugs had limited efficacy suggesting that alternative, and more potent and specific therapies targeting CD123 are required to observe anti-leukemic activity.

[0008] A possibly more potent alternative therapy for the treatment of Leukemia could be the use of immune cells expressing chimeric antigen receptors (CARs) that selectively direct immune cell specificity towards cell surface tumor associated antigens (TAAs) in an MHC-independent manner (Jena, Dotti et al. 2010) and destroy them.

[0009] CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single or multiple fusion molecule(s). In general, the binding moiety of a CAR consists of an antigen-binding domain of a single-chain antibody (scFv), comprising the light and variable fragments of a monoclonal antibody joined by a flexible linker. Binding moieties based on receptor or ligand domains have also been used successfully. The signaling domains for first generation CARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. First generation CARs have been shown to successfully redirect T cell cytotoxicity, however, they failed to provide prolonged expansion and anti-tumor activity in vivo. Signaling domains from co-stimulatory molecules including OX-40 (CD134), and 4-1BB (CD137) have been added alone or in combination to enhance survival and increase proliferation of CAR modified T cells in vitro. However, not all combination of domains results in a CAR that can direct immune cells to target cells in vivo.

[0010] In mouse models of human cancer, CARs can redirected the CAR-expressing cells against antigens expressed at the surface of tumor cells with an efficiency dependent on the nature and length of each domain (Condomine M. et al., 2015 Plos One 10(6):e0130518). So far autologous transfer of CAR-expressing specific T cells, alone, has been shown to be successful in treating specific forms of cancer despite several side effects such as cytokine storm, non specific destruction of cell populations, or unwanted specific immune reactions (Park, Rosenberg et al. 2011). It is not known whether allogenic CD123 CAR engineered T cells can be used safely and efficiently for the treatment of AML (ClinicalTrials.gov ID: NCT02159495).

[0011] Thus, to broaden the population of patients that may benefit such treatments so called allogenic T cells expressing CAR have been prepared for their use in human suffering cancer. in that case, immune cells are isolated from healthy donors, engineered, and then used as a treatment in several different selected patients in need thereof.

[0012] For that purpose and to reduce the risk of potential graft versus host disease, selected genes were carefully knocked out to provide engineered CAR-expressing immune cells with reduced expression of molecules involved in the immune response, for example that of MHC molecules or subunits of the TCR molecules. The extent to which such engineered cells still proliferate and survive in any hosts remains largely unknown, especially in patients already treated with chemotherapy agents.

[0013] The use of such CAR expressing immune T cell targeting CD123 in combination with cytotoxic chemotherapy agents as a treatment usually employed as anti-cancer treatments remains a problem as anti-cancer treatments also affect the proliferation and/or survival of T cells.

Thus, there is also a need of developing T cells targeting CD123 that would be specific efficient and compatible with the use of drugs, in particular of anti-cancer chemotherapies, such as those affecting cell proliferation, that would still be able to proliferate and survive to reach their target (cancer cells).

[0014] To use "off-the-shelf" allogeneic therapeutic cells in conjunction with chemotherapy, the inventors identified means to provide allogenic engineering T-cell, less allogenic and permissive for the immune system of patients suffering a cancer and/or already treated with chemotherapeutic agents. The therapeutic benefits are provided by the synergistic effects between chemotherapy and immunotherapy of the claimed objects.

[0015] The present invention is also drawn to anti-CD123 chimeric antigen receptors (anti-CD123 CAR), which extracellular binding domain (scFv) is modified in such a way to allow both cell sorting and cell depletion. The structure allowing this is an epitope recognized by a monoclonal antibody (named mAb-driven sorting/depletion system or epitope recognized by a specific monoclonal antibody or mimotope) and comprises a selected epitope inserted into the extracellular domain within the scFv and/or the hinge. This epitope has the specificity to be recognized by a specific antibody (preferably a monoclonal antibody (mAb), optionally humanized). Given the fact that mainly the external ligand binding domain of the CAR is modified to include the epitope, different CAR architectures can be envisioned: single-chain or multi-chain as disclosed in PCTUS2013/058005.

[0016] The chimeric scFv of the invention, which is formed of the VH and VL polypeptides and the specific epitope(s) may itself have different structures depending on the position of insertion of the epitope and the use of linkers (FIG. **3**). The present invention also relates to the resulting method for sorting and/or depleting the engineered immune cells endowed with the modified CARs.

[0017] Several epitope-mAb couples can be used to generate such system; in particular those already approved for medical use, such as CD20/rituximab as a non-limiting example.

[0018] Finally, the invention encompasses therapeutic methods where number, activity and survival of the engineered immune cells endowed with anti-CD123 CARs is modulated by depleting the cells by using an antibody that directs the external ligand binding domain of said CARs.

SUMMARY OF THE INVENTION

[0019] Interleukin 3 receptor alpha chain (CD123) has been identified as being frequently over-expressed on Leukemia tumor cells, especially in the case of acute myeloid leukemia (AML), compared to normal cells of the same lineage.

[0020] The inventors have generated an immune cell engineered to express a CD123 specific CAR comprising a scFV from KLON43 antibody, a hinge from FcγRIIIα, and intracellular domains conferring host cells the capacity to proliferate in vivo, reach CD123 target cells and alter their survival, said cell comprising additional marker or suicide domain allowing their specific destruction once target cells are contained. These CD123 specific CAR are designated CD123 specific CAR or anti-CD123 CAR, or 123 CAR, or "CAR of the invention" indiscriminately.

[0021] In the present invention, a specific and selective tolerogenic TCR KO CD123 CAR expressing T cell was prepared using one antibody specific for this IL-3 receptor subunit, namely klon43 or humanized sequences derived from this klon43 antibody for the treatment of patients suffering AML, B-cell lymphoproliferative disorder (BC-LPD) or BPDCN.

[0022] Concomitantly, these cells allow the destruction precancerous cells stopping the progression and emergence of refractory/relapsed cancer. Due to their capacity to proliferate in vivo and reach tissues or niches these cells acts faster than cancerous cells themselves and can eradicate even aggressive lymphoproliferative disorder.

[0023] Following non-specific activation in vitro (e.g. with anti CD3/CD28 coated beads and recombinant IL2), T-cells from donors have been transformed with polynucleotides expressing CARs of the invention using viral transduction. The T-cells were further engineered to create less-alloreactive T-cells, by disruption of a component of the T cell receptor TCR (αβ-T-Cell receptors) to reduce Graft versus host reaction.

[0024] In a preferred embodiment, T-cells were further engineered by deleting specific combination(s) of genes identified in table 9, to create tolerogene T cells resistant to anti-cancer drugs, to be used in combination with said classical anti-cancer drugs, namely purine analogs.

[0025] The resulting engineered T-cells displayed reactivity against CD123 positive cells showing that the CARs of the present invention contribute to antigen dependent activation, and also proliferation, of the T-cells, making them useful for immunotherapy.

[0026] The resulting engineered T-cells displayed reactivity in-vivo against CD123 positive cells and significantly reduce the number of cancer cells in vivo.

[0027] In a particular embodiment, several administrations of the engineered T-cells of the invention can be performed, making them useful for immunotherapy as a first treatment (induction), as a consolidation treatment, as a treatment in combination with classical anticancer chemotherapy.

[0028] The polypeptides and polynucleotide sequences encoding the CARs of the present invention are detailed in the present specification.

[0029] The engineered immune cells of the present invention are particularly useful for therapeutic applications such as B-cell lymphoma or leukemia treatments and can be selectively eliminated from the organism. R

BRIEF DESCRIPTION OF THE FIGURES

[0030] FIG. **1**: Schematic representation of an engineered immune cell according to the invention. The engineered immune cell presented in this figure is a T-cell transduced with a retroviral polypeptide encoding CAR. This T-cell is further engineered to allow a better and safer engraftment into the patient, which is part of the frame of the present invention. X gene is a gene expressing a component of TCR (TCRalpha or TCRbeta gene), Y a gene involved into the sensitivity of T-cells to purine analogues, dCK.

[0031] FIG. **2**: Schematic representation of the two CAR of the invention (123 CAR) comprising a CD123 scfv, optionally humanized, a hinge from FcRIII or CD8alpha, a transmembrane domain from CD8alpha, and two intracellular domains from 4-1BB and CD3zeta.

[0032] FIG. **3**: Shows examples of a CAR according to the invention.

[0033] L: linker between VH and VL of (GGGGS)n with n=1 to 4, preferably n=3 (SEQ ID NO.: 10)

[0034] Li: linker sequence corresponding to GGGS or SGGGGS or GSGGGGS, TM: transmembrane domain.

[0035] A CD123 CAR of the invention comprising a VH from Klon 43, optionally humanized, a linker L, a VL from Klon 43, optionally humanized, a suicide domain (e.g. two copies of a CD20 mimotope of sequence CPYSNPSLCS (SEQ ID NO. 161), and a copy of SEQ ID NO 169), said mimotopes are located preferably in the scfv, a CD8 hinge or part of it, a transmembrane domain (CD8 TM) from CD8alpha, a co-stimulatory domain (4-113B) and a stimulatory domain (ITAM CD3 zeta), was prepared.

[0036] In a CD123 CAR of the invention, two copies of a CD20 mimotope of sequence CPYSNPSLCS, linked to each other and to the VL by a linker Li, were inserted between the anti-CD123 scFv and a hinge from CD8alpha, an optional linker LI joins the mimotopes to the hinge (SEQ ID NO 160).

[0037] Other possibilities of epitope (eg in scfv) insertion are contemplated and illustrated as a circle in FIG. **3**.

[0038] FIG. **4**: Degranulation activity of different a scFv according to the invention for one architecture (v3: CD8-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD123 expressing cells (RPM18226), or with cells that do not express CD123

[0039] FIG. **5**: IFN gamma release by T-cells when co-cultured for 24 h with cells expressing different levels of (K562). CD123 (KG1a or RPM18226), or with cells that do not express CD123 (K562)

[0040] FIG. **6**: Dose-response of the specific cytolytic activity of CAR-T cells in vivo in mice treated with PNA (20 mg/kg) ip.

[0041] FIG. **7**: Dose-response of the specific cytolytic activity of CAR-T cells in vivo in mice treated with PNA (20 mg/kg) ip.

[0042]

TABLE 1

Functional domains	SEQ ID #	amino acid sequence
CD8α signal peptide	SEQ ID NO. 1	MALPVTALLLPLALLLHAARP
Alternative signal peptide	SEQ ID NO. 2	METDTLLLWVLLLWVPGSTG
FcγRIIIα hinge	SEQ ID NO. 3	GLAVSTISSFFPPGYQ
CD8α hinge	SEQ ID NO. 4	TTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACD
IgG1 hinge	SEQ ID NO. 5	EPKSPDKTHTCPPCPAPPVAGPSVFLFPP KPKDTLMIARTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALH NHYTQKSL SLSPGK
CD8α transmembrane domain	SEQ ID NO. 6	IYIWAPLAGTCGVLLLSLVITLYC
41BB transmembrane domain	SEQ ID NO. 7	IISFFLALTSTALLFLLFFLTLRFSVV
41BB intracellular domain	SEQ ID NO. 8	KRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCEL
CD3ζ intracellular domain	SEQ ID NO. 9	RVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQA LPPR
Linker	SEQ ID NO. 10	GGGGSGGGGSGGGGS

TABLE 2

Sequence of the antibody fragments from Klon 43 used in the anti-CD123 scfv of the invention for the CD123 CAR of the invention

ScFv sequences	SEQ ID #	amino acid sequence
Klon43 light chain variable region	SEQ ID NO. 11	MADYKDIVMTQSHKFMSTSVGDRVNITCKA SQNVDSAVAWYQQKPGQSPKALIYSASYRY SGVPDRFTGRGSGTDFTLTISSVQAEDLAVYY CQQYYSTPWTFGGGTKLEIKR
Klon43 heavy chain variable region	SEQ ID NO. 12	EVKLVESGGGLVQPGGSLSLSCAASGFTFTD YYMSWVRQPPGKALEWLALIRSKADGYTTE YSASVKGRFTLSRDDSQSILYLQMNALRPEDS ATYYCARDAAYYSYYSPEGAMDYWGQGTS VTVSS

TABLE 3

Sequence of the humanized antibody fragments from Klon 43 used in the anti-CD123 scfv of the invention for the CD123 CAR of the invention

Functional domains	SEQ ID #	Raw amino acid sequence
Humanized scFv Klon43 Variant VL1	SEQ ID NO. 18	MADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VL2	SEQ ID NO. 19	MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VL3	SEQ ID NO. 20	MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKALIYSASYRYSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VL4	SEQ ID NO. 21	MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKLLIYSASYRYSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VL5	SEQ ID NO. 22	MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKLLIYSASYRQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VL6	SEQ ID NO. 23	MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDS AVAWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEI KR
Humanized scFv Klon43 Variant VH1	SEQ ID NO. 24	EVKLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGLIRSKADGYTTEYSAS VKGRFTISRDDSKSILYLQMNSLKTEDTAVYYC ARDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH2	SEQ ID NO. 25	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGLIRSKADGYTTEYSA SVKGRFTISRDDSKSILYLQMNSLKTEDTAVY YCARDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH3	SEQ ID NO. 26	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGLIRSKADGYTTEYSAS VKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCA RDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH4	SEQ ID NO. 27	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGFIRSKADGYTTEYSAS VKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCA RDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH5	SEQ ID NO. 28	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGFIRSKADGYTTEYAAS VKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCA RDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH6	SEQ ID NO. 29	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDY YMSWVRQAPGKGLEWVGLIRSKADGYTTEYAA SVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYY CARDAAYYSYYSPEGAMDYWGQGTLVTVSS
Humanized scFv Klon43 Variant VH7	SEQ ID NO. 30	EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSW VRQAPGKGLEWVGFIRSKADGYTTEYAASVKGRFTIS RDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPE GAMDYWGQGTLVTVSS

TABLE 4

CAR of structure V-1

CAR Designation V-1	CAR Structure: signal peptide	VH	VL	FcγRIIIα hinge	CD8α TM	41BB-IC	CD3ζ CD
Klo43-1 (SEQ ID NO: 31)	SEQ ID NO. 1	SEQ ID NO. 12	SEQ ID NO. 11	SEQ ID NO. 3	SEQ ID NO. 6	SEQ ID NO. 8	SEQ ID NO. 9

TABLE 5

CAR of structure V-3

CAR Designation V-3	CAR Structure: signal peptide	VH	VL	CD8α hinge	CD8α TM	41BB-IC	CD3ζ CD
Klo43-3 (SEQ ID NO: 32)	SEQ ID NO. 1	SEQ ID NO. 12	SEQ ID NO. 11	SEQ ID NO. 4	SEQ ID NO. 6	SEQ ID NO. 8	SEQ ID NO. 9

TABLE 6

CAR of structure V-5

CAR Designation V-5	CAR Structure: signal peptide	VH	VL	IgG1 hinge	CD8α TM	41BB-IC	CD3ζ CD
Klo43-5 (SEQ ID NO: 33)	SEQ ID NO. 1	SEQ ID NO. 12	SEQ ID NO. 11	SEQ ID NO. 5	SEQ ID NO. 6	SEQ ID NO. 8	SEQ ID NO. 9

TABLE 7

Examples of mAb-specific epitopes also called mimotope (and their corresponding mAbs) that can be used in the extracellular domain of the CAR of the invention such as for example mimotopes and epitope with their corresponding mAb

Rituximab			
Mimotope	SEQ ID NO 161	CPYSNPSLC	
Palivizumab			
Epitope	SEQ ID NO 162	NSELLSLINDMPITNDQKKLMSNN	
Cetuximab			
Mimotope 1	SEQ ID NO 163	CQFDLSTRRLKC	
Mimotope 2	SEQ ID NO 164	CQYNLSSRALKC	
Mimotope 3	SEQ ID NO 165	CVWQRWQKSYVC	
Mimotope 4	SEQ ID NO 166	CMWDRFSRWYKC	
Nivolumab			
Epitope 1	SEQ ID NO 167	SFVLNWYRMSPSNQTDKLAAFPEDR	
Epitope 2	SEQ ID NO 168	SGTYLCGAISLAPKAQIKE	

TABLE 7-continued

Examples of mAb-specific epitopes also called mimotope (and their corresponding mAbs) that can be used in the extracellular domain of the CAR of the invention such as for example mimotopes and epitope with their corresponding mAb

QBEND-10			
Epitope	SEQ ID NO 169	ELPTQGTFSNVSTNVSPAKPTTTA	
Alemtuzumab			
Epitope	SEQ ID NO 170	GQNDTSQTSSPS	

[0043] Sequences of the anti-CD123 CAR of the invention (SEQ ID NO.:31-160), preferred sequences are SEQ ID NO.:31, 32, 160 and 34-117, among 34-117 more preferred are 76 to 117, even more preferred SEQ ID NO. 32, SEQ ID NO. 89, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97.

DETAILED DESCRIPTION OF THE INVENTION

[0044] The present invention provides:

[0045] 1. A CD123 specific chimeric antigen receptor (CD123 CAR) comprising

[0046] an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH optionally humanized a linker, preferably a linker of sequence (GGGGS)n with n=1-4, preferably n=3, and a VL optionally humanized a hinge,

[0047] a transmembrane domain and

[0048] a cytoplasmic domain.

[0049] at least one epitope specific for a monoclonal antibody (mimotope).

[0050] 2. the CD123 CAR according to 1 comprising

[0051] an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH selected from SEQ ID NO 12, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29 and SEQ ID NO 30, optionally humanized a linker, preferably a linker of sequence (GGGGS)n with n=1-4, preferably n=3, and a VL selected from SEQ ID NO 11, 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, optionally humanized a hinge,

[0052] a transmembrane domain from CD8 alpha, and

[0053] a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0054] 3. The CD123 CAR according to 1 or 2 comprising no sequence having identity the human CD28 NP_006130.1.

[0055] 4. The CD123 CAR according to any one of 1 to 3 comprising a sequence selected from SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174, SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO

184, SEQ ID NO 185, SEQ ID NO 186 and SEQ ID NO 187, optionally further comprising at least one SEQ ID No 161.

[0056] 5. the CD123 CAR according to any one of 1 to 4 wherein said extracellular domain comprises at least one epitope specific for a monoclonal antibody (mimotope), selected from the list consisting of SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169 and SEQ ID NO 170, preferably of SEQ ID NO 161 and of SEQ ID NO 169.

[0057] 6. the CD123 CAR according to any one of 1 to 5 comprising a sequence selected from SEQ ID NO 160, SEQ ID NO 171, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 195, SEQ ID NO 196, and SEQ ID NO 197.

[0058] 7. A polynucleotide encoding a CD123 specific chimeric antigen receptor (CD123 CAR) according to any one of claims **1** to **6**.

[0059] 8. An expression vector comprising a polynucleotide according to claim **7**.

[0060] 9. An expression vector comprising a backbone and at least one sequence coding any one of the CD123 CAR defined in any one of claims **1** to **6**.

[0061] 10. An expression vector comprising a backbone, an EF1 promotor, an RQR8 open reading frame (RQR8 ORF), a sequence coding any one of the CD123 CAR of 1 to 6.

[0062] 11. A T Cell Receptor (TCR) knock-out (KO) or TCR and human deoxycytidine kinase (dCK) KO engineered immune cell expressing at the cell surface membrane a CD123 CAR according to any one of 1 to 6.

[0063] 12. A TCR KO or TCR and dCK KO engineered immune cell comprising a polynucleotide coding a CD123 specific chimeric antigen receptor (CD123 CAR) according to any one of 1 to 6.

[0064] 13. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 4 further expressing a suicide domain at the cell surface.

[0065] 14. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to any one of the 11 to 13 wherein the expression of at least one MHC protein, is suppressed.

[0066] 15. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to any one of 11 to 14 for use in therapy.

[0067] 16. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 15 wherein the condition is acute myelogenous leukemia (AML), preferably refractory/relapsed AML, BPDNL, or for use during or before bone marrow transplant.

[0068] 17. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 15, for use as a treatment, preferably as a treatment for a lymphoproliferative disorder, more preferably for leukemia of lymphoma or for a treatment selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome and BPDNL.

[0069] 18. A TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 15, for use as a treatment for AML.

[0070] The present invention also provides:

1a. A polypeptide encoding chimeric antigen receptor (CAR) specific for CD123 comprising at least one extracellular binding domain, said extracellular domain comprising at least one scFv said scfv is comprising at least a VH chain and a VL chain binding specifically to CD123, wherein said extracellular binding domain comprises at least one mAb-specific epitope or mimotope.

2a. The polypeptide according to 1a, wherein said mAb-specific epitope is located between the VH and VL chains, or before the VH and the VL.

[0071] In one embodiment, the polypeptide encoding a chimeric antigen receptor (CAR) specific for CD123 is comprising a transmembrane domain (TM) and a hinge and said mAb-specific epitope is located between the scfv and the hinge.

[0072] The present invention provides:

3a. a polypeptide according to 1a or 2a, wherein said VH and VL chains, and mAb specific-epitope are bound together by at least one linker and by a hinge to the transmembrane domain of said CAR.

4a. The polypeptide according to 3a, wherein the mAb-epitope is joined to the VH and VL chains by two linkers.

5a. The polypeptide according to any one of 1a to 3a wherein the mAb-specific epitope is an epitope to be bound by an epitope-specific mAb for in vitro cell sorting and/or in vivo cell depletion of T cells expressing a CAR comprising such epitope.

6a. The polypeptide according to any one of 1a to 5a, wherein the polypeptide comprises one extracellular binding domain, a transmembrane domain, and an intracellular domain, wherein said extracellular binding domain comprises at least one mAb-specific epitope.

7a. The polypeptide according to any one of 1a to 6a, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

8a. The polypeptide according to any one of 1a to 7a, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.

9a. The polypeptide according to any one of 1a to 8a, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes.

10a. The polypeptide according to any one of 1a to 9a, wherein the extracellular binding domain comprises the following sequence

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

wherein,

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

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

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

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

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

11a. The polypeptide according to 10a, wherein the extracellular binding domain comprises the following sequence V_1-L_1-V_2-L-Epitope1; V_1-L_1-V_2-L-Epitope1-L; V_1-L_1-V_2-L-Epitope1-L-Epitope2; V_1-L_1-V_2-L-Epitope1-L-Epitope2-L; V_1-L_1-V_2-L-Epitope1-L-Epitope2-L-Epitope3; V_1-L_1-V_2-L-Epitope1-L-Epitope2-L-Epitope3-L; V_1-L_1-V_2-Epitope1; V_1-L_1-V_2-Epitope1-L; V_1-L_1-V_2-Epitope1-L-Epitope2; V_1-L_1-V_2-Epitope1-L-Epitope2-L; V_1-L_1-V_2-Epitope1-L-Epitope2-L-Epitope3; V_1-L_1-V_2-Epitope1-L-Epitope2-L-Epitope3-L; Epitope1-V_1-L_1-V_2; Epitope1-L-V_1-L_1-V_2; L-Epitope1-V_1-L_1-V_2; L-Epitope1-L-V_1-L_1-V_2; Epitope1-L-Epitope2-V_1-L_1-V_2; Epitope1-L-Epitope2-L-V_1-L_1-V_2; L-Epitope1-L-Epitope2-V_1-L_1-V_2; L-Epitope1-L-Epitope2-L-V_1-L_1-V_2; Epitope1-L-Epitope2-L-Epitope3-V_1-L_1-V_2; Epitope1-L-Epitope2-L-Epitope3-L-V_1-L_1-V_2; L-Epitope1-L-Epitope2-L-Epitope3-V_1-L_1-V_2; L-Epitope1-L-Epitope2-L-Epitope3-L-V_1-L_1-V_2; V_1-L-Epitope1-L-V_2; L-Epitope1-L-V_1-L-Epitope2-L-V_2; V_1-L-Epitope1-L-V_2-L-Epitope2-L; V_1-L-Epitope1-L-V_2-L-Epitope2-L-Epitope3; V_1-L-Epitope1-L-V_2-L-Epitope2-Epitope3; V_1-L-Epitope1-L-V_2-L-Epitope2-L-Epitope3-Epitope4; L-Epitope1-L-V_1-L-Epitope2-L-V_2-L-Epitope3-L; Epitope1-L-V_1-L-Epitope2-L-V_2-L-Epitope3-L; L-Epitope1-L-V_1-L-Epitope2-L-V_2-L-Epitope3; L-Epitope1-L-V_1-L_1-V_2-L-Epitope2-L; L-Epitope1-L-V_1-L_1-V_2-L-Epitope2-L-Epitope3; L-Epitope1-L-V_1-L_1-V_2-L-Epitope2-Epitope3, or Epitope1-L-V_1-L_1-V_2-L-Epitope2-L-Epitope3-Epitope4 wherein

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

L_1 is 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 occurrences of L in the same extracellular binding domain, and,

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

12a. The polypeptide according to 10a, wherein L_1 is a linker comprising Glycine and/or Serine.

13a. The polypeptide according to 12a, wherein L_1 is a linker comprising the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$ or $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is 1, 2, 3, 4 or 5 or a linker comprising the amino acid sequence $(Gly_4Ser)_4$ or $(Gly_4Ser)_3$.

14a. The polypeptide according to any one of 10a to 13a wherein L is a linker comprising Glycine and/or Serine.

15a. The polypeptide according to 14a wherein L is a linker having an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGGS, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGGSGGGGS.

16a. The polypeptide according to 14a wherein L is a SGGGG, GGGGS or SGGGGS.

17a. The polypeptide according to any one of 10a to 16a wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10 and ustekinumab.

[0073] In a preferred embodiment said Epitope 1, Epitope 2, are specifically recognized by rituximab and epitope 3 is specifically recognized by QBEND-10.

18a. The polypeptide according to any one of 10a to 16a wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes having an amino acid sequence of SEQ ID NO 161 to 170.

19a. The polypeptide according to any one of 10a to 18a wherein Epitope 1 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 161.

20a. The polypeptide according to any one of claims **10**a to **19**a wherein Epitope 2 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 161.

21a. The polypeptide according to any one of claims **10**a to **20**a wherein Epitope 3 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 161 or SEQ ID NO 169.

22a. The polypeptide according to any one of claims **10**a to **21**a wherein Epitope 4 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 161.

23a. The polypeptide according to claim **22**a wherein Epitope 1, Epitope 2 and Epitope 4 are a mAb-specific epitope having an amino acid sequence of SEQ ID NO 161 and Epitope 3 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 169.

24a. The polypeptide according to anyone of 1a to 9a, wherein the mAb-specific epitope is from one polypeptide selected from those listed in Table 7.

25a. The polypeptide according to any one of 1a to 9a wherein the mAb-specific epitope is 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 and ustekinumab.

26a. The polypeptide according to any one of 1a to 9a wherein the mAb-specific epitope is selected from mAb-specific epitope having an amino acid sequence of SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169 or SEQ ID NO 170.

27a. The polypeptide according to any one of 1a to 9a wherein the mAb-specific epitope is has an amino acid sequence of SEQ ID NO 160.

28a. The polypeptide according to anyone of 1a to 27a, wherein said VH and VL chains have an antigenic target sequence of over 80% identity, preferably over 90%, and more preferably over 95% with SEQ ID NO 11 (CD123 antigen VH), SEQ ID NO 12 (CD123 antigen VL).

29a. The polypeptide according to any one of 1a to 27a wherein said CD123 antigen is a cell surface marker antigen.

30a. The polypeptide according to any one of 1a to 27a wherein said CD123 antigen is a tumor-associated surface antigen.

31a. The polypeptide according to any one of 1a to 27a wherein said antigen is CD123,

32a. The polypeptide according to any one of 1a to 27a wherein VH and VL are selected from a VH of SEQ ID NO 11, SEQ ID NO 24 to SEQ ID NO 30 and a VL of SEQ ID NO 12; SEQ ID NO 18 to SEQ ID NO 23.

33a. The polypeptide according to any one of 2a to 32a wherein the hinge comprises a CD8alpha hinge or a FcγRIII alpha hinge.

34a. The polypeptide according to any one of 2a to 3a3 wherein the transmembrane domain comprises the transmembrane region(s) CD8,

35a. The polypeptide according to any one of 2a to 33a wherein the transmembrane domain comprises the transmembrane region(s) of CD8 alpha.

36a. The polypeptide according to any one of 2a to 33a wherein the transmembrane domain comprises the transmembrane region(s) of CD8 alpha and a hinge from CD8 alpha.

37a. The polypeptide according to any one of 2a to 37a wherein the intracellular domain comprises a CD3zeta signaling domain.

38a. The polypeptide according to any one of 2a to 37a wherein the intracellular domain comprises a 4-1BB domain.

39a. A polypeptide according to anyone of 1a to 38a, wherein the CAR is a single-chain CAR.

40a. A polypeptide according to anyone of 1a to 38a wherein the CAR is a multi-chain CAR.

40a bis A polypeptide according to anyone of 1a to 39a wherein the CAR a sequence selected from SEQ ID NO 189 to SEQ ID NO 197.

41a. A polynucleotide encoding a polypeptide according to anyone of 1a to 40a.

42a. A polynucleotide encoding a chimeric antigen receptor according to anyone of 1a to 40a, wherein said CAR comprises a CD3 zeta signaling domain and co-stimulatory domain from 4-113B.

43a. An expression vector comprising a nucleic acid of 41a or 42a.

44a. An engineered immune cell expressing at its cell surface a polypeptide according to anyone of 1a to 40a.

45a. The engineered immune cell according to 44a, wherein said cell is derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes (CTL), regulatory T-lymphocytes or helper T-lymphocytes, preferably a CTL cell.

46a. The engineered immune cell according to 44a or 45a for use as a medicament.

47a. A method for engineering an immune cell of anyone of 44a-46a, comprising:

(a) Providing an immune cell;

(b) Introducing into said cell at least one polynucleotide encoding the chimeric antigen receptor according to anyone of 1a-40a.

(c) Expressing said polynucleotide into said cell.

48a. The method for engineering an immune cell of 47a, wherein immune cell is a T-cell.

49a. A method for in vitro sorting engineered immune cell expressing at its cell surface a polypeptide comprising at least one mAb-specific epitope according to anyone of claims **1**a to **40**a comprising

[0074] contacting a population of immune cells comprising said engineered immune cells with a monoclonal antibody specific for the mAb-specific epitope;

[0075] selecting the cells that bind to the monoclonal antibody to obtain a population of cells enriched in engineered immune cell.

50a. The method according to 49a wherein the monoclonal antibody specific for the mAb-specific epitope is conjugated to a fluorophore and the step of selecting the cells that bind to the monoclonal antibody is done by Fluorescence Activated Cell Sorting (FACS).

51a. The method according to 49a wherein the monoclonal antibody specific for the mAb-specific epitope is conjugated to a magnetic particle and the step of selecting the cells that bind to the monoclonal antibody is done by Magnetic Activated Cell Sorting (MACS).

52a. The method according to any one of 49 to 51 wherein the polypeptide comprises an mAb-specific epitope having an amino acid sequence of SEQ ID NO 160 and the monoclonal antibody is rituximab.

53a. The method according to any one of 49a to 51a wherein the polypeptide comprises an mAb-specific epitope having an amino acid sequence of SEQ ID NO 169 and the antibody used to contact the population of immune cells is QBEND-10.

54a. The method according to any one of 49a to 53a wherein the population of cells enriched in engineered immune cell comprises at least 70%, 75%, 80%, 85%, 90%, 95% of CAR-expressing immune cells.

55a. A method for in vivo depleting an engineered immune cell expressing at its cell surface a polypeptide comprising at least one mAb-specific epitope according to anyone of 1a to 40a in a patient, comprising contacting said engineered immune cell with at least one epitope-specific mAb.

56a. The method according to 56a wherein the mAb-specific epitope is a CD20 epitope or mimotope and the epitope-specific mAb is rituximab.

57a. The method according to 57a wherein the mAb-specific epitope has an amino acid sequence of SEQ ID NO 160.

58a. The method according to any one of 56a to 58a wherein the epitope-specific mAb is conjugated with a molecule able to activate the complement system.

59a. The method according to any one of 56a to 58a wherein, wherein a cytotoxic drug is coupled to the epitope-specific mAb.

60a. A method for in vivo depleting an engineered immune cell expressing at its cell surface a polypeptide comprising

at least one mAb-specific epitope according to anyone of 1a to 40a in a patient, comprising contacting said engineered immune cell with bi-specific mAb (BsAb) able to bind both the mAb-specific epitope borne on said cells and to an surface antigen borne on an effector (and cytotoxic) cell.

61a. A method according to any one of 47a to 60a, wherein said immune cell is a T-cell.

[0076] specifically, the present invention also provides 1b a CD123 specific chimeric antigen receptor (CD123 CAR) comprising

[0077] an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH selected from SEQ ID NO 12, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29 and SEQ ID NO 30, optionally humanized at least one linker, preferably a linker of sequence (GGGGS)n with n=1-4, more preferably n=3, and a VL selected from SEQ ID NO 11, 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, optionally humanized a hinge,

[0078] a transmembrane domain from CD8 alpha, and

[0079] a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0080] A CD123 specific chimeric antigen receptor (CD123 CAR) comprising

[0081] an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH selected from SEQ ID NO 12, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29 and SEQ ID NO 30, a linker of SEQ ID NO. 10, and a VL selected from SEQ ID NO 11, 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, a hinge,

[0082] a transmembrane domain from CD8 alpha, and

[0083] a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

2b the present invention provides a CD123 CAR according to 1b comprising no sequence having identity the human CD28 NP_006130.1.

3b The present invention provides a CD123 CAR according to 1b or 2b wherein said extracellular domain comprises at least one epitope specific for a monoclonal antibody of sequence selected from SEQ ID NO 161 to SEQ ID NO 170, or a combination thereof, preferably at least one epitope specific for a monoclonal antibody of SEQ ID NO 161.

[0084] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence selected from SEQ ID NO 189 to SEQ ID NO 197.

[0085] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 190

[0086] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 191

[0087] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 192

[0088] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 193

[0089] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 194

[0090] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 195

[0091] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 196

[0092] The present invention provides a CD123 CAR wherein the CD123 CAR comprises a sequence of SEQ ID NO 197.

[0093] The present invention provides a CD123 CAR according to 1b or 2b wherein said extracellular domain comprises an scfv comprising at least one epitope specific for a monoclonal antibody of sequence selected from SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168 and SEQ ID NO 169, and SEQ ID NO 170.

[0094] Preferably, the present invention provides a CD123 CAR according to 1b or 2b wherein said extracellular domain comprises an scfv comprising two epitopes specific for a monoclonal antibody recognizing SEQ ID NO 161 and one other two epitopes specific for a monoclonal antibody recognizing SEQ ID NO 169.

4b. The present invention provides a CD123 CAR according to 3b comprising a sequence of SEQ ID NO 171.

5b The present invention provides a CD123 specific chimeric antigen receptor (CD123 CAR) according to 1b having a sequence selected from SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO. 33 and SEQ ID No 34 to SEQ ID No 160.

6b The present invention provides a CD123 specific chimeric antigen receptor (CD123 CAR) according to any one of 1b to 5b having a sequence selected from SEQ ID No 34 to SEQ ID No 160.

7b The present invention provides a polynucleotide encoding a CD123 specific chimeric antigen receptor (CD123 CAR) according to any one of 1b to 6b.

8b The present invention provides an expression vector comprising a polynucleotide according to 7.

9b) The present invention provides an expression vector comprising a backbone and a sequence coding any one of the CD123 CAR defined in any one of 1b to 6b.

10b) The present invention provides an expression vector comprising a backbone, preferably a backbone comprising an EF1 promotor, an RQR8 open reading frame (RQR8 ORF), a sequence coding any one of the CD123 CAR of 1 to 8 embodiments above.

11b) The present invention provides a T Cell Receptor (TCR) knock-out (KO) or TCR and human deoxycytidine kinase (dCK) KO engineered immune cell expressing at the cell surface membrane a CD123 CAR according to any one of 1b to 9b.

12b) The present invention provides a TCR KO or TCR and dCK KO engineered immune cell comprising a polynucleotide coding a CD123 specific chimeric antigen receptor (CD123 CAR) according to any one of 1b to 6b.

[0095] A TCR KO or TCR and dCK KO engineered immune cell comprising an expression vector comprising a polynucleotide encoding a CD123 specific chimeric antigen receptor (CD123 CAR) of the invention.

[0096] A TCR KO or TCR and dCK KO engineered immune cell comprising an expression vector comprising a backbone and a sequence comprising a polynucleotide

encoding a CD123 specific chimeric antigen receptor (CD123 CAR) of the invention.

[0097] A TCR KO or TCR and dCK KO engineered immune cell comprising an expression vector comprising a backbone, an EF1 promotor, an RQR8 open reading frame (RQR8 ORF), a sequence coding any one of the CD123 CAR of the invention.

13b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 11b or 12b further expressing a suicide domain at the cell surface.

(14b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune T cell according to any one of 10b to 13b wherein said suicide domain at the cell surface is inserted into the CD123 CAR extracellular domain.

[0098] A TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to any one of 11b to 14b, wherein the dCK gene is deleted conferring resistance to purine nucleotide analogs (PNA).

(15b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR—expressing engineered immune cell according to any one of the 11b to 14b wherein expression of at least one MHC protein, is suppressed.

16b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to any one of (11b) to (15b) for use in therapy.

[0099] The present invention provides a pharmaceutical composition comprising a CD123 CAR as in 1b) to 6b).

[0100] The present invention provides a pharmaceutical composition comprising a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to any one of 11b to 15b.

17b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 16b wherein the condition is acute myelogenous leukemia (AML), preferably refractory/relapsed AML, BPDNL, or for use during bone marrow transplant.

18b) The present invention provides a TCR KO or TCR KO and dCK KO CD123 CAR-expressing engineered immune cell according to 16b, for use as a treatment, preferably as a treatment for a lymphoproliferative disorder, more preferably for leukemia of lymphoma or for a treatment selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome and BPDNL.

[0101] The present invention provides a pharmaceutical composition as above for use in therapy.

[0102] The present invention provides a pharmaceutical composition as above for use in therapy for the treatment of acute myelogenous leukemia (AML), preferably refractory/relapsed AML, BPDNL, or for use during bone marrow transplant.

[0103] The present invention provides a pharmaceutical composition for use as a treatment, preferably as a treatment for a lymphoproliferative disorder, more preferably for leukemia of lymphoma or for a treatment selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome and BPDNL.

[0104] 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, pharmacology, immunology, biochemistry, genetics, and molecular biology.

[0105] 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.

[0106] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Current Protocols in Molecular Biology (Frederick M. AUSUBEL, 2000, Wiley and son Inc, Library of Congress, USA); Molecular Cloning: A Laboratory Manual, Third Edition, (Sambrook et al, 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Harries & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the series, Methods In ENZYMOLOGY (J. Abelson and M. Simon, eds.-in-chief, Academic Press, Inc., New York), specifically, Vols. 154 and 155 (Wu et al. eds.) and Vol. 185, "Gene Expression Technology" (D. Goeddel, ed.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); and Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

[0107] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific chimeric antigen receptor ("CD123 CAR" or "CAR") comprising an extra cellular ligand binding-domain comprising a VH and a VL from a monoclonal anti-CD123 antibody KLON43 or humanized VH and humanized VL sequence thereof, a hinge from CD8 alpha of from FcγRIIIα, a transmembrane domain from CD8 alpha, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB, said 123 CAR having sequence identity with either SEQ ID NO. 31, SEQ ID NO. 32, or SEQ ID NO. 33, or any one of SEQ ID NO. 34 to SEQ ID NO. 117, SEQ ID NO. 160 or preferably, SEQ ID NO 188 to SEQ ID NO 197.

[0108] Preferably the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR of SEQ ID No 31, 32 or 160, more preferably an engineered immune cell (TCR KO) expressing a CD123 specific CAR of SEQ ID No 31, and even more preferably an engineered immune cell (TCR KO and dck KO) expressing a CD123 specific CAR of SEQ ID No 32.

[0109] Advantageously said the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR of SEQ ID No 31, 32 or 160 and a suicide domain, more preferably an engineered immune cell (TCR KO) expressing a CD123 specific CAR of SEQ ID No 31 and a suicide domain, and even more preferably an engineered immune cell (TCR KO and dck KO) expressing a CD123 specific CAR of SEQ ID No 32, and even more more preferably an engineered immune cell (TCR KO and dck KO) expressing a CD123 specific CAR of SEQ ID No 160, or of one of the following sequences SEQ ID NO 188 to SEQ ID NO 197.

[0110] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR having one of the polypeptide structure selected from V1, V3 as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD123 antibody KLON 43, a hinge from CD8alpha or FcγRIIIα, a transmembrane domain from CD8 alpha, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB, and no sequence from CD28 said 123 CAR having at least 80% sequence identity with either SEQ ID NO. 31, SEQ ID NO. 32, preferably SEQ ID NO. 32.

[0111] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR having one of the polypeptide structure selected from V1, V3 as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD123 antibody KLON 43, a hinge from CD8alpha or FcγRIIIα, a transmembrane domain from CD8 alpha, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB, and no sequence from CD28 said 123 CAR having at least 80% sequence identity with either SEQ ID NO. 34 to SEQ ID NO. 159, preferably SEQ ID NO. 34 to SEQ ID NO. 117, preferably SEQ ID NO. 76 to SEQ ID NO. 117.

[0112] In one embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR having a sequence selected from SEQ ID NO. 34, SEQ ID NO. 76, SEQ ID NO. 36, SEQ ID NO. 78; SEQ ID NO. 37, SEQ ID NO. 79, SEQ ID NO. 41, SEQ ID NO. 83, SEQ ID NO. 42, SEQ ID NO. 8), SEQ ID NO. 43, SEQ ID NO. 85, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91. SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97.

[0113] Preferably, the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR having a sequence selected from SEQ ID NO. 34, SEQ ID NO. 76, SEQ ID NO. 36, SEQ ID NO. 78; SEQ ID NO. 43, SEQ ID NO. 85, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96 SEQ ID NO. 97.

[0114] More preferably, the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR having a sequence selected from SEQ ID NO. 47, SEQ ID NO. 89, SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97.

[0115] And even more preferably the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence selected from SEQ ID NO. 32, SEQ ID NO. 89, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97.

[0116] The most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 172 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0117] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 173 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0118] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 174 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0119] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 175 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0120] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 176 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0121] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 177 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0122] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 178 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0123] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 179 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO.

161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0124] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 180 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0125] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 181 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0126] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 182 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0127] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 183 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0128] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 184 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0129] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 185 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0130] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 186 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0131] Another most preferred embodiment discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 187 comprising at least one epitope recognized by a specific monoclonal antibody selected from SEQ ID NO. 161 to SEQ ID NO. 170, preferably two of SEQ ID NO 161 and one from SEQ ID NO 169.

[0132] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 188

[0133] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 189.

[0134] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 190.

[0135] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 191.

[0136] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 192.

[0137] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 193.

[0138] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 194

[0139] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 195.

[0140] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 196.

[0141] In another most preferred embodiment the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR having a sequence of SEQ ID NO. 197.

[0142] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific 123 CAR which extracellular binding domain is modified in such a way to allow both cell sorting and cell depletion. This structure named "mAb-driven sorting/depletion system" or "epitope specific for a monoclonal antibody" or "mimotope" is a selected epitope inserted within the extracellular domain of the anti-CD123 CAR of the invention, in particular into the anti-CD123 scFv; or between the TM and the hinge; this epitope having a specificity to be recognized by a specific antibody (preferably mAb). Given the fact that mainly the external ligand binding domain of the CAR is modified to include the epitope, different CAR architectures can be envisioned: single-chain or multi-chain. The chimeric scFv of the invention, which is formed of the VH and VL polypeptides and of the specific epitope(s) may itself have different structures depending on the position of insertion of the epitope and the use of linkers. The present invention also relates to the resulting method for sorting and/or depleting the engineered immune cells endowed with the modified CARs.

[0143] In some embodiments, the extracellular binding domain of the anti-CD123 CAR comprises the following sequence (including mimotopes) (Nterm is located on the left hand side):

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

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

wherein,

V_1 and V_2 are V_H and V_L of an ScFv anti-CD123 (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_1 is any linker suitable to link the VH chain to the VL chain in a 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 is 0 or 1 and each occurrence of x is independently from the others; and,

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

[0144] In some embodiments, the extracellular binding domain comprises the following sequence (N-term is located on the left hand side):

V_H-L_1-V_1-L-Epitope1-L-Epitope2-L;
L-Epitope1-L-V_H-L-Epitope2-L-V_1-L-Epitope3-L;
V_1-L^1-V_H-L-Epitope1-L-Epitope2-L; or,
L-Epitope1-L-V_1-L-Epitope2-L-V_H-L-Epitope3-L.

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

[0145] L_1 is a linker comprising Glycine and/or Serine. In some embodiment, L_1 is a linker comprising the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$ or $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is 1, 2, 3, 4 or 5. In some embodiments L_1 is $(Gly_4Ser)_4$ or $(Gly_4Ser)_3$.

[0146] L is a flexible linker, preferably comprising Glycine and/or Serine. In some embodiments, L has an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGGS, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGGSGGGGS preferably SGG, SGGS, SSGGS, GGGG, SGGGGS, SGGGGGS, SGGGGG, GSGGGGS or SGGGGSGGGGS. In some embodiment, when the extracellular binding domain comprises several occurrences of L, all the Ls are identical. In some embodiments, when the extracellular binding domain comprises several occurrences of L, the Ls are not all identical. In some embodiments, L is SGGGGS, In some embodiments, the extracellular binding domain comprises several occurrences of L and all the Ls are SGGGGS.

[0147] 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 as in Table 7.

[0148] In a preferred embodiments, Epitope 1, Epitope 2 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, preferably those already approved for medical use, such as rituximab as a non-limiting example.

[0149] Finally, the invention encompasses therapeutic methods where the number, activation and/or survival of the engineered immune cells endowed with a CAR is controlled by using an antibody that directly binds to at least one epitope specific for a monoclonal antibody in the CD123 CARs at the cell surface.

[0150] The present invention encompasses an embodiment disclosing an engineered immune cell (TCR KO and/or dck KO) expressing individually any one of the CD123 specific CAR discloses herein, preferably one of the following sequences SEQ ID NO 188 to SEQ ID NO 197.

[0151] The present invention encompasses an embodiment disclosing vectors encoding allowing the preparation of engineered immune cell (TCR KO and/or dck KO) expressing individually any (each) one of the CD123 specific CAR discloses above. In particular the present invention encompasses an embodiment disclosing vectors encoding (each) one of the CD123 specific CAR discloses above, preferably comprising a backbone.

[0152] The present invention encompasses an embodiment disclosing vectors encoding allowing the preparation of engineered immune cell (TCR KO and/or dck KO) expressing individually any (each) one of the CD123 specific CAR discloses above, preferably one of the following sequences SEQ ID NO 188 to SEQ ID NO 197.

[0153] The present invention encompasses an embodiment disclosing a pharmaceutical composition comprising an engineered immune cell (TCR KO and/or dck KO) expressing individually any (each) one of the CD123 specific CAR discloses above and a pharmaceutically acceptable vehicle.

[0154] The present invention encompasses an embodiment disclosing a pharmaceutical composition comprising an engineered immune cell (TCR KO and/or dck KO) expressing individually any (each) one of the CD123 specific CAR discloses above and a pharmaceutically acceptable vehicle for use as a medicament.

[0155] The present invention encompasses an embodiment disclosing a pharmaceutical composition comprising between from 104 to from 1010/kg engineered immune cells (TCR KO and/or dck KO) expressing individually any (each) one of the CD123 specific CAR discloses above and a pharmaceutically acceptable vehicle for use as a medicament.

[0156] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a specific CD123 CAR having a polypeptide structure V3 as illustrated in FIG. **2**, and described above wherein said CD123 CAR has at least 80% sequence identity with SEQ ID NO. 31.

[0157] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a specific CD123 CAR having a polypeptide structure V3 as illustrated in FIG. **2**, and described above wherein said CD123 CAR has at least 80% sequence identity with SEQ ID NO. 32.

[0158] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a specific CD123 CAR having a polypeptide structure V3 as illustrated in FIG. **2**, and described above wherein said CD123 CAR has at least 80% sequence identity with SEQ ID NO. 33.

[0159] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a specific CD123 CAR having a polypeptide structure V3 as illustrated in FIG. **2**, and described above wherein said CD123 CAR has at least 80% sequence identity with SEQ ID NO. 160.

[0160] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a specific CD123 CAR having one of the following sequences SEQ ID NO 188 to SEQ ID NO 197.

[0161] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 CAR as described above, wherein said extra cellular ligand binding-domain VH and VL from a monoclonal anti-CD123 antibody respectively comprise at least one of the following sequences:

(Variant VH1: SEQ ID NO. 24):
EVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH2: SEQ ID NO. 25):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH3: SEQ ID NO. 26):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH4: SEQ ID NO. 27):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH5: SEQ ID NO. 28):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH6: SEQ ID NO. 29):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(Variant VH7: SEQ ID NO. 30):
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

Variant VH8
EVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS

Variant VH9:
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS

Variant VH10:
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS

Variant VH11:
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS

and one of the following sequences:

Variant VL1: SEQ ID NO. 18):
MADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

Variant VL2: SEQ ID NO. 19):
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

Variant VL3: SEQ ID NO. 20):
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

Variant VL4: SEQ ID NO. 21):
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

Variant VL5: SEQ ID NO. 22):
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,
and

Variant VL6: SEQ ID NO. 23):
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

Variant VL1a:
DIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL2a:
DIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL3a:
DIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL4a:

DIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKLLIYS

ASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKR

-continued

Variant VL6a:
DIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKLLIYS

ASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL7a:
DIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL8a:
DIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQ

GTKVEIKR

Variant VL9a:
DIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYS

ASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQ

GTKVEIKR

[0162] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said extra cellular ligand binding-domain VH and VL from a monoclonal anti-CD123 antibody respectively comprise at least one of the following sequences:

(SEQ ID NO. 24)
EVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 25)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 26)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 27)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 28)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 29)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

-continued

(SEQ ID NO. 30)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 18)
MADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 19)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 20)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 21)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 22)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,
and

(SEQ ID NO. 23)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR, or a combination thereof.

[0163] Advantageously, the present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said extra cellular ligand binding-domain VH and VL from a monoclonal anti-CD123 antibody respectively comprise at least one of the following sequences:

(SEQ ID No 24)
EVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

(SEQ ID No 25)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

-continued

(SEQ ID No 26)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

(SEQ ID No 27)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

(SEQ ID No 28)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

(SEQ ID No 29)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

(SEQ ID No 30)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,.

and at least one of the following sequences:

(SEQ ID NO 18)
MADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,.

(SEQ ID NO 172)
GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDV

LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK

GHDGLYQGLSTATKDTYDALHMQALPPR

huK43-VH9/VL6

(SEQ ID NO 173)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKLLIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

-continued

huK43-VH10/VL3

(SEQ ID NO 174)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

huK43-VH9/VL8

(SEQ ID NO 175)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

huK43-VH2/VL3

(SEQ ID NO 176)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

huK43-VH10/VL9

(SEQ ID NO 177)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PDRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKR

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TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

huK43-VH9/VL3

(SEQ ID NO 178)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

huK43-VH2/VL1

(SEQ ID NO 179)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR

GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL

YQGLSTATKDTYDALHMQALPPR

[0164] In another embodiment the present invention provides an engineered immune cell endowed with the following CAR

V5

huK43-VH10/VL1

(SEQ ID NO 180)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV

SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

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KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR

huK43-VH9/VL6

(SEQ ID NO 181)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKLLIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTSPWTFGQGTKVEIK

REPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVD

VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN

GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSL

VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV

KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD

ALHMQALPPR

huK43-VH10/VL3

(SEQ ID NO 182)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTSPWTFGQGTKVEIK

REPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVD

VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN

GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSL

VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV

KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD

ALHMQALPPR

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huK43-VH9/VL8

(SEQ ID NO 183)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKR

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV

SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR

huK43-VH2/VL3

(SEQ ID NO 184)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYVGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV

SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR

huK43-VH10/VL9

(SEQ ID NO 185)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

TRSKADGYTTETAASVKGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSP

SSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PDRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKR

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV

SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

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CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR

huK43-VH9/VL3

(SEQ ID NO 186)

EVQLVESGGGLVQPGRSLRLSCTASGFTEFTDYYMSWVRQAPGKGLEWVG

LIRSKADGYTTEYSASVKGRFTISRDDSQSTAYLQMNSLKTEDTAVYYCA

RDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQS

PSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSG

VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIK

REPKSPDKTFITCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVV

DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL

NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS

LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLS

LVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR

VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRR

KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY

DALHMQALPPR

huK43-VH2/VL1

(SEQ ID NO 187)

EVQLVESGGGLVQPGRSLRLSCTASGETFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIVMTQSP

SSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYSGV

PSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKR

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV

SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLV

ITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA

LHMQALPPR.

[0165] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said structure V3 (see FIG. **2**) comprises a CD8 alpha hinge and a CD8 alpha transmembrane domain, preferably and no CD28 sequence.

[0166] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said structure V3 comprises a CD8 alpha hinge, a 4-1BB cytoplasmic domain and a CD8 alpha transmembrane domain.

[0167] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said structure V3 comprises a CD8 alpha hinge and a 4-1BB transmembrane domain and no sequence from CD28.

[0168] The present invention discloses an engineered immune cell expressing a CD123 specific CAR as above and further comprising another extracellular ligand binding domain which is not specific for CD123. In a preferred embodiment, another extracellular ligand binding domain which is not specific for CD123 is a suicide domain, more preferably more preferably a suicide domain as any one disclosed in patent application PA 2015 70044 table 2).

[0169] The present invention discloses an engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above, wherein said CD123 specific CAR comprises a suicide domain comprising at least one of SEQ ID NO.: 161, preferably at least two SEQ ID NO.: 161, bound by a linker Li comprising G and S.

[0170] In one embodiment said suicide domain is integrated into the hinge domain, of a CD123 CAR of the invention. In one more preferred embodiment, the present invention provides a CD123 CAR of SEQ ID NO.:160 or having at least 95% identity with SEQ ID NO:160.

[0171] Other suicide domains as those described in table 2 of patent application PA201570044 which is incorporated herein by reference in its entirety are suitable for the present invention.

[0172] The present invention discloses an engineered immune cell as above, wherein expression of at least one MHC protein, preferably β2m or HLA, is suppressed in said engineered immune cell. β2m stands for beta 2 microglobulin and HLA for human leukocyte antigen. The MHC protein is a MHC protein of Class I or of class II.

[0173] The present invention discloses an engineered immune cell as above, wherein said engineered immune cell is engineered to confer resistance to at least one immune suppressive drug, chemotherapy drug, or anti-cancer drug, preferably to purine analogs.

[0174] The present invention discloses a composition comprising a pharmaceutically acceptable vehicle and any one of the engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above.

[0175] The present invention discloses a composition comprising a pharmaceutically acceptable vehicle and any one of the engineered immune cell (TCR KO and/or dck KO) expressing a CD123 specific CAR as described above and another drug, preferably a purine analogues and more preferably a FLAG treatment.

[0176] Examples of purine analogues according to the invention may be pentostatin, fludarabine 2-deoxyadenosine, cladribine, clofarabine, Nelarabine, preferably pentostatin, fludarabine monophosphate, and 2-chlorodeoxyadenosine (2-CDA).

[0177] Examples of FLAG treatments that may be associated with the CD123 T cells of the invention are as follows: Standard FLAG without additions, FLAG-IDA, Mito-FLAG, FLAMSA.

[0178] An Example of FLAG treatment according to the invention may be Standard FLAG without additions

Drug	Dose	Mode	Days
(FL)udarabine	30 mg/m^2	a IV infusion over 30 min, every 12 hours in 2 day divided doses	Days 1-5
(A)ra-C	2000 mg/m^2	IV infusion over 4 hours, every 12 hours in 2 divided doses, starting 4 hours after the end of fludarabine infusion	Days 1-5
(G)-CSF	5 µg/kg	SC	From day 6 till neutrophil recovery
FLAG-IDA			
(FL)udarabine	30 mg/m^2	a IV infusion over 30 min, every 12 hours in 2 day divided doses	Days 1-5
(A)ra-C	2000 mg/m^2	a IV infusion over 4 hours, every 12 hours in 2 day divided doses, starting 4 hours after the end of fludarabine infusion	Days 1-5
(IDA)rubicin	10 mg/m^2	IV bolus	Days 1-3
(G)-CSF	5 µg/kg	SC	From day 6 till neutrophil recovery
Mito-FLAG			
(FL)udarabine	30 mg/m^2	IV infusion over 30 min, every 12 hours in 2 divided doses	Days 1-5
(A)ra-C	2000 mg/m^2	IV infusion over 3 hours, every 12 hours in 2 divided doses, starting 4 hours after the end of fludarabine infusion	Days 1-5
(Mito)xantrone	7 mg/m^2	IV infusion	Days 1, 3 and 5
(G)-CSF	5 µg/kg	SC	From day 6 till neutrophil recovery
FLAMSA			
(FL)udarabine	30 mg/m^2	IV infusion over 30 min, every 12 hours in 2 divided doses	Days 1-4
(A)ra-C	2000 mg/m^2	IV infusion over 4 hours, every 12 hours in 2 divided doses, starting 4 hours after the end of fludarabine infusion	Days 1-4
(AMSA)crine	100 mg/m^2	IV infusion	Days 1-4
Filgrastim	5 µg/kg	SC	From transplant day (or from day 5 if FLAMSA is not a part of conditioning) till neutrophil recovery

[0179] The following combination treatments are disclosed herein:

[0180] The present invention discloses an engineered immune cell as any one described above, a composition comprising said engineered immune cell as disclosed above, for use in therapy.

[0181] The present invention discloses an engineered immune cell of the invention, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the patient is a human.

[0182] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the condition is a pre-malignant or malignant cancer condition characterized by CD123-expressing cells.

[0183] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the condition is a condition which is characterized by an overabundance of CD123-expressing cells.

[0184] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the malignant cancer condition is a haematological cancer condition.

[0185] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the haematological cancer condition is leukemia or malignant lymphoproliferative disorders.

[0186] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein said leukemia is selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

[0187] The present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein the leukemia is acute myelogenous leukemia (AML), preferably refractory/relapsed AML.

[0188] In one embodiment, the present invention discloses an engineered immune cell, a composition comprising said engineered immune cell as disclosed above, for use in therapy as above, wherein said hematologic cancer is a malignant lymphoproliferative disorder.

[0189] The present invention discloses an engineered immune cell for use in therapy as above, wherein said malignant lymphoproliferative disorder is lymphoma.

[0190] The present invention discloses an engineered immune cell for use in therapy as above, wherein said lymphoma is selected from the group consisting of multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

[0191] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention, from 10^4 to 10^8 cells/kg, in combination with a FLAG treatment without addition, for use in the treatment of AML, preferably refractory relapsed AML.

[0192] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention, from 10^4 to 10^8 cells/kg, in combination with a FLAG treatment without addition, for use in the treatment of BPDCN.

[0193] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention from 10^4 to 10^8 cells/kg in combination with a FLAG treatment without addition for use as a treatment before bone marrow transplant as a bridge of transplant.

[0194] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention (from 104 to 108 cells/kg) in combination with fludarabine (from 20 mg/kg to 50 mg/kg), for use in the treatment of AML, preferably refractory relapsed AML.

[0195] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention (from 10^4 to 10^8 cells/kg) in combination with fludarabine (from 20 mg/kg to 50 mg/kg), for use in the treatment of BPDCN.

[0196] CD123 CAR of SEQ ID No 31 or SEQ ID No 32 or SEQ ID No 160 expressed in TCR KO and dck KO T cells of the invention (from 104 to 108 cells/kg) in combination with fludarabine (from 20 mg/kg to 50 mg/kg), for use as a treatment before bone marrow transplant as a bridge of transplant.

[0197] The present invention discloses a method of impairing a hematologic cancer cell comprising contacting said hematologic cancer cell with an engineered cell according to the invention in an amount effective to cause impairment of said cancer cell (from 10^4 to 10^8 cells/kg).

[0198] The present invention discloses a method of engineering an immune cell comprising:

[0199] 1. Providing an immune cell from a donor,

[0200] 2. Knocking out the TCR gene,

[0201] 3. Expressing at the surface of said cell at the CD123 specific chimeric antigen receptor according to the invention as any one of the above.

[0202] A donor may the patient suffering a cancer himself (for autologous adoptive transfer) or another individual (for adoptive transfer of allogenic T cells). The present invention discloses a method of engineering an immune cell as above comprising:

[0203] 1. Providing an immune cell from a donor,

[0204] 2. Knocking out the TCR gene, and the dck gene

[0205] 3. Expressing at the surface of said cell the CD123 specific chimeric antigen receptor according to any one of the above by introducing into said cell at least one polynucleotide encoding said CD123 specific chimeric antigen receptor,

[0206] In a more preferred embodiment, said method comprises expressing at the cell surface a suicide domain.

[0207] The present invention discloses a method of engineering an immune cell as above comprising:

[0208] 1. Providing an immune cell,

[0209] 2. Knocking out a TCR gene, using half TALE-nuclease TALEN of SEQ ID NO: 16 and SEQ ID NO: 17 and the dck gene

[0210] 3. Expressing at the surface of said cell any one of CD123 specific chimeric antigen receptor according to the above by introducing into said cell at least one polynucleotide encoding said CD123 specific chimeric antigen receptor.

[0211] In a preferred embodiment, said method comprises expressing at the cell surface a suicide domain, preferably a suicide domain recognized by one of the following antibodies 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 and ustekinumab.

[0212] In another embodiment said method further comprises a step of binding said engineered immune cell of the invention to a specific monoclonal antigen as those disclosed herein selected from 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 and ustekinumab.

[0213] The present invention discloses a method of treating a subject in need thereof comprising:

[0214] 1. Providing an immune cell expressing at the surface a CD123 specific Chimeric Antigen Receptor according to any one of the above or a composition comprising it

[0215] 2. Administrating said immune cells to said patient. In a preferred embodiment, said composition further comprises a purine analogue, fludarabine

[0216] In another embodiment, said composition is associated to a FLAG treatment, a FLAG treatment without addition.

[0217] In one embodiment said subject in need thereof suffers AML, preferably refractory relapsed AML, BPDNL, or must have bone marrow transplantation.

[0218] The present invention discloses a method of treating a subject in need thereof as above, wherein an immune cell is provided from a donor.

[0219] The present invention discloses a method of treating a subject in need thereof as above, wherein said immune cell is provided from the patient himself.

CD123 Specific Chimeric Antigen Receptors

[0220] The present invention relates to new anti-CD123 chimeric antigen receptor (CAR) comprising an extracellular ligand-binding domain from or derived from KLON 43 antibody, a transmembrane domain from CD8 alpha, a hinge from CD8 alpha or from FcγRIIIα, a suicide domain and a signaling transducing domain. In a preferred embodiment said suicide domain is integrated into the hinge domain. In a more preferred embodiment said suicide domain comprises at least two sequences of SEQ ID NO 161 integrated into the hinge domain.

[0221] In a preferred embodiment, said anti-CD123 CAR of the invention is a polypeptide of SEQ ID NO.: 31, 32, or 160,

[0222] In another embodiment, said anti-CD123 CAR of the invention is a polypeptide of SEQ ID NO.: 34 to SEQ ID NO.:159, SEQ ID NO.:34 to SEQ ID NO.:117, SEQ ID NO.:76 to SEQ ID NO.:117.

[0223] The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding CD123. Preferably, the extracellular ligand-binding domain may be chosen to recognize CD123 that acts as a cell surface marker on target cells associated with a particular disease state. More preferably, the extracellular ligand-binding domain may be chosen to recognize

CD123 that acts as a cell surface marker on target cells associated with AML, BPDCN or a CD123-expressing cell involved in a cancer state.

[0224] In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising the light (VL) and the heavy (VH) variable fragment of a target antigen specific monoclonal anti CD-123 antibody KLON 43 joined by a flexible linker. Said V_L and VH are preferably selected from the sequences disclosed in Table 1 to 2, more preferably an scfv comprising a VH, a linker and VL from or derived from Klon43 (humanized VH and VL as described in table 2). They are preferably linked together by a flexible linker of sequence (GGGGS)n wherein n=1 to 4, more preferably n=3 comprising the sequence SEQ ID NO. 10. In other words, said CARs preferentially comprise an extracellular ligand-biding domain comprising a polypeptide sequence 100% identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 12 for VH and SEQ ID NO: 11 for VL and SEQ ID NO: 18 to SEQ ID NO: 30 for humanized fragments (see Table 2).

[0225] By the term "recombinant antibody" as used herein, is meant an antibody or antibody fragment which is generated using recombinant DNA technology, such as, for example, an antibody or antibody fragment expressed by a bacteriophage, a yeast expression system or a mammalian cell expression system. The term should also be construed to mean an antibody or antibody fragment which has been generated by the synthesis of a DNA molecule encoding the antibody or antibody fragment and which DNA molecule expresses an antibody or antibody fragment protein, or an amino acid sequence specifying the antibody or antibody fragment, wherein the DNA or amino acid sequence has been obtained using recombinant or synthetic DNA or amino acid sequence technology which is available and well known in the art.

[0226] As used herein, the term "conservative sequence modifications" or "humanization" or "humanized antibody" or "humanized antibody fragment", "humanized VH or humanized VL" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the CAR and/or that do not significantly affect the activity of the CAR containing the modified amino acid sequence and reduce or abolish a human anti-mouse antibody (HAMA) response.

[0227] In a preferred embodiment, amino acid modifications significantly improve the binding characteristics of the CAR and/or significantly improve the activity of the CAR containing the modified amino acid sequence and reduce or abolish a human anti-mouse antibody (HAMA) response.

[0228] Such conservative modifications include amino acid substitutions, additions and deletions in said antibody fragment in said CAR and/or any of the other parts of said CAR molecule. Modifications can be introduced into an antibody, into an antibody fragment or in any of the other parts of the CAR molecule of the invention by standard techniques known in the art, such as site-directed mutagenesis, PCR-mediated mutagenesis or by employing optimized germline sequences.

[0229] Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered CAR can be tested for the ability to bind CD 123 using the functional assays described herein.

[0230] In one embodiment said scfv comprises at least one, preferably two epitopes binding to a monoclonal antibody. Examples of such epitopes are disclosed in table 7.

[0231] The signal transducing domain or intracellular signaling domain of a CAR according to the present invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "signal transducing domain" refers to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

[0232] Preferred examples of signal transducing domain for use in a CAR can be the cytoplasmic sequences of the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivate or variant of these sequences and any synthetic sequence that has the same functional capability. Signal transduction domain 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 CAR can comprise the CD3zeta signaling domain which has amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97% or 99% or 100% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 9.

[0233] In particular embodiment the signal transduction domain of the CAR of the present invention comprises a co-stimulatory signal molecule. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient immune response. "Co-stimulatory ligand" refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a

signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83. A "co-stimulatory molecule" refers to the cognate binding partner on a T-cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83.

[0234] In one embodiment, the signal transduction domain of the CAR of the present invention in particular the co-stimulatory molecules do not include and CD28 (NP_006130.1).

[0235] In one preferred embodiment, the CAR of the present invention does not include a sequence of human CD28 (NP_006130.1) and/or from any other CD28.

[0236] In another preferred embodiment, the signal transduction domain of the CAR of the present invention comprises a part of co-stimulatory signal molecule selected from the group consisting of fragments of 4-1BB (GenBank: AAA53133.). In particular the signal transduction domain of the CAR of the present invention comprises amino acid sequence which comprises at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97% or 99% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8.

[0237] A CAR according to the present invention is expressed on the surface membrane of the cell. Thus, such CAR further comprises a transmembrane domain. The distinguishing features of appropriate transmembrane domains comprise the ability to be expressed at the surface of a cell, preferably in the present invention an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and to interact together for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T-cell receptor such as α, β, γ or δ, polypeptide constituting CD3 complex, IL2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fcγ receptor III or CD proteins. Alternatively the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine.

[0238] In a preferred embodiment said transmembrane domain (TM) is derived from the human CD8 alpha chain (e.g. NP_001139345.1), IgG1, IgG4, FcγRIIIα.

[0239] In a more preferred embodiment said TM domain comprises a sequence or part of the SEQ ID NO 6. A CD123 CAR according to the invention generally further comprises a transmembrane domain (TM) from CD8α, showing at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptides of SEQ ID NO. 6.

[0240] In one embodiment the CD123 CAR of the invention does not comprise a TM domain from 4-1BB, preferably of sequence IISFFLALTSTALLFLLFFLTLRFSVV (SEQ ID NO. 7) In a more preferred embodiment said Hinge is of SEQ ID NO 171.

[0241] A CAR according to the present invention comprises a hinge region between said extracellular ligand-binding domain and said transmembrane domain. The term "hinge region" used herein generally means any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, hinge region are used to provide more flexibility and accessibility for the extracellular ligand-binding domain. A hinge region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Hinge region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4, or from all or part of an antibody constant region.

[0242] In one embodiment, said hinge region comprises at least one epitopes recognized by a monoclonal antibody, as disclosed in table 7.

[0243] In one embodiment, said hinge region comprises at least two sequences of SEQ ID No 161 and preferably is of SEQ ID No 171.

[0244] In one embodiment said CD123 CAR of the invention comprises at least one sequence of SEQ ID No 161 in the scfv.

[0245] In one embodiment said CD123 CAR of the invention comprises two sequences of SEQ ID No 161 and a sequence of SEQ ID NO 169 in the scfv, preferably at the N-terminal end of the scfv.

[0246] Alternatively, the hinge region may be a synthetic sequence that corresponds to a naturally occurring hinge sequence, or may be an entirely synthetic hinge sequence. In a preferred embodiment said hinge domain comprises a part of human CD8 alpha chain, FcγRIIIα receptor or IgG1 respectively referred to in this specification as SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5, or hinge polypeptides which display preferably at least 80%, more preferably at least 90%, 95% 97% or 99% sequence identity with these polypeptides.

[0247] In a more preferred embodiment, said hinge domain comprises a part of human CD8 alpha chain, or of FcγRIIIα receptor, more preferably said hinge domain comprises a sequence of SEQ ID NO. 3 or of SEQ ID NO. 4 or with at least 80%, more preferably at least 90%, 95% 97% or 99% sequence identity with SEQ ID NO. 3 or SEQ ID NO. 4.

[0248] Other Additional Scfv

[0249] 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 CD123 specific CAR according to the invention can comprise another extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In one embodiment, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In another embodiment, said different extracellular ligand-binding domains can be placed on different transmembrane polypeptides composing the CAR. In another embodiment, the present invention relates to a population of CARs comprising each one different extracellular ligand binding domains. In a particular, the present invention relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of said cell a population of CAR each one comprising different extracellular ligand binding domains. In another particular embodiment, the present invention relates to a method of engineering an immune cell comprising providing an immune cell and introducing into said cell polynucleotides encoding polypeptides composing a population of CAR each one comprising different extracellular ligand binding domains. By population of CARs, it is meant at least two, three, four, five, six or more CARs each one comprising different extracellular ligand binding domains. The different extracellular ligand binding domains according to the present invention can preferably simultaneously bind different elements in target thereby augmenting immune cell activation and function.

[0250] The present invention also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand binding domains.

[0251] In a preferred embodiment, a CD123 CAR according to the invention comprises a polypeptide of SEQ ID NO. 31 or a polypeptide of SEQ ID NO. 32, more preferably a CD123 CAR according to the invention comprises a polypeptide with at least 80% identity, preferably 80% to 99% identity with SEQ ID NO. 31 or a polypeptide having 80 to 99% identity with SEQ ID NO. 32. Even more preferably a CAR according to the invention comprises a polypeptide having 85 to 99% identity with a polypeptide of SEQ ID NO. 31 or with SEQ ID NO. 32.

[0252] In a preferred embodiment, a CD123 CAR according to the invention comprises a polypeptide having the following sequences SEQ ID NO. 31.

[0253] In a preferred embodiment, a CD123 CAR according to the invention comprises a polypeptide having the following sequence selected from SEQ ID NO. 32, SEQ ID NO. 31 and SEQ ID NO. 160.

[0254] In one preferred embodiment, a CAR according to the invention comprises at least one polypeptide selected from the following sequences:

(SEQ ID NO. 24)
EVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

-continued

(SEQ ID NO. 25)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 26)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 27)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYSASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 28)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS,

(SEQ ID NO. 29)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGL

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCAR

DAAYYSYYSPEGAMDYWGQGTLVTVSS
and

(SEQ ID NO. 30)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTR

DAAYYSYYSPEGAMDYWGQGTLVTVSS

and at least one sequence selected from the following sequences

(SEQ ID NO. 18)
MADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 19)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 20)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

ALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

(SEQ ID NO. 21)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR,

-continued

(SEQ ID NO. 22)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR
and

(SEQ ID NO. 23)
MADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPK

LLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTP

WTFGQGTKVEIKR.

[0255] In one embodiment, a CD123 CAR according to the invention comprises one polypeptide selected from the following sequences: SEQ ID NO. 24, SEQ ID NO. 25, SEQ ID NO. 26, SEQ ID NO. 27, SEQ ID NO. 28, SEQ ID NO. 29, and SEQ ID NO. 30 and a peptide selected from the following sequences: 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.

[0256] In one embodiment, a CD123 CAR according to the invention comprises a polypeptide having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with a polypeptide of SEQ ID NO. 11, or having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with a polypeptide of SEQ ID NO. 12.

[0257] In a more preferred embodiment, a CD123 CAR according to the invention comprises a polypeptide comprising 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with SEQ ID NO. 11+SEQ ID NO. 10+SEQ ID NO. 12.

[0258] According to the invention, the immune cells expressing the anti-CD123 CAR of the invention trigger an anti-cancer immune response, no or reduce GVHD and proliferate even in the presence of purine analogue of FLAG treatment.

[0259] In a preferred embodiment, the immune cells expressing the CAR of the invention endowed with the anti-CD123 CAR of the invention does trigger an immune response which does not comprise a human anti-mouse antibody (HAMA) response.

[0260] According to the invention, an efficient amount of the engineered immune cell of the invention can be administered to a patient in need thereof at least once, alone or in combination with another treatment.

[0261] Polynucleotides, Vectors

[0262] The present invention also relates to polynucleotides, vectors encoding the above described CAR according to the invention.

[0263] 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).

[0264] In a particular embodiment, the different nucleic acid sequences can be included in one polynucleotide or vector which comprises a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By "codon" is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

[0265] To direct transmembrane polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading 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. Pat. No. 5,037,743; Holland et al., U.S. Pat. No. 5,143,830). In a preferred embodiment the signal peptide comprises the amino acid sequence SEQ ID NO: 1 and 2 or at least 90%, 95% 97% or 99% sequence identity with SEQ ID NO: 1 or 2, preferably SEQ ID NO: 1.

[0266] 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.

[0267] In one embodiment, the different nucleic acid sequences encoding a CD123 CAR of the invention can be included in one polynucleotide or vector.

[0268] In a more preferred embodiment the claimed invention is directed to a vector allowing a stable expression of the CAR of the invention. Stable means here that the CAR of the invention is detected at the cell surface of engineered cells at least 1 year after injection.

[0269] In another embodiment the claimed invention is directed to a vector, allowing a stable expression of a CD123 CAR of the invention, preferably of SEQ ID NO. 31, SEQ ID NO 32 or SEQ ID NO 160.

[0270] In a preferred embodiment, the present invention provides a pCLS27333 vector comprising a sequence encoding any one of the CD123 CAR of the invention preferably a CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO. 160.

[0271] Cells

[0272] Cell according to the present invention refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response. Cell according to the present invention is preferably a T-cell obtained from a donor. Said T 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, totipotent stem cells or hematopoietic stem cells. In a preferred embodiment, cells are human cells, in particular human stem cells. In a more preferred embodiment, cells are human T cells, in particular human engineered T cells.

[0273] Representative human stem cells are CD34+ cells. Said isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T-cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In another embodiment, said cell can be derived from the group consisting of CD4+T-lymphocytes and CD8+T-lymphocytes. In a preferred embodiment, said cell can be derived from the group consisting of engineered CD4+T-lymphocytes and engineered CD8+T-lymphocytes.

[0274] 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 is preferably derived from a healthy donor. In another embodiment, said cell is part of a mixed population of cells which present different phenotypic characteristics.

[0275] Preferably, isolation and preparation of stem cells does not require the destruction of at least one human embryo. The immune cells can originate from the patient, in view of operating autologous treatments, or from one or several donors in view of producing allogeneic cells, which can be used in allogeneic treatments.

[0276] More preferably the engineered immune cell of the invention express an anti-CD123 CAR corresponding to SEQ ID NO 31, SEQ ID NO 32, or SEQ ID NO 33 at the cell surface, even more preferably the engineered immune cell of the invention express an humanized anti-CD123 CAR corresponding to humanized SEQ ID NO 31, SEQ ID NO 32, or SEQ ID NO 32.

[0277] In one embodiment the engineered immune cell of the invention express an anti-CD123 CAR corresponding to SEQ ID NO. 34 to SEQ ID NO. 159, preferably SEQ ID NO. 34 to SEQ ID NO. 117, more preferably SEQ ID NO. 76 to SEQ ID NO. 117.

[0278] Among these anti-CD123 CARs, those comprising

VH1/VL1, VH1/VL3; VH1/VL4;

VH2/VL2, VH2/VL3, VH2/VL4;

VH3/VL1, VH3/VL2, VH3/VL3, VH3/VL4

VH4/VL1, VH4/VL2, VH4/VL3, VH4/VL4

[0279] are preferred;

VH1/VL1, VH1/VL3;

VH2/VL4;

VH3/VL1, VH3/VL2, VH3/VL3,

[0280] VH4/VL1, VH4/VL2, VH4/VL3, VH4/VL4 are more preferred,
and those comprising VH3/VL2, VH4/VL1, VH4/VL2, VH4/VL3, VH4/VL4 are even more preferred.

[0281] The most preferred are those having a humanized Klone 43 (huK43) and

VH10a/VL1a

VH9a/VL6a

VH10a/VL3a

VH9a/VL8a

VH2a/VL3a

VH10a/VL9a

VH9a/VL3a

VH2a/VL1a

[0282] In one embodiment the engineered immune cell of the invention express an anti-CD123 CAR corresponding to SEQ ID NO. 160 and SEQ ID NO. 172 to SEQ ID NO. 187

Methods of Engineering Immune Cells Endowed with CARs:

[0283] The present invention encompasses the method of preparing immune cells for immunotherapy comprising introducing ex-vivo into said immune cells the polynucleotides or vectors encoding the CD123 CAR previously described in WO2014/130635 WO2013176916, WO2013176915 and incorporated herein by reference.

[0284] In a preferred embodiment, said polynucleotides are included in lentiviral vectors in view of being stably expressed in the immune cells.

[0285] In a more preferred embodiment, said polynucleotide is included in a lentiviral vector in view of being stably expressed in the immune cells.

[0286] According to further embodiments, said method further comprises the step of genetically modifying said cell to make them more suitable for allogeneic transplantation and to reduce GVHD response.

Modifying T-Cell by Inactivating at Least One Gene Encoding a T-Cell Receptor (TCR) Component.

[0287] According to a first aspect, the immune cell can be made less allogeneic, for instance, by inactivating at least one gene expressing one or more component of T-cell receptor (TCR) as described in WO 2013/176915, which can be combined with the inactivation of a gene encoding or regulating HLA or β2m protein expression. Accordingly the risk of graft versus host syndrome and graft rejection is significantly reduced.

[0288] Accordingly, when the immune cells are T-cells, the present invention also provides methods to engineer T-cells that are less allogeneic.

[0289] Methods of making cells less allogenic can comprise the step of inactivating at least one gene encoding a T-Cell Receptor (TCR) component, in particular TCRalpha, TCRbeta genes.

[0290] Methods disclosed in WO2013/176915 to prepare CAR expressing immune cell suitable for allogeneic transplantation, by inactivating one or more component of T-cell receptor (TCR), are all incorporated herein by reference.

[0291] The present invention encompasses an anti-CD123 CAR expressing immune cell wherein at least one gene expressing one or more component of T-cell receptor (TCR)

has been inactivated. Thus, the present invention provides an anti-CD123 CAR expressing T cell wherein the CAR is derived from Klon 43, in particular having at least 80% identity with SEQ ID No 31 and wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated.

[0292] The present invention encompasses an anti-CD123 CAR expressing immune cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) has been inactivated. Thus, the present invention provides an anti-CD123 CAR expressing T cell wherein the CAR is derived from Klon 43, in particular having at least 80% identity with SEQ ID No 32 and wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated.

[0293] The present invention encompasses an anti-CD123 CAR expressing immune cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) has been inactivated. Thus, the present invention provides an anti-CD123 CAR expressing T cell wherein the CAR is derived from Klon 43, in particular having at least 80% identity with SEQ ID No 160 and wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated.

[0294] According to the invention, anti-CD123 CAR immune cells with one or more component of T-cell receptor (TCR) inactivated, are intended to be used as a medicament.

[0295] By inactivating a TCR gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. The nucleic acid strand breaks caused by the rare-cutting endonuclease are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). However, NHEJ is an imperfect repair process that often results in changes to the DNA sequence at the site of the cleavage. Mechanisms involve rejoining of what remains of the two DNA ends through direct re-ligation (Critchlow and Jackson 1998) or via the so-called microhomology-mediated end joining (Betts, Brenchley et al. 2003; Ma, Kim et al. 2003). Repair via non-homologous end joining (NHEJ) often results in small insertions or deletions and can be used for the creation of specific gene knockouts. Said modification may be a substitution, deletion, or addition of at least one nucleotide. Cells in which a cleavage-induced mutagenesis event, i.e. a mutagenesis event consecutive to an NHEJ event, has occurred can be identified and/or selected by well-known method in the art. In a particular embodiment, the step of inactivating at least a gene encoding a component of the T-cell receptor (TCR) into the cells of each individual sample comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one gene encoding a component of the T-cell receptor (TCR). In a more particular embodiment, said cells of each individual sample are transformed with nucleic acid encoding a rare-cutting endonuclease capable of disrupting at least one gene encoding a component of the T-cell receptor (TCR), and said rare-cutting endonuclease is expressed into said cells.

[0296] Said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease, CRISPR/Cas9 nuclease, Argonaute nuclease, a TALE-nuclease or a MBBBD-nuclease. In a preferred embodiment, said rare-cutting endonuclease is a TALE-nuclease. By TALE-nuclease is intended a fusion protein consisting of a DNA-binding domain derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence (Boch, Scholze et al. 2009; Moscou and Bogdanove 2009; Christian, Cermak et al. 2010; Cermak, Doyle et al. 2011; Geissler, Scholze et al. 2011; Huang, Xiao et al. 2011; Li, Huang et al. 2011; Mahfouz, Li et al. 2011; Miller, Tan et al. 2011; Morbitzer, Romer et al. 2011; Mussolino, Morbitzer et al. 2011; Sander, Cade et al. 2011; Tesson, Usal et al. 2011; Weber, Gruetzner et al. 2011; Zhang, Cong et al. 2011; Deng, Yan et al. 2012; Li, Piatek et al. 2012; Mahfouz, Li et al. 2012; Mak, Bradley et al. 2012). In the present invention new TALE-nucleases have been designed for precisely targeting relevant genes for adoptive immunotherapy strategies.

[0297] Preferred TALE-nucleases recognizing and cleaving the target sequence are described in PCT/EP2014/075317. In particular, additional catalytic domain can be further introduced into the cell with said rare-cutting endonuclease to increase mutagenesis in order to enhance their capacity to inactivate targeted genes. More particularly, said additional catalytic domain is a DNA end processing enzyme. Non limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5' flap endonucleases, helicases, phosphatase, hydrolases and template-independent DNA polymerases. Non limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivate of the protein domain selected from the group consisting of hExol (EXO1_HUMAN), Yeast Exol (EXO1_YEAST), *E. coli* Exol, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In a preferred embodiment, said additional catalytic domain has a 3'-5'-exonuclease activity, and in a more preferred embodiment, said additional catalytic domain is TREX, more preferably TREX2 catalytic domain (WO2012/058458). In another preferred embodiment, said catalytic domain is encoded by a single chain TREX2 polypeptide. Said additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein according to the invention optionally by a peptide linker.

[0298] Endonucleolytic breaks are known to stimulate the rate of homologous recombination. Thus, in another embodiment, the genetic modification step of the method further comprises a step of introduction into cells of an exogeneous nucleic acid comprising at least a sequence homologous to a portion of the target nucleic acid sequence, such that homologous recombination occurs between the target nucleic acid sequence and the exogeneous nucleic acid. In particular embodiments, said exogenous nucleic acid comprises first and second portions which are homologous to region 5' and 3' of the target nucleic acid sequence, respectively. Said exogenous nucleic acid in these embodiments also comprises a third portion positioned between the first and the second portion which comprises no homology with the regions 5' and 3' of the target nucleic acid sequence. Following cleavage of the target nucleic acid sequence, a homologous recombination event is stimulated between the target nucleic acid sequence and the exogenous nucleic acid. Preferably, homologous sequences of at least 50 bp, prefer-

ably more than 100 bp and more preferably more than 200 bp are used within said donor matrix. In a particular embodiment, the homologous sequence can be from 200 bp to 6000 bp, more preferably from 1000 bp to 2000 bp. Indeed, shared nucleic acid homologies are located in regions flanking upstream and downstream the site of the break and the nucleic acid sequence to be introduced should be located between the two arms.

Drug Resistant T-Cells

[0299] The inventor sought to engineer TCR KO T-cell for immunotherapy, in particular to engineer TCR KO anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing immune cell that can be used in combination with a therapeutic agent (anti-cancer drug).

[0300] As used herein, a cell which is "resistant or tolerant" to an agent means a cell which has been genetically modified so that the cell proliferates and is active in the presence of an amount of an agent that inhibits or prevents proliferation of a cell without the genetic modification.

[0301] By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiment, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. In a particular embodiment, the step of inactivating at least one drug sensitizing gene comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one drug sensitizing gene. In a more particular embodiment, said cells are transformed with nucleic acid encoding a rare-cutting endonuclease capable of disrupting a drug sensitizing gene, and said rare-cutting endonuclease is expressed into said cells. Said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease, CRISPR/Cas9 nuclease, A MBBBD-nuclease or a TALE-nuclease. In a preferred embodiment, said rare-cutting endonuclease is a TALE-nuclease.

[0302] In a preferred embodiment, a drug sensitizing gene which can be inactivated to confer drug resistance to the T-cell is the human deoxycytidine kinase (dCK) gene. This enzyme is required for the phosphorylation of the deoxyribonucleosides deoxycytidine (dC), deoxyguanosine (dG) and deoxyadenosine (dA). Purine nucleotide analogs (PNAs) are metabolized by dCK into mono-, di- and triphosphate PNA. Their triphosphate forms and particularly clofarabine triphosphate compete with ATP for DNA synthesis, acts as proapoptotic agent and are potent inhibitors of ribonucleotide reductase (RNR) which is involved in trinucleotide production.

[0303] Preferably, the inactivation of dCK in T cells is mediated by TALE nuclease. To achieve this goal, several pairs of dCK TALE-nuclease have been designed, assembled at the polynucleotide level and validated by sequencing. Examples of TALE-nuclease pairs which can be used according to the invention are depicted in PCT/EP2014/075317.

[0304] This dCK inactivation in T cells confers resistance to purine nucleoside analogs (PNAs) such as clofarabine and fludarabine.

[0305] In a more preferred embodiment, the dCK inactivation in T cells is combined with an inactivation of TRAC genes rendering these double knock out (KO) T cells both resistant to drug such as clofarabine and less allogeneic than the same cell with an intact TCR. This double features is particularly useful for a therapeutic goal, allowing "off-the-shelf" allogeneic cells for immunotherapy in conjunction with chemotherapy to treat patients with cancer preferably refractory relapsed AML, or BPDNL.

[0306] This double KO inactivation dCK/TRAC can be performed simultaneously or sequentially before or after CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expression. One example of TALE-nuclease dCK/TRAC pairs which gave success in the invention is described in PCT/EP2014/075317, in particular, the target sequences in the 2 loci (dCK and TRAC).

[0307] According to another aspect, the CD123 CAR expressing T-cell of the invention (TCR KO anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160-expressing immune cells) can be further genetically engineered to improve its resistance to immunosuppressive drugs or chemotherapy treatments, which are used as standard care for treating cancer.

[0308] Several cytotoxic agents (anti-cancer drugs) such as anti-metabolites, alkylating agents, anthracyclines, DNA methyltransferase inhibitors, platinum compounds and spindle poisons have been developed to kill cancer cells. However, the introduction of these agents with novel therapies, such as immunotherapies, is problematic as these drugs affect the functioning/survival of immune T cells. For example, chemotherapy agents can be detrimental to the establishment of robust anti-tumor immunocompetent cells due to the agents' non-specific toxicity profiles. Small molecule-based therapies targeting cell proliferation pathways may also hamper the establishment of anti-tumor immunity. If chemotherapy regimens that are transiently effective can be combined with novel immunocompetent cell therapies then significant improvement in anti-neoplastic therapy might be achieved (for review (Dasgupta, McCarty et al. 2011).

[0309] To improve cancer therapy and selective engraftment of allogeneic TCR KO, CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T-cells, drug resistance is conferred to said allogeneic T cells to protect them from the toxic side effects of chemotherapy agent. The drug resistance of T-cells also permits their enrichment in or ex vivo, as T-cells which express the drug resistance gene will survive and multiply relative to drug sensitive cells.

[0310] Methods for engineering T-cells resistant to chemotherapeutic agents are disclosed in PCT/EP2014/075317 which is fully incorporated by reference herein.

[0311] In particular, the present invention discloses a method of engineering allogeneic TCR KO, CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T-cells, suitable for immunotherapy wherein at least one gene encoding a T-cell receptor (TCR) component is inactivated and at least one gene is modified to confer drug resistance, preferably the dCK gene is inactivated.

[0312] A method of engineering allogeneic CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T-cells suitable for combination therapy with purine analogues comprising:

[0313] Providing an anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 160 expressing T cell,

[0314] Modifying said anti-CD123 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

[0315] Modifying said anti-CD123 CAR expressing T-cell to confer drug resistance to said anti-CD123 CAR expressing T-cell; preferably to confer resistance to purine analogues

[0316] Expanding said engineered anti-CD123 CAR expressing T-cell in the presence of said drug said drug is a purine analogue selected from pentostatin, fludarabine 2-deoxyadenosine, cladribine, clofarabine, Nelarabine, preferably pentostatin, fludarabine monophosphate, and 2-chlorodeoxyadenosine (2-CDA).

[0317] Alternatively, the present invention relates to a method comprising:

[0318] Providing an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell,

[0319] Modifying said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell to confer drug resistance to said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell; by deleting the dck gene, preferably said drug is a purine analogue

[0320] Modifying said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

[0321] Expanding said engineered anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell in the presence of said drug, said drug is a purine analogue selected from pentostatin, fludarabine 2-deoxyadenosine, cladribine, clofarabine, Nelarabine, preferably pentostatin, fludarabine monophosphate, and 2-chlorodeoxyadenosine (2-CDA).

[0322] In particular, the present invention relates to a method of engineering allogeneic cells suitable for immunotherapy wherein a gene encoding a T-cell receptor (TCR alpha) component is inactivated and the dCK gene is modified to confer resistance to purine analogues comprising:

[0323] Providing an anti-CD123 CAR expressing T-cell; in particular an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell,

[0324] Modifying said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell by inactivating the gene encoding the T-cell receptor (TCR alpha) component;

[0325] Inactivating the dCK gene in said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell to confer resistance to purine analogues;

[0326] Expanding said engineered anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell in the presence of said drug.

[0327] Alternatively, the present invention relates to a method comprising:

[0328] Providing an anti-CD123 CAR expressing T-cell; in particular an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell,

[0329] Inactivating the dCK gene in said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T-cell to confer resistance purine analogues

[0330] Modifying said anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR alpha) component;

[0331] Expanding said engineered anti-CD123 CAR expressing T-cell in the presence of purine analogues.

[0332] In a preferred embodiment dCK KO, TCR KO CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 CAR expressing T-cells provided are resistant to a drug selected from pentostatin, fludarabine 2-deoxyadenosine, cladribine, clofarabine, or Nelarabine, preferably to fludarabine monophosphate, or to 2-chlorodeoxyadenosine (2-CDA).

Gene Expression Conferring Drug Resistance to Anti-CD123 CAR-Expressing Immune Cells

[0333] In a particular embodiment, said drug resistance can be conferred to the T-cell of the invention by the expression of at least one drug resistance gene. Said drug resistance gene refers to a nucleic acid sequence that encodes "resistance" to an agent, such as a chemotherapeutic agent (e.g. methotrexate). In other words, the expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent to a greater extent than the proliferation of a corresponding cell without the drug resistance gene or survival in the presence of said drug. The expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent and does not affect its activity. A drug resistance gene of the invention can encode resistance to anti-metabolite, methotrexate, vinblastine, cisplatin, alkylating agents, anthracyclines, cytotoxic antibiotics, anti-immunophilins, their analogs or derivatives.

[0334] In one embodiment, a drug resistance gene confers resistance to a drug (or an agent), in particular an anti-cancer drug selected from Aracytine, Cytosine Arabinoside, amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

[0335] Several drug resistance genes have been identified that can potentially be used to confer drug resistance the CD123 CAR expressing T cells of the invention (Takebe, Zhao et al. 2001; Sugimoto, Tsukahara et al. 2003; Zielske, Reese et al. 2003; Nivens, Felder et al. 2004; Bardenheuer, Lehmberg et al. 2005; Kushman, Kabler et al. 2007).

[0336] One example of drug resistance gene can also be a mutant or modified form of Dihydrofolate reductase (DHFR) encoding gene. DHFR is an enzyme involved in regulating the amount of tetrahydrofolate in the cell and is essential to DNA synthesis. Folate analogs such as methotrexate (MTX) inhibit DHFR and are thus used as antineoplastic agents in clinic. Different mutant forms of DHFR which have increased resistance to inhibition by anti-folates used in therapy have been described. In a particular embodiment, the drug resistance gene according to the present invention can be a nucleic acid sequence encoding a mutant form of human wild type DHFR (GenBank: AAH71996.1) which comprises at least one mutation conferring resistance to an anti-folate treatment, such as methotrexate. In particular embodiment, mutant form of DHFR comprises at least one mutated amino acid at position G15, L22, F31 or F34, preferably at positions L22 or F31 (Schweitzer, Dicker et al. 1990); International application WO94/24277; U.S. Pat. No.

6,642,043). In a particular embodiment, said DHFR mutant form comprises two mutated amino acids at position L22 and F31. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type DHFR polypeptide set forth in GenBank: AAH71996.1. In a particular embodiment, the serine residue at position 15 is preferably replaced with a tryptophan residue. In another particular embodiment, the leucine residue at position 22 is preferably replaced with an amino acid which will disrupt binding of the mutant DHFR to antifolates, preferably with uncharged amino acid residues such as phenylalanine or tyrosine. In another particular embodiment, the phenylalanine residue at positions 31 or 34 is preferably replaced with a small hydrophilic amino acid such as alanine, serine or glycine.

[0337] As used herein, "antifolate agent" or "folate analogs" refers to a molecule directed to interfere with the folate metabolic pathway at some level. Examples of antifolate agents include, e.g., methotrexate (MTX); aminopterin; trimetrexate (Neutrexin™); edatrexate; N10-propargyl-5,8-dideazafolic acid (CB3717); ZD1694 (Tumodex), 5,8-dideazaisofolic acid (IAHQ); 5,10-dideazatetrahydrofolic acid (DDATHF); 5-deazafolic acid; PT523 (N alpha-(4-amino-4-deoxypteroyl)-N delta-hemiphthaloyl-L-ornithine); 10-ethyl-10-deazaaminopterin (DDATHF, lomatrexol); piritrexim; 10-EDAM; ZD1694; GW1843; Pemetrexate and PDX (10-propargyl-10-deazaaminopterin).

[0338] Another example of drug resistance gene can also be a mutant or modified form of ionisine-5'-monophosphate dehydrogenase II (IMPDH2), a rate-limiting enzyme in the de novo synthesis of guanosine nucleotides. The mutant or modified form of IMPDH2 is an IMPDH inhibitor resistance gene. IMPDH inhibitors can be mycophenolic acid (MPA) or its prodrug mycophenolate mofetil (MMF). The mutant IMPDH2 can comprises at least one, preferably two mutations in the MAP binding site of the wild type human IMPDH2 (NP_000875.2) that lead to a significantly increased resistance to IMPDH inhibitor. The mutations are preferably at positions T333 and/or S351 (Yam, Jensen et al. 2006; Sangiolo, Lesnikova et al. 2007; Jonnalagadda, Brown et al. 2013). In a particular embodiment, the threonine residue at position 333 is replaced with an isoleucine residue and the serine residue at position 351 is replaced with a tyrosine residue. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human IMPDH2 polypeptide set forth in NP_000875.2.

[0339] Another drug resistance gene is the mutant form of calcineurin. Calcineurin (PP2B), an ubiquitously expressed serine/threonine protein phosphatase that is involved in many biological processes and which is central to T-cell activation. Calcineurin is a heterodimer composed of a catalytic subunit (CnA; three isoforms) and a regulatory subunit (CnB; two isoforms). After engagement of the T-cell receptor, calcineurin dephosphorylates the transcription factor NFAT, allowing it to translocate to the nucleus and active key target gene such as IL2. FK506 in complex with FKBP12, or cyclosporine A (CsA) in complex with CyPA block NFAT access to calcineurin's active site, preventing its dephosphorylation and thereby inhibiting T-cell activation (Brewin, Mancao et al. 2009). The drug resistance gene of the present invention can be a nucleic acid sequence encoding a mutant form of calcineurin resistant to calcineurin inhibitor such as FK506 and/or CsA. In a particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer a at positions: V314, Y341, M347, T351, W352, L354, K360, preferably double mutations at positions T351 and L354 or V314 and Y341. In a particular embodiment, the valine residue at position 341 can be replaced with a lysine or an arginine residue, the tyrosine residue at position 341 can be replaced with a phenylalanine residue; the methionine at position 347 can be replaced with the glutamic acid, arginine or tryptophane residue; the threonine at position 351 can be replaced with the glutamic acid residue; the tryptophane residue at position 352 can be replaced with a cysteine, glutamic acid or alanine residue, the serine at position 353 can be replaced with the histidine or asparagines residue, the leucine at position 354 can be replaced with an alanine residue; the lysine at position 360 can be replaced with an alanine or phenylalanine residue of a sequence corresponding to GenBank: ACX34092.1. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer a polypeptide set forth in (GenBank: ACX34092.1).

[0340] In another particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer b at positions: V120, N123, L124 or K125, preferably double mutations at positions L124 and K125. In a particular embodiment, the valine at position 120 can be replaced with a serine, an aspartic acid, phenylalanine or leucine residue; the asparagine at position 123 can be replaced with a tryptophan, lysine, phenylalanine, arginine, histidine or serine; the leucine at position 124 can be replaced with a threonine residue; the lysine at position 125 can be replaced with an alanine, a glutamic acid, tryptophan, or two residues such as leucine-arginine or isoleucine-glutamic acid can be added after the lysine at position 125 in the amino acid sequence corresponding to GenBank: ACX34095.1. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer b polypeptide set forth in (GenBank: ACX34095.1).

[0341] Another drug resistance gene is 0(6)-methylguanine methyltransferase (MGMT) encoding human alkyl guanine transferase (hAGT). AGT is a DNA repair protein that confers resistance to the cytotoxic effects of alkylating agents, such as nitrosoureas and temozolomide (TMZ). 6-benzylguanine (6-BG) is an inhibitor of AGT that potentiates nitrosourea toxicity and is co-administered with TMZ to potentiate the cytotoxic effects of this agent. Several mutant forms of MGMT that encode variants of AGT are highly resistant to inactivation by 6-BG, but retain their ability to repair DNA damage (Maze, Kurpad et al. 1999). In a particular embodiment, AGT mutant form can comprise a mutated amino acid of the wild type AGT position P140, in the amino acid sequence SEQ ID NO: 18 (UniProtKB: P16455). In a preferred embodiment, said proline at position 140 is replaced with a lysine residue.

[0342] Another drug resistance gene can be multidrug resistance protein 1 (MDR1) gene. This gene encodes a membrane glycoprotein, known as P-glycoprotein (P-GP) involved in the transport of metabolic byproducts across the cell membrane. The P-Gp protein displays broad specificity towards several structurally unrelated chemotherapy agents.

[0343] Overexpressing multidrug resistance protein 1 has been described to confer resistance to drugs such as Mitoxantrone (Charles S. Morrow, Christina Peklak-Scott, Bimjhana Bishwokarma, Timothy E. Kute, Pamela K. Smitherman, and Alan J. Townsend. Multidrug Resistance Protein 1 (MRP1, ABCC1) Mediates Resistance to Mitoxantrone via Glutathione-Dependent Drug Efflux *Mol Pharmacol* April 2006 69:1499-1505).

[0344] Thus, drug resistance can be conferred to cells by the expression of nucleic acid sequence that encodes MDR-1 (NP_000918).

[0345] Still another way of preparing drug resistant cells according to the invention is to prepare cells with specific mutation (s) such as mutations at Arg486 and Glu571 in the Human Topoisomerase II gene, to confer resistance to amsacrine (S. PATEL, B. A. KELLER, and L. M. FISHER. 2000. MOLECULAR PHARMACOLOGY. Vol 57: p784-791 (2000).

[0346] Still another way of preparing drug resistant cells according to the invention is to prepare cells overexpressing microRNA-21 to confer resistance to Daunorubicine (Involvement of miR-21 in resistance to daunorubicin by regulating PTEN expression in the leukaemia K562 cell line Bai, Haitao et al. FEBS Letters, Volume 585, Issue 2, 402-408).

[0347] In a preferred embodiment, cells of the invention are bearing such a drug resistance conferring mRNA or protein and also comprise an inhibitory mRNA or a gene, the expression of which is conditioned by another drug, allowing the selective destruction of said drug resistant cells of the invention in the presence of said other drug or upon administration of said other drug.

[0348] Drug resistance gene can also confer resistance to cytotoxic antibiotics, and can be ble gene or mcrA gene. Ectopic expression of ble gene or mcrA in an immune cell gives a selective advantage when exposed to the chemotherapeutic agent, respectively the bleomycine or the mitomycin C.

[0349] The most practical approach to gene therapy is the addition of a gene to engineer T-cell by using efficient gene delivery with vectors, preferably viral vector. Thus, in a particular embodiment, the present invention provides a method of conferring drug resistance to the CD123 immune cells of the invention by introducing a transgene preferably encoded by at least one vector into a cell.

[0350] The random insertion of genes into the genome may lead to the inappropriate expression of the inserted gene or the gene near the insertion site. Specific gene therapy using homologous recombination of exogenous nucleic acid comprising endogenous sequences to target genes to specific sites within the genome can allow a secure engineering of T-cells. As described above, the genetic modification step of the method according to the invention can comprise a step of introduction into cells of an exogeneous nucleic acid comprising at least a sequence encoding the drug resistance gene and a portion of an endogenous gene such that homologous recombination occurs between the endogenous gene and the exogeneous nucleic acid. In a particular embodiment, said endogenous gene can be the wild type "drug resistance" gene, such that after homologous recombination, the wild type gene is replaced by the mutant form of the gene which confers resistance to the drug.

[0351] Endonucleolytic breaks are known to stimulate the rate of homologous recombination. Thus, in a particular embodiment, the method of the invention further comprises the step of expressing in the cell a rare-cutting endonuclease which is able to cleave a target sequence within an endogenous gene. Said endogenous gene can encode for examples DHFR, IMPDH2, calcineurin or AGT. Said rare-cutting endonuclease can be a TALE-nuclease, a Zinc finger nuclease, a CRISPR/Cas9 endonuclease, a MBBBD-nuclease or a meganuclease.

[0352] Another example of enzyme which can be inactivated is human hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene (Genbank: M26434.1). In particular HPRT can be inactivated in engineered T-cells to confer resistance to a cytostatic metabolite, the 6-thioguanine (6TG) which is converted by HPRT to cytotoxic thioguanine nucleotide and which is currently used to treat patients with cancer, in particular leukemias (Hacke, Treger et al. 2013). Guanines analogs are metabolized by HPRT transferase that catalyzes addition of phosphoribosyl moiety and enables the formation of TGMP Guanine analogues including 6 mercapthopurine (6MP) and 6 thioguanine (6TG) are usually used as lymphodepleting drugs to treat ALL. They are metabolized by HPRT (hypoxanthine phosphoribosyl transferase that catalyzes addition of phosphoribosyl moiety and enables formation TGMP. Their subsequent phosphorylations lead to the formation of their triphosphorylated forms that are eventually integrated into DNA. Once incorporated into DNA, thio GTP impairs fidelity of DNA replication via its thiolate groupment and generate random point mutation that are highly deleterious for cell integrity.

[0353] In another embodiment, the inactivation of the CD3 normally expressed at the surface of the T-cell can confer resistance to anti-CD3 antibodies such as teplizumab.

Combination Treatment

[0354] The terms "therapeutic agent", "chemotherapeutic agent", or "drug" or "anti-cancer drug" as used herein refers to a medicament, preferably a compound or a derivative thereof that can interact with a cancer cell, thereby reducing the proliferative status of the cell and/or killing the cell. Examples of chemotherapeutic agents or "anti-cancer drug" include, but are not limited to, alkylating agents (e.g., Busulfan, Carboplatine, Chlorambucil, Cisplatine, Cyclophosphamide, Ifosfamide, Melphalan, Mechlorethamine, Oxaliplatine, Uramustine, •Temozolomide, Fotemustine), metabolic antagonists (e.g., purine nucleoside antimetabolite such as clofarabine, fludarabine or 2'-deoxyadenosine, methotrexate (MTX), 5-fluorouracil or derivatives thereof, Azathioprine, Capecitabine, Cytarabine, •Floxuridine, •Fluorouracile, •Gemcitabine, •Methotrexate, Pemetrexed), antitumor antibiotics (e.g., mitomycin, Adriamycin, Bleomycine, •Daunorubicine, •Doxorubicine, •Epirubicine, •Hydroxyurea, •Idarubicine, •Mitomycin C, •Mitoxantrone), plant-derived antitumor agents (e.g., vincristine, vindesine, Taxol, Vinblastine, •(Vinorelbine), •Docetaxel, •Paclitaxel), topoisomerase inhibitor (Irinotecan, •Topotecan, •Etoposide).

[0355] In a preferred embodiment, a therapeutic agent, a chemotherapy drug as used herein refers to a compound or a derivative thereof that may be used to treat cancer, in particular to treat a hematopoietic cancer cell and more particularly AML, and even more particular refractory relapsed AML thereby reducing the proliferative status of the cancer cell and/or killing the cancer cell.

[0356] Other Examples of chemotherapeutic agents include, but are not limited to Aracytine, Cytosine Arabinoside, Amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof.

[0357] In other embodiments of the present invention, cells of the invention are administered to a patient in conjunction with a drug (or an agent) selected from Aracytine, Cytosine Arabinoside, amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

[0358] Such agents may further include, but are not limited to, the anti-cancer agents TRIMETHOTRIXATE™ (TMTX), TEMOZOLOMIDE™, RALTRITREXED™, S-(4-Nitrobenzyl)-6-thioinosine (NBMPR), 6-benzyguanidine (6-BG), bis-chloronitrosourea (BCNU) and CAMPTOTHECIN™, or a therapeutic derivative of any thereof.

[0359] In a more preferred embodiment an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell with a TCR and a dck KO gene, is administered to a patient, in combination with at least one therapeutic agent selected from Aracytine, Cytosine Arabinoside, Amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid and combination thereof.

[0360] An anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell with a TCR and a dck KO gene, in combination with at least one therapeutic agent selected from Aracytine, Cytosine Arabinoside, Amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid and combination thereof is used as a therapeutic agent.

[0361] Preferably, an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell with a TCR and a dck KO gene, in combination with a purine analogue (fludarabine), is used as a therapeutic agent.

[0362] Preferably, an anti-CD123 CAR of SEQ ID NO 31, of SEQ ID NO 32, or of SEQ ID NO 160 expressing T cell with a TCR and a dck KO gene, in combination with a FLAG treatment, is used as a therapeutic agent.

[0363] In one embodiment, said CD123 CAR is selected from SEQ ID NO 76 to SEQ ID NO. 117 and expressed in a double TCR dCK KO T cells and combined to a purine analogue or to FLAG as a therapeutic agent.

[0364] In the present invention a combination treatment comprises infusion of a mAb for sorting and/or depletion purpose(s). In a preferred embodiment, said mAb is Rixutimab.

[0365] The maximum dose of Rituximab to be administered preferably by intravenous route is 2,250 mg/m2. It is no administered as an intravenous push or bolus.

Multiple Drug Resistance of Anti-CD123 CAR-Expressing Immune Cells

[0366] In another particular embodiment, the inventors sought to develop an "off-the shelf" immunotherapy strategy, using allogeneic T-cells, in particular allogenic anti-CD123 CAR expressing T-cell resistant to multiple drugs to mediate selection of engineered T-cells when the patient is treated with different drugs. The therapeutic efficiency can be significantly enhanced by genetically engineering multiple drug resistance allogeneic T-cells. Such a strategy can be particularly effective in treating tumors that respond to drug combinations that exhibit synergistic effects. Moreover multiple resistant engineered T-cells can expand and be selected using minimal dose of drug agents.

[0367] Thus, the method according to the present invention can comprise modifying T-cell of the invention to confer multiple drug resistance to said T-cell of the invention. Said multiple drug resistance can be conferred by either expressing more than one drug resistance gene or by inactivating more than one drug sensitizing gene. In another particular embodiment, the multiple drug resistance can be conferred to said T-cell by expressing at least one drug resistance gene and inactivating at least one drug sensitizing gene. In particular, the multiple drug resistance can be conferred to said T-cell by expressing at least one drug resistance gene such as mutant form of DHFR, mutant form of IMPDH2, mutant form of calcineurin, mutant form of MGMT, the ble gene, and the mcrA gene and inactivating at least one drug sensitizing gene such as HPRT gene. In a preferred embodiment, multiple drug resistance can be conferred by inactivating HPRT gene and expressing a mutant form of DHFR; or by inactivating HPRT gene and expressing a mutant form of IMPDH2; or by inactivating HPRT gene and expressing a mutant form of calcineurin; by inactivating HPRT gene and expressing a mutant form of MGMT; by inactivating HPRT gene and expressing the ble gene; by inactivating HPRT gene and expressing the mcrA gene.

[0368] In one embodiment, the present invention provides allogenic anti-CD123 CAR expressing T-cell expressing more than one drug resistance gene or wherein more than one drug sensitizing gene is inactivated.

[0369] Suicide Genes in Anti-CD123 CAR-Expressing Immune Cells of the Invention

[0370] In some instances, since engineered T-cells can expand and persist for years after administration, it is desirable to include a safety mechanism to allow selective deletion of administrated T-cells. Thus, in some embodiments, the method of the invention comprises the transformation of said T-cells with a recombinant suicide gene. Said recombinant suicide gene is used to reduce the risk of direct toxicity and/or uncontrolled proliferation of said T-cells once administrated in a subject (Quintarelli C, Vera F, blood 2007; Tey S K, Dotti G., Rooney C M, boil blood marrow transplant 2007). Suicide genes enable selective deletion of transformed cells in vivo. In particular, the suicide gene has the ability to convert a non-toxic pro-drug into cytotoxic drug or to express the toxic gene expression product. In other words, "Suicide gene" is a nucleic acid coding for a product, wherein the product causes cell death by itself or in the presence of other compounds.

[0371] A representative example of such a suicide gene is one which codes for thymidine kinase of herpes simplex virus. Additional examples are thymidine kinase of varicella zoster virus and the bacterial gene cytosine deaminase which can convert 5-fluorocytosine to the highly toxic compound 5-fluorouracil. Suicide genes also include as non limiting examples caspase-9 or caspase-8 or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID). Suicide genes can also be polypeptides that are expressed at the surface of the cell and can make the cells sensitive to therapeutic monoclonal antibod-

ies. As used herein "prodrug" means any compound useful in the methods of the present invention that can be converted to a toxic product. The prodrug is converted to a toxic product by the gene product of the suicide gene in the method of the present invention. A representative example of such a prodrug is ganciclovir which is converted in vivo to a toxic compound by HSV-thymidine kinase. The ganciclovir derivative subsequently is toxic to tumor cells. Other representative examples of prodrugs include acyclovir, FIAU [1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil], 6-methoxypurine arabinoside for VZV-TK, and 5-fluorocytosine for cytosine deaminase.

[0372] One preferred suicide gene system of the invention employs a recombinant antigenic polypeptide comprising antigenic motif recognized by the anti-CD20 mAb Rituximab, especially QBen10, such as in the so-called RQR8 polypeptide described in WO2013153391. Rituximab, an authorized antibody drug, can then be used for cell depletion when needed.

[0373] In one embodiment, the present invention provides a TCR KO-dCK KO anti-CD123 CAR of SEQ ID NO. 31 or 32 expressing T-cell, expressing a RQR8 suicide gene allowing said cells to be selectively destroyed.

[0374] More preferably, the present invention provides a TCR KO-dCK KO anti-CD123 CAR expressing T-cell, wherein said CD123 CAR comprises a suicide domain allowing CD123 CAR expressing cells to be selectively destroyed, preferably said suicide domain comprised at least two domains of SEQ ID NO 161, more preferably said CD123 CAR comprises a sequence of SEQ ID NO 171.

CD123+/Luc+ Drug Resistant Daudi Cells for Testing the Cytotoxicity of Drug Resistant Allogenic CART Cells

[0375] The present invention encompasses also a method for manufacturing target cells which express both CD123 and are resistant to purine analogues. These target cells are particularly useful for testing the cytotoxicity of CD123 CAR T cells of the invention. These cells are readily resistant to clinically relevant dose of clofarabine and harbor luciferase activity. This combination of features enable traking them in vivo in a mice model or destroy them when required.

[0376] More particularly, they can be used to assess the cytotoxicity properties drug resistant T cells in mice in the presence of clofarabine or other PNAs. Clofarabine resistant Daudi cells mimick the physiological state of acute lymphoblastic leukemia (ALL) patients relapsing form induction therapy, that harbor drug resistant B cell malignancies. Thus, these cells are of great interest to evaluate the reliability and cytotoxicity of drug resistant CAR T cells. Preferably, these target cells are CD123+ Luciferase+ Daudi cells.

Isolated Cell

[0377] The present invention relates to an isolated cell expressing a CD123 CAR which binds to CD123. Thus, the invention relates to an anti-CD123 CAR expressing cell. In a particular embodiment, said anti-CD123 CAR expressing cell is resistant to at least one drug and/or comprises at least one disrupted gene encoding a T-cell receptor component.

[0378] In a preferred embodiment, the present invention relates to an isolated T cell expressing a CAR which binds to CD123 and is resistant to at least one purine analogue and comprises a disrupted gene encoding a T-cell receptor alpha component.

[0379] In a more preferred embodiment, the present invention relates to an isolated T cell expressing a CAR of SEQ ID NO 31, 32 or 160 which binds to CD123 and destroys CD123 expressing cells and is resistant to at least one purine analogue and comprises a disrupted gene encoding a T-cell receptor alpha component.

[0380] In a more preferred embodiment, the present invention relates to an isolated T cell expressing a CAR of SEQ ID NO 160 which binds to CD123 and destroys CD123 expressing cells and is resistant to at least one purine analogue and comprises a disrupted gene encoding a T-cell receptor alpha component.

[0381] In a more preferred embodiment, the present invention relates to an isolated T cell expressing a CAR of SEQ ID NO 160 which binds to CD123 and destroys CD123 expressing cells and is resistant to at least one purine analogue and comprises a disrupted gene encoding a T-cell receptor beta component.

[0382] In another particular embodiment, said anti-CD123 CAR expressing T cell comprises at least one disrupted drug sensitizing gene such as dCK or HPRT gene. In a more particular embodiment, said isolated anti-CD123 CAR T-cell comprises a disrupted HPRT gene and express a DHFR mutant; said isolated anti-CD123 CAR T-cell comprises a disrupted HPRT gene and express a IMPDH2 mutant; said isolated anti-CD123 CAR T-cell comprises a disrupted HPRT gene and express a calcineurin mutant; said isolated anti-CD123 CAR T-cell comprises a disrupted HPRT gene and express a AGT mutant.

[0383] In particular, the present invention relates to an isolated T-cell, in particular an isolated allogeneic anti-CD123 CAR expressing T-cell, and preferably an isolated allogeneic anti-CD123 CAR expressing T-cell comprising a peptide having a sequence of SEQ ID NO. 31 32 or 160, said isolated allogeneic anti-CD123 CAR expressing T-cell is more particularly resistant to a purine analogue, and specifically suitable for immunotherapy in the presence of purine analogues.

[0384] In one aspect, the present invention provides methods for engineering an isolated immune cells to make it resistant to purine analogs (purine nucleotide analogs or PNA), such a clorofarabine or fludarabine, so that they can be used in cancer immunotherapy treatments in patients pre-treated with these conventional chemotherapies.

[0385] The resistance to drugs can be conferred to the isolated T-cell of the invention by inactivating one or more gene(s) responsible for the cell's sensitivity to the drug (drug sensitizing gene(s)), such as the dcK and/or HPRT genes.

Immune Check Points

[0386] The present invention provides allogeneic dCK KO T-cells expressing an anti-CD123 CAR, in particular an anti-CD123 CAR of SEQ ID No 31, or of SEQ ID No 32, or of SEQ ID No 160 wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated and/or one gene selected from the genes CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10,

SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, is inactivated as referred to in WO2014/184741.

[0387] According to further aspect of the invention, the immune cells can be further manipulated to make them more active or limit exhaustion, by inactivating genes encoding proteins that act as "immune checkpoints" that act as regulators of T-cells activation, such as the following gene selected from CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, preferably, said gene is PDCD1 or CTLA-4. Examples of genes, which expression could be reduced or suppressed in T cells of the invention are indicated in Table 9.

[0388] In one embodiment said gene is a gene that acts as a regulator of T-cells activation coding the beta 2 microglobulin protein.

[0389] According to a further aspect of the invention, the anti-CD123 CAR-immune cells of the invention can be further manipulated to make them resistant to a drug, in particular to a drug used during chemotherapy against cancer, in particular a CD123-expressing cell-mediated cancer such as AML. This can be achieved by introducing a gene conferring resistance to said drug. This same gene may be turned on and off by using a gene inducible inhibition/expression system as previously described (Garcia E L, Mills A A (2002) Getting around lethality with inducible Cre-mediated excision. Semin Cell Dev Biol 13:151-8, Lewandoski M (2001) Conditional control of gene expression in the mouse. Nat Rev Genet 2:743-55; Scharfenberger L, Hennerici T, Kirly G et al. (2014) Transgenic mouse technology in skin biology: Generation of complete or tissue-specific knockout mice. J Invest Dermatol 134:e16; Schwenk F, Kuhn R, Angrand P O et al. (1998) Temporally and spatially regulated somatic mutagenesis in mice. Nucleic Acids Res 26:1427-32.

[0390] Thus, anti-CD123 CAR of sequence corresponding to SEQ ID NO 31, to SEQ ID NO 32 or to SEQ ID NO 160-expressing, PNA resistant immune T cell, wherein (i) at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated (ii) at least one gene conferring resistance to a drug (other than PNA) is incorporated or a gene conferring sensitivity to said drug (other than PNA) is deleted or mutated to be inactivated (iii) another gene selected from the gene disclosed in table 9 is inactivated—is an object of the present invention.

[0391] The present invention encompasses the isolated anti-CD123 CAR-immune cells or cell lines obtainable by the method of the invention, more particularly isolated cells comprising any of the proteins, polypeptides, allelic variants, altered or deleted genes or vectors described herein.

[0392] This object is provided for the treatment of a cancer, in particular AML, refractory relapse AML, BPDNL.

[0393] The immune cells of the present invention or cell lines can further comprise exogenous recombinant polynucleotides, in particular CARs or suicide gene(s) (encoding SEQ ID NO. 161) or they can comprise altered or deleted genes coding for checkpoint proteins or ligands thereof that contribute to their efficiency as a therapeutic product, ideally as an "off the shelf" product.

[0394] In another aspect, the present invention concerns the method for treating or preventing cancer in the patient by administrating at least once an engineered immune cell obtainable by the above methods.

TABLE 5

List of genes encoding immune checkpoint proteins modified in engineered T cells of the invention

Pathway		Genes that can be inactivated In the pathway
Co-inhibitory receptors	CTLA4 (CD152)	CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22 (prefered)
	PDCD1 (PD-1, CD279)	PDCD1 (prefered and more prefered)
	CD223 (lag3)	LAG3
	HAVCR2 (tim3)	HAVCR2
	BTLA(cd272)	BTLA
	CD160(by55)	CD160
	IgSF family	TIGIT
		CD96
		CRTAM
	LAIR1(cd305)	LAIR1
	SIGLECs	SIGLEC7
		SIGLEC9
	CD244(2b4)	CD244
Death receptors	TRAIL	TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7
	FAS	FADD, FAS
Cytokine signalling	TGF-beta signaling	TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1
	IL10 signalling	IL10RA, IL10RB, HMOX2
	IL6 signalling	IL6R, IL6ST
Prevention of TCR signalling		CSK, PAG1
		SIT1
Induced Treg Transcription factors controlling exhaustion	induced Treg transcription factors controlling exhaustion	FOXP3
		PRDM1 (=blimp1, heterozygotes mice control chronic viral infection better than wt or conditional KO) Stat 5
		BATF
Hypoxia mediated tolerance	iNOS induced guanylated cyclase	GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3

[0395] One, preferably two alleles, of above genes are modified in engineered T cells of the invention the locus is verified for each preparation of TALEN used to KO said gene/allele.

[0396] In a preferred embodiment said method of further engineer the immune cells involves introducing into said T cells polynucleotides, in particular mRNAs, encoding specific rare-cutting endonuclease to selectively inactivate the genes mentioned above by DNA cleavage. In a more preferred embodiment said rare-cutting endonucleases are TALE-nucleases or Cas9 endonuclease. TAL-nucleases have so far proven higher specificity and cleavage efficiency over the other types of rare-cutting endonucleases, making them the endonucleases of choice for producing of the engineered immune cells on a large scale with a constant turn-over.

Delivery Methods

[0397] The different methods described above involve expressing a protein of interest such as drug resistance gene,

rare-cutting endonuclease, Chimeric Antigen Receptor (CAR), in particular an anti-CD123 CAR and more particularly, a CAR comprising a SEQ ID NO. 31, or 32 or 160 and/including a suicide domain into an isolated cell.

[0398] As non-limiting example, said protein of interest can be expressed in the cell by its introduction as a transgene preferably encoded by at least one plasmid vector. Polypeptides may be expressed 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.

[0399] Methods for introducing a polynucleotide construct into cells are known in the art and include as non limiting examples stable transformation methods wherein the polynucleotide construct 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.

[0400] Said polynucleotides may be introduced into a cell by for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, transient transformation methods include for example microinjection, electroporation or particle bombardment, cell fusion. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells. Said plasmid vector can comprise a selection marker which provides for identification and/or selection of cells which received said vector.

[0401] Different transgenes can be included in one vector. Said vector can comprise a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see Donnelly et al., J. of General Virology 82: 1013-1025 (2001); Donnelly et al., J. of Gen. Virology 78: 13-21 (1997); Doronina et al., Mol. And. Cell. Biology 28(13): 4227-4239 (2008); Atkins et al., RNA 13: 803-810 (2007)).

[0402] 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.

[0403] In a more preferred embodiment of the invention, polynucleotides encoding polypeptides according to the present invention can be mRNA which is introduced directly into the cells, for example by electroporation. The inventors determined the optimal condition for mRNA electroporation in T-cell. The inventor used the cytoPulse technology which allows, by the use of pulsed electric fields, to transiently permeabilize living cells for delivery of material into the cells. The technology, based on the use of PulseAgile (BTX Havard Apparatus, 84 October Hill Road, Holliston, Mass. 01746, USA) electroporation waveforms grants the precise control of pulse duration, intensity as well as the interval between pulses (U.S. Pat. No. 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.

[0404] The different methods described above involve introducing a CD123 CAR of SEQ ID NO. 31, SEQ ID NO 32 or SEQ ID NO 160 into a cell. As non-limiting example, said CAR can be introduced as transgenes encoded by one plasmid vector. Said plasmid vector can also contain a selection marker which provides for identification and/or selection of cells which received said vector.

[0405] Polypeptides may be synthesized in situ in the cell as a result of the introduction of polynucleotides encoding said polypeptides into the cell. Alternatively, said polypeptides could be produced outside the cell and then introduced thereto. Methods for introducing a polynucleotide construct into cells are known in the art and including as non limiting examples stable transformation methods wherein the polynucleotide construct is integrated into the genome of the cell, transient transformation methods wherein the polynucleotide construct is not integrated into the genome of the cell and virus mediated methods. Said polynucleotides may be introduced into a cell by for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, transient transformation methods include for example microinjection, electroporation or particle bombardment. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells.

[0406] Engineered Immune Cells

[0407] The present invention also relates to isolated cells or cell lines susceptible to be obtained by said method to engineer cells. In particular said isolated cell comprises at least one CAR as described above. In another embodiment, said isolated cell comprises a population of CARs each one comprising different extracellular ligand binding domains. In particular, said isolated cell comprises exogenous polynucleotide sequence encoding CAR. Genetically modified immune cells of the present invention are activated and proliferate independently of antigen binding mechanisms.

[0408] 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, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34+ cells. Said isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T-cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In another embodiment, said cell can be derived from the group consisting of CD4+T-lymphocytes and CD8+T-lymphocytes. Prior to expansion and genetic modification of the cells of the invention, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources,

including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available and known to those skilled in the art, may be used. In another embodiment, said cell can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In another embodiment, said cell is part of a mixed population of cells which present different phenotypic characteristics. In the scope of the present invention is also encompassed a cell line obtained from a transformed T-cell according to the method previously described. Modified cells resistant to an immunosuppressive treatment and susceptible to be obtained by the previous method are encompassed in the scope of the present invention.

[0409] As a preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a CD123 CAR having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160 as described above, that do not express functional TCR are resistant to PNA and that a reactive towards CD123 positive cells, for their adoptive transfer into patients, preferably into patients suffering AML, refractory relapse AML, BPDNL or a lymphoproliferative disorder, or as a treatment before bone marrow transplantation.

[0410] As a more preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a CD123 CAR CD123 CAR having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160 and that a reactive towards CD123 positive cells as described above, that do not express a functional TCR and are resistant to a selected PNA, for their allogeneic transplantation into patients treated with said selected PNA.

[0411] In an even more preferred embodiment, the present invention provides T-cells or a population of TRC KO dCK KO T-cells endowed with an anti-CD123 CAR comprising a polypeptide of CD123 CAR having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160 in combination with another treatment.

[0412] Activation and Expansion of T Cells

[0413] Whether prior to or after genetic modification of the T cells, even if the genetically modified immune cells of the present invention are activated and proliferate independently of antigen binding mechanisms, the immune cells, particularly T-cells of the present invention can be further activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 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.

[0414] 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.

[0415] As non-limiting examples, T cell populations may be stimulated in vitro such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-g, 1L-4, 1L-7, GM-CSF, -10, -2, 1L-15, TGFp, and TNF- or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanoi. Media can include RPMI 1640, A1M-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in 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

[0416] 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.

Pharmaceutical Compositions

[0417] Provided herein are pharmaceutical compositions comprising the genetically engineered immune cells of the invention, e.g., genetically engineered TCR KO-dCK KO CD123 CAR T cells having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160.

[0418] Provided herein are pharmaceutical compositions comprising the genetically engineered immune cells of the invention, e.g., genetically engineered TCR KO-dCK KO CD123 CAR T cells having a sequence corresponding to any one of the sequences selected from SEQ ID NO 188 to SEQ ID NO 197.

[0419] In another embodiment, pharmaceutical compositions comprising the genetically engineered immune T cells of the invention, e.g., genetically engineered TCR KO dCK KO CD123 CAR having any one of the sequence selected from having a sequence selected from SEQ ID NO. 34 to SEQ ID NO. 159, preferably from SEQ ID NO. 34, SEQ ID NO. 76, SEQ ID NO. 36, SEQ ID NO. 78; SEQ ID NO. 37, SEQ ID NO. 79, SEQ ID NO. 41, SEQ ID NO. 83, SEQ ID NO. 42, SEQ ID NO. 8), SEQ ID NO. 43, SEQ ID NO. 85, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91, SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, and SEQ ID NO. 97, T cells are provided.

[0420] In accordance with this disclosure, the term "pharmaceutical composition" relates to a composition for administration to an individual. In a preferred embodiment, the pharmaceutical composition comprises a composition for parenteral, transdermal, intraluminal, intra-arterial, intrathecal or intravenous administration (iv) or for direct injection into a cancer. It is in particular envisaged that said pharmaceutical composition is administered to the individual via infusion or injection iv. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous (iv), subcutaneous, intraperitoneal, intramuscular, topical or intradermal administration.

[0421] The pharmaceutical composition of the present disclosure may further comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, etc. Compositions comprising such carriers can be formulated by well-known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose.

[0422] The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. An example of a dosage for administration might be in the range of 0.24 μg to 48 mg of cells according to the invention, preferably 0.24 μg to 24 mg, more preferably 0.24 μg to 2.4 mg, even more preferably 0.24 μg to 1.2 mg and most preferably 0.24 μg to 240 mg units per kilogram of body weight per day. Particularly preferred dosages are recited herein below. Progress can be monitored by periodic assessment.

[0423] The CAR cell compositions of the disclosure may be administered locally or systemically. Administration will generally be parenteral, e.g., intravenous; DNA may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. In a preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. In addition, the pharmaceutical composition of the present disclosure might comprise proteinaceous carriers, like, e.g., serum albumin or immunoglobulin, preferably of human origin. It is envisaged that the pharmaceutical composition of the disclosure might comprise, in addition to the proteinaceous bispecific single chain antibody constructs or nucleic acid molecules or vectors encoding the same (as described in this disclosure), further biologically active agents, depending on the intended use of the pharmaceutical composition.

[0424] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, one or more cells according to the invention for use in cell therapy and/or the reagents to generate one or more cells for use in cell therapy that harbors recombinant expression vectors may be comprised in a kit. The kit components are provided in suitable container means.

[0425] Some components of the kits may be packaged either in aqueous media or in lyophilized form and under frozen packages. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits also will typically include a means for containing the components in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained.

[0426] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly useful. In some cases, the container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit.

[0427] In a preferred embodiment one and/or more liquid solutions is cryoresistant.

[0428] However, the components of the kit or part of it may be provided as dried powder(s) or as a tablet. When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

[0429] In particular embodiments, cells that are to be used for cell therapy are provided in a kit, and in some cases the cells are essentially the sole component of the kit. The kit may comprise reagents and materials to make the desired cell. In specific embodiments, the reagents and materials include primers for amplifying desired sequences, nucleotides, suitable buffers or buffer reagents, salt, and so forth, and in some cases the reagents include vectors and/or DNA that encodes a CAR molecule as described herein and/or regulatory elements therefor.

[0430] In particular embodiments, there are one or more apparatuses in the kit suitable for extracting one or more samples from an individual. The apparatus may be a syringe, scalpel, and so forth.

[0431] In some cases, the kit, in addition to cell therapy embodiments, also includes a second cancer therapy, such as chemotherapy, hormone therapy, and/or immunotherapy, for example. The kit(s) may be tailored to a particular cancer for an individual and comprise respective second cancer thera-

pies for the individual. Preferably, said other cancer therapy is a purine analogue, a FLAG treatment.

[0432] Therapeutic uses of engineered T-cells Comprising a CD123 CAR of the invention.

[0433] In various embodiments CAR constructs, nucleic acid sequences, vectors, host cells, as contemplated herein and/or pharmaceutical compositions comprising the same are used for the prevention, treatment or amelioration of a cancerous disease, such as a tumorous disease. In particular embodiments, the pharmaceutical composition of the present disclosure may be particularly useful in preventing, ameliorating and/or treating cancer, including cancer having solid tumors, for example.

[0434] In particular embodiments, provided herein are a method of treating an individual for cancer, comprising the step of providing a therapeutically effective amount of a plurality of any of cells of the disclosure to the individual.

[0435] In certain aspects, the cancer is a solid tumor, and the tumor may be of any size, but in specific embodiments, the solid tumors are about 2 mm or greater in diameter. In certain aspects, the method further comprises the step of providing a therapeutically effective amount of an additional cancer therapy to the individual.

[0436] As used herein "treatment" or "treating," includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, and may include even minimal reductions in one or more measurable markers of the disease or condition being treated, e.g., cancer. Treatment can involve optionally either the reduction or amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. "Treatment" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

[0437] As used herein, "prevent," and similar words such as "prevented," "preventing" etc., indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition, e.g., cancer. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

[0438] In particular embodiments, the present invention contemplates, in part, cells, CAR constructs, nucleic acid molecules and vectors that can administered either alone or in any combination using standard vectors and/or gene delivery systems, and in at least some aspects, together with a pharmaceutically acceptable carrier or excipient. In certain embodiments, subsequent to administration, said nucleic acid molecules or vectors may be stably integrated into the genome of the subject.

[0439] In specific embodiments, viral vectors may be used that are specific for certain cells or tissues and persist in said cells. Suitable pharmaceutical carriers and excipients are well known in the art. The compositions prepared according to the disclosure can be used for the prevention or treatment or delaying the above identified diseases.

[0440] Furthermore, the disclosure relates to a method for the prevention, treatment or amelioration of a tumorous disease comprising the step of administering to a subject or individual in the need thereof an effective amount of immune cells, e.g., T cells or cytotoxic T lymphocytes, harboring a CD123 CAR of SEQ ID NO. 31 or a CD123 CAR of SEQ ID NO. 32 or a CD123 CAR of SEQ ID NO. 160; a nucleic acid sequence encoding a the same; a vector comprising a nucleotide sequence encoding said CD123 CAR, as described herein and/or produced by a process as described herein.

[0441] Possible indications for administration of the composition(s) of the exemplary CD123 CAR cells are cancerous diseases, including tumorous diseases, including:

[0442] The administration of the composition(s) of the disclosure is useful for all stages and types of lymphoproliferative disorder, including for minimal residual disease, early cancer, advanced cancer, and/or relapsed and/or refractory cancer.

[0443] The disclosure further encompasses co-administration protocols with other compounds, e.g. bispecific antibody constructs, targeted toxins or other compounds, which act via immune cells.

[0444] The clinical regimen for co-administration of the inventive compound(s) may encompass co-administration at the same time, before or after the administration of the other component. Particular combination therapies include chemotherapy, radiation, surgery, hormone therapy, or other types of immunotherapy.

[0445] Particular doses for therapy may be determined using routine methods in the art. However, in specific embodiments, the engineered CD123 T cells of the invention are delivered to an individual in need thereof once, although in some cases it is multiple times, including 2, 3, 4, 5, 6, or more times. When multiple doses are given, the span of time between doses may be of any suitable time, but in specific embodiments, it is days or weeks or months between the doses. Doses and origins of the T cell donor are selected to avoid any undesirable side effects. The time between doses may vary in a single regimen and may depend on the recipient (patient in need thereof). In particular embodiments, the time between doses is 2, 3, 4, 5, 6, 7, 8, 9, 10, or more days or weeks. In specific cases, it is between 4-8 or 6-8 weeks, for example.

[0446] In specific embodiments, one regimen includes one of the following dose regimen
of CD123 T cell of the invention:
$11\times10^4/m^2$,
$11\times10^5/m^2$,
$11\times10^6/m^2$,
$23\times10^6/m^2$,
$31\times10^7/m^2$ $43\times10^7/m^2$,
$51\times10^8/m^2$,
or from 10^4 to from 10^{10} cells/kg.

[0447] In an alternative embodiment, the T cells are provided to the individual in the following dose regimen:

Dose Level CD123 CTL Dose

[0448] $11\times10^6/m^2$,
$21\times10^7/m^2$,
$31\times10^8/m^2$,
6 10^4 to from $5\cdot10^8$ cells/kg,

[0449] An efficient amount of the CD123 CAR engineered immune cell of the invention corresponds to such dose that may be adapted depending on the status of each treated individual.

[0450] Therapeutic Applications

[0451] In another embodiment, isolated cell(s) obtained by the different methods or cell derived (after one to 50 in vitro passages) from said isolated cell of the invention can be used as a medicament. Genetically engineered TCR KO dCK KO CD123 CART cells of the invention that can be used as a medicament can be those having a CD123 CAR having a sequence corresponding to any one of SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO 160, SEQ ID NO. 34, to SEQ ID NO. 159.

[0452] In a preferred embodiment a genetically engineered TCR KO dCK KO CD123 CAR T cells having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160. is provided for use as a medicament.

[0453] In another preferred embodiment, a, genetically engineered TCR KO dCK KO CD123 CAR T cells having any one of the sequence selected from having a sequence selected from SEQ ID NO. 34, SEQ ID NO. 76, SEQ ID NO. 36, SEQ ID NO. 78, SEQ ID NO. 37, SEQ ID NO. 79, SEQ ID NO. 41, SEQ ID NO. 83, SEQ ID NO. 42, SEQ ID NO. 8), SEQ ID NO. 43, SEQ ID NO. 85, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91, SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96, SEQ ID NO. 97, is provided for use as a medicament.

[0454] In another preferred embodiment, a genetically engineered TCR KO dCK KO CD123 CAR T cells having any one of the sequence selected from SEQ ID NO. 160, SEQ ID NO. 188, SEQ ID NO. 189, SEQ ID NO. 190, SEQ ID NO. 191; SEQ ID NO. 192, SEQ ID NO. 193, SEQ ID NO. 194, SEQ ID NO. 195, SEQ ID NO. 196, SEQ ID NO. 197 is provided for use as a medicament.

[0455] In another embodiment, said medicament can be used for treating cancer, particularly for the treatment of B-cell lymphomas and leukemia in a patient in need thereof.

[0456] In another embodiment, said isolated cell according to the invention or cell line derived from said isolated cell can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

[0457] In a particular embodiment, an anti-CD123 CAR expressing T cell is provided as a medicament for the treatment of AML, of an AML subtype, of an AML-related complication, of an AML-related condition. In a preferred embodiment, an anti-CD123 CAR expressing T cell wherein said anti-CD123 CAR comprises SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 is provided as a medicament.

[0458] In another embodiment, said medicament can be used for treating a CD123-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a CD123-expressing cell. In other words, the invention is related to an anti-CD123 CAR expressing T cell comprising 80% to 100% of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 for its use as a medicament to treat a condition linked to the detrimental activity of CD123-expressing cells, in particular to treat a condition selected from AML, AML subtype, AML-related complication, and AML-related conditions;

[0459] 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:

[0460] (a) providing an immune-cell obtainable by any one of the methods previously described;

[0461] (b) Administrating said transformed immune cells to said patient,

[0462] On one embodiment, said T cells of the invention can undergo robust in vivo T cell expansion and can persist for an extended amount of time.

[0463] In a preferred embodiment said T cell is destroyed by administration of a drug or an antibody that selectively destroys said T cells.

[0464] In a more preferred embodiment genetically engineered TCR KO dCK KO CD123 CAR T cells of the invention having a CD123 CAR having a sequence corresponding to any one of SEQ ID NO. 31, SEQ ID NO. 32, SEQ ID NO 160, SEQ ID NO. 34, to SEQ ID NO. 159 are uses as a medicament/a treatment and then cleared up from the patient using an antibody recognizing the suicide domain RQR8, or CD20 domain, or SEQ ID NO 161—using preferably rituximab.

[0465] 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.

[0466] Cells of the invention that can be used with the disclosed methods are described in the previous section. Said treatment can be used to treat patients diagnosed wherein a pre-malignant or malignant cancer condition characterized by CD123-expressing cells, especially by an overabundance of CD123-expressing cells. Such conditions are found in hematologic cancers, such as leukemia or malignant lymphoproliferative disorders. Lymphoproliferative disorder can be lymphoma, in particular multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

[0467] Cancers that may be treated using the cells of the invention or a pharmaceutical composition comprising said cell may comprise nonsolid tumors (such as hematological tumors, including but not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma, BPDNL, AML, refractory relapse AML, or before bone marrow transplantation.

[0468] Types of cancers to be treated with the CD123 cell expressing CARs of the invention include, but are not limited leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0469] In one embodiment, the present invention provides a pharmaceutical composition for its use in the treatment of a CD123 expressing cells-mediated disease, in particular a CD123 expressing cells—mediated hematologic cancer, said composition comprising said anti-CD123 CAR expressing T cell of the invention, preferably said anti-CD123 CAR is of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160, more preferably having a sequence selected from SEQ ID NO. 160, SEQ ID NO. 188, SEQ ID NO. 189, SEQ ID NO. 190, SEQ ID NO. 191; SEQ ID NO. 192, SEQ ID NO. 193, SEQ ID NO. 194, SEQ ID NO. 195, SEQ ID NO. 196, SEQ ID NO. 197 Any other CD123-mediating or CD123-involving malignant lymphoproliferative disorders disclosed herein may be improved with the anti-CD123 CAR-expressing cells of the present invention.

[0470] In a preferred embodiment, the cancer that may be treated using the anti-CD123 CAR-expressing cells of the present invention is leukemia (AML), a disease associated to leukemia or a complication thereof.

[0471] Leukemias that can be treated using the anti-CD123 CAR-expressing cells of the present invention can be acute myelogenous leukemia (AML), chronic myelogenous leukemia, melodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

[0472] AML or AML subtypes that may be treated using the anti-CD123 CAR-expressing cells of the present invention may be in particular, acute myeloblastic leukemia, minimally differentiated acute myeloblastic leukemia, acute myeloblastic leukemia without maturation, acute myeloblastic leukemia with granulocytic maturation, promyelocytic or acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia (M5a) or acute monocytic leukemia (M5b), acute erythroid leukemias, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b), acute megakaryoblastic leukemia, acute basophilic leukemia, acute panmyelosis with myelofibrosis, whether involving CD123-positive cells.

[0473] Subtypes of AML that may be treated using the anti-CD123 CAR-expressing cells of the present invention also include, hairy cell leukemia, philadelphia chromosome-positive acute lymphoblastic leukemia.

[0474] AML may be classified as AML with specific genetic abnormalities. Classification is based on the ability of karyotype to predict response to induction therapy, relapse risk, survival.

[0475] Accordingly, AML that may be treated using the anti-CD123 CAR-expressing cells of the present invention of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 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.

[0476] The present invention is particularly useful for the treatment of AML associated with these particular cytogenetic markers.

[0477] The present invention also provides an anti-CD123 CAR expressing T cell of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 for the treatment of patients with specific cytogenetic subsets of AML, such as patients with t(15; 17)(q22; q21) identified using all-trans retinoic acid (ATRA) 16-19 and for the treatment of patients with t(8; 21)(q22; q22) or inv(16)(p13q22)/t(16; 16)(p13; q22) identified using repetitive doses of high-dose cytarabine.

[0478] Preferably, the present invention provides an anti-CD123 CAR expressing T cell of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 for the treatment of 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 combination with a FLAG.

Group of Patients

[0479] In a preferred embodiment, the invention provides an anti-CD123 CAR expressing T cell of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 as a treatment for AML in patients over 60 years or in patients of less than 20 years.

[0480] In a more preferred embodiment, the present invention provides an anti-CD123 CAR expressing T cell of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 for use as a pediatric treatment, in particular a pediatric treatment against AML, or AML-related diseases or complications.

[0481] In still another preferred embodiment, the present invention is used as a treatment in AML patients with low, poor or unfavorable status that is to say with a predicted survival of less than 5 years survival rate. In this group, patients suffering AML with the following cytogenetic characteristics: −5; 5q; −7; 7q-; 11q23; non t(9; 11); inv(3); t(3; 3); t(6; 9); t(9; 22) is associated with poor-risk status (Byrd J. C. et al., Dec. 15, 2002; Blood: 100 (13) and is especially contemplated to be treated according to the present invention or with an object of the present invention.

[0482] In one embodiment, the anti-CD123 CAR expressing T cell of present invention may be used as induction therapy, as post remission therapy of AML or as a consolidation therapy in patient with AML. Preferably, TRC KO cells or TCR KO and dck KO T cells expressing an anti-CD123 CAR of SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO. 160 are used as post remission therapy of AML or as a consolidation therapy in patient with AML.

[0483] In one embodiment, the TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell of the present invention may be used in case of AML relapse, or in case of refractory or resistant AML. Preferably, said TCR KO T dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell of the present invention are used in patients with AML relapse, or with refractory or resistant AML, more preferably, in combination with at least one other anti-cancer drug

[0484] In another preferred embodiment, TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell of the present invention is used for preventing cancer cells development occurring in particular after anti-cancer treatment, during bone marrow depletion or before bone marrow transplantation, after bone marrow destruction.

AML Complications

[0485] In one particular embodiment the invention provides a medicament that improves the health condition of a patient, in particular a patient undergoing a complication related to AML. More preferably, said TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell is used as a medicament for the treatment of a complication related to AML, preferably with FLAG.

[0486] A complication or disease related to AML may include a preceding myelodysplasia phase, secondary leukemia, in particular secondary AML, high white blood cell count, and absence of Auer rods. Among others, leukostasis and involvement of the central nervous system (CNS), Hyperleukocytosis, residual disease, are also considered as a complication or disease related to AML.

AML Associated Diseases

[0487] In one embodiment, the present invention also provides a TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell for the treatment of a pathological condition related to AML. Preferably, the present invention provides a TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell for the treatment of a pathological condition related to AML.

[0488] The present invention provides a therapy (a TCR KO, dck KO, anti-CD123 CAR of SEQ ID NO 31, SEQ ID NO 32 or SEQ ID NO 160 expressing T cell, preferably having a sequence selected from SEQ ID NO. 160, SEQ ID NO. 188, SEQ ID NO. 189, SEQ ID NO. 190, SEQ ID NO. 191; SEQ ID NO. 192, SEQ ID NO. 193, SEQ ID NO. 194, SEQ ID NO. 195, SEQ ID NO. 196, SEQ ID NO. 197) for AML related myeloid neoplasms, for acute myeloid leukemia and myelodysplastic syndrome, a treatment of relapsed or refractory acute myeloid leukemia, a treatment of relapsed or refractory acute promyelocytic leukemia in adults, a treatment for acute promyeloid leukaemia, a treatment of acute myeloid leukemia in adults over 60 years.

[0489] According to another aspect, the present invention provides a composition for the treatment of AML associated diseases, in particular hematologic malignancy related to AML.

[0490] Hematologic malignancy related to AML conditions include myelodysplasia syndromes (MDS, formerly known as "preleukemia") which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AM

[0491] In another embodiment, the invention provides a medicament that improves the health state of a patient suffering multiple myeloma.

[0492] Other pathological conditions or genetic syndromes associated with the risk of AML can be improved with the adequate use of the present invention, said genetic syndromes include Down syndrome, trisomy, Fanconi anemia, Bloom syndrome, Ataxia-telangiectasia, Diamond-Blackfan anemia, Schwachman-Diamond syndrome, Li-Fraumeni syndrome, Neurofibromatosis type 1, Severe congenital neutropenia (also called Kostmann syndrome)

Other CD123-Mediated Pathological Conditions

[0493] According to another aspect, the present invention provides a composition for the treatment of CD123+ cell-mediated diseases. These CD123+ cell mediated diseases include inflammation, autoimmune diseases.

[0494] In particular, the present invention can be used for the treatment of CD123+ cell mediated diseases such as inflammation of the gastrointestinal mucosae and more particularly, inflammatory bowel diseases, nasal allergy, inflammation of the skin such as juvenile dermatomyositis, hematodermia.

[0495] The present invention can be used as a medicament for the treatment of CD123+ cell mediated diseases such as autoimmune diseases in particular Kikushi disease.

[0496] Preferably, the present invention provides a treatment for a recurrent infection including infection due to viruses such as Epstein-Barr virus, herpes simplex virus, in particular oncogenic viruses, HHV-8, HHV-6, HTLV or HIV, parasitic infection such as infection due to *plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, or *Plasmodium malariae.*

[0497] In particular, the present invention provides a treatment for Epstein-Barr virus lymphadenitis, herpes simplex virus lymphadenitis.

[0498] In another aspect, the present invention provides a composition for the treatment of systemic lupus erythematosus lymphadenitis, tuberculosis, cystic fibrosis, hepatitis, biliary atresia, in particular virus-induced hepatitis or biliary atresia in children, autoimmune hepatitis; primary biliary cirrhosis.

[0499] Composition Comprising an Engineered T Cells According to the Invention for Use as a Medicament and Method

[0500] The present invention also provides a composition comprising a genetically engineered immune TCR KO-dCK KO-CD123 CAR having a sequence corresponding to SEQ ID NO. 31, SEQ ID NO. 32 or SEQ ID NO 160 T cells, or endowed with a sequence selected from SEQ ID NO. 160, SEQ ID NO. 188, SEQ ID NO. 189, SEQ ID NO. 190, SEQ ID NO. 191; SEQ ID NO. 192, SEQ ID NO. 193, SEQ ID NO. 194, SEQ ID NO. 195, SEQ ID NO. 196, SEQ ID NO. 197 or

a genetically engineered immune TCR KO-dCK KO CD123 CAR having any one of the sequence selected from SEQ ID NO. 34 to SEQ ID NO. 159, preferably from SEQ ID NO. 34, SEQ ID NO. 76, SEQ ID NO. 36, SEQ ID NO. 78; SEQ ID NO. 37, SEQ ID NO. 79, SEQ ID NO. 41, SEQ ID NO. 83, SEQ ID NO. 42, SEQ ID NO. 8), SEQ ID NO. 43, SEQ ID NO. 85, SEQ ID NO. 46, SEQ ID NO. 47, SEQ ID NO. 48, SEQ ID NO. 49, SEQ ID NO. 88, SEQ ID NO. 89, SEQ ID NO. 90, SEQ ID NO. 91, SEQ ID NO. 52, SEQ ID NO. 53, SEQ ID NO. 54, SEQ ID NO. 55, SEQ ID NO. 94, SEQ ID NO. 95, SEQ ID NO. 96 and SEQ ID NO. 97, T cells.

[0501] Preferably, the present invention provides a composition comprising a genetically engineered immune TCR KO-dCK KO-CD123 CAR having a sequence corresponding to SEQ ID NO. 31 T cells.

[0502] Preferably, the present invention provides a composition comprising a genetically engineered immune TCR KO-dCK KO-CD123 CAR having a sequence corresponding to SEQ ID NO. 32 T cells.

[0503] Preferably, the present invention provides a composition comprising a genetically engineered immune TCR KO-dCK KO-CD123 CAR having a sequence corresponding to SEQ ID NO. 160 T cells

[0504] Or any one of the sequence selected from SEQ ID NO. 160, SEQ ID NO. 188, SEQ ID NO. 189, SEQ ID NO. 190, SEQ ID NO. 191; SEQ ID NO. 192, SEQ ID NO. 193, SEQ ID NO. 194, SEQ ID NO. 195, SEQ ID NO. 196, SEQ ID NO. 197,

for its use or a method for treating a cancer such as BPDNL or as a treatment before bone marrow transplant. In one aspect, the disease is a hematologic cancer, in particular a stem cell cancer including but is not limited to leukemia (such as acute myelogenous leukemia (AML), chronic myelogenous leukemia, acute lymphoid leukemia, chronic lymphoid leukemia and myelodysplasia syndrome) and malignant lymphoproliferative conditions, including lymphoma (such as multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and small cell- and large cell-follicular lymphoma), or a complication (relapse refractor AML), thereof.

[0505] The present invention also provides a composition as above for its use or a method for inhibiting the proliferation or reducing a CD123-expressing cell population or activity in a patient. An exemplary method includes contacting a population of cells comprising a CD123-expressing cell with a CD 123 CART cell of the invention that binds to the CD123-expressing cell.

[0506] In a more specific aspect, the present invention provides a composition for its use or a method for inhibiting the proliferation or reducing the population of cancer cells expressing CD 123 in a patient, the methods comprising contacting the CD123-expressing cancer cell population with a CD 123 CART cell of the invention that binds to the CD123-expressing cell, binding of a CD 123 CART cell of the invention to the CD123-expressing cancer cell resulting in the destruction of the CD123-expressing cancer cells

[0507] In certain aspects, the CD 123 CART cell of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% (to undetectable level) in a subject with or animal model for myeloid leukemia or another cancer associated with CD123-expressing cells, relative to a negative control.

[0508] The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disorder or condition associated with CD123-expressing cells (e.g., associated with a hematologic cancer), the methods comprising administering to a subject in need a CD 123 CART cell of the invention that binds to the CD123-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with CD123-expressing cells include autoimmune disorders (such as lupus), inflammatory disorders (such as allergies, IBD, and asthma) and cancers (such as hematological cancers, in particular AML or AML complications).

[0509] The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disease associated with CD123-expressing cells, the method comprising administering to a subject in need a CD 123 CART cell of the invention that binds to the CD123-expressing cell. In one aspect, the subject is a human. Non-limiting examples of diseases associated with CD123-expressing cells include Acute Myeloid Leukemia (AML), myelodysplasia, B-cell Acute Lymphoid Leukemia, T-cell Acute Lymphoid Leukemia, hairy cell leukemia, blastic plasmacytoid dendritic cell neoplasm (BPDCN), chronic myeloid leukemia, Hodgkin lymphoma.

[0510] The present invention provides a composition for its use or a method for treating or preventing relapse of cancer associated with CD123-expressing cells, the method comprising administering to a subject in need thereof a CD 123 CART cell of the invention that binds to the CD 123-expressing cell. In another aspect, the methods comprise administering to the subject in need thereof an effective amount of a CD 123 CART cell of the invention that binds to the CD123-expressing cell in combination with an effective amount of another therapy.

[0511] In one aspect, CD 123 is considered to be a "cancer stem cell" marker in AML. Therefore, a CD 123 CART cell of the invention can prevent relapse of AML, or even treat AML that is mostly CD 123-negative but with a "stem" population of CD 123+ cells (a CD123-expressing cells).

[0512] In one aspect, the invention provides compositions and methods for treating subjects that have undergone treatment for a disease or disorder associated with elevated expression levels of CD 19, and exhibits a disease or disorder associated with elevated levels of CD123.

[0513] In one aspect, B-cell acute lymphoid leukemia (ALL) is an example of disease requiring a serial treatment using CART cells. For example, treatment with anti-CD 19 CAR T cells can sometimes result in CD19-negative relapse, which can be treated with anti-CD123 CAR T cells of the invention. Alternatively, the present invention includes dual targeting of B-ALL using CART cells comprising an anti-CD 19 CAR and an anti-CD 123 CAR.

[0514] The treatment with the engineered immune cells according to the invention may be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

[0515] Preferably, the treatment with the engineered immune cells according to the invention may be administered in combination (e.g., before, simultaneously or following) with one or more therapies against cancer selected from Aracytine, Cytosine Arabinoside, amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof.

[0516] 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.

[0517] The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermaly, 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.

[0518] The administration of the cells or population of cells can consist of the administration of from 10^4-10^9 cells per kg body weight, preferably 10^5 to 10^6 cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of

effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage 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.

[0519] 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.

[0520] In certain embodiments of the present invention, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizimab treatment for MS patients or efaliztimab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycoplienolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH, In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. In that case, the CD123 CAR expressed in T cell of the invention does not consist in SEQ ID 160, unless it is administered before Rituxan.

[0521] 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.

[0522] In certain embodiments of the present invention, anti-CD123 CAR expressing cells are administered to a patient in conjunction (e.g., before, simultaneously or following) with a drug selected from Aracytine, Cytosine Arabinoside, amsacrine, Daunorubicine, Idarubicine, Novantrone, Mitoxantrone, Vepeside, Etoposide (VP16), arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof. In these embodiments anti-CD123 CAR expressing cells may be resistant to the particular drug or combination of drugs that is (are) administered in conjunction with anti-CD123 CAR expressing cells.

[0523] In other embodiments of the present invention, anti-CD123 CAR expressing cells are administered to a patient in conjunction with a drug selected from cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

Other Definitions

[0524] Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one. Amino acid residues in a polypeptide sequence are designated herein according to the one-letter code, in which, for example, Q means Gln or Glutamine residue, R means Arg or Arginine residue and D means Asp or Aspartic acid residue.

[0525] Amino acid substitution means the replacement of one amino acid residue with another, for instance the replacement of an Arginine residue with a Glutamine residue in a peptide sequence is an amino acid substitution.

[0526] Nucleotides are designated as follows: one-letter code is used for designating the base of a nucleoside: an is adenine, t is thymine, c is cytosine, and g is guanine. For the degenerated nucleotides, r represents g or a (purine nucleotides), k represents g or t, s represents g or c, w represents a or t, m represents a or c, y represents t or c (pyrimidine nucleotides), d represents g, a or t, v represents g, a or c, b represents g, t or c, h represents a, t or c, and n represents g, a, t or c.

[0527] "As used herein, "nucleic acid" or "polynucleotides" refers to nucleotides and/or polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Nucleic acids can be either single stranded or double stranded.

[0528] By chimeric antigen receptor (CAR) is intended molecules that combine a binding domain against a component present on the target cell, for example an antibody-based specificity for a desired antigen (e.g., tumor antigen) with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific anti-target cellular immune activity. Generally, CAR consists of an extracellular single chain antibody (scFv Fc) fused to the intracellular signaling domain of the T cell antigen receptor complex zeta chain (scFv Fc:ζ) and have the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity. One example of CAR

used in the present invention is a CAR directing against CD123 antigen and can comprise as non limiting example the amino acid sequences: SEQ ID NO: 23 to 48 and 160.

[0529] The term "endonuclease" refers to any wild-type or variant enzyme capable of catalyzing the hydrolysis (cleavage) of bonds between nucleic acids within a DNA or RNA molecule, preferably a DNA molecule. Endonucleases do not cleave the DNA or RNA molecule irrespective of its sequence, but recognize and cleave the DNA or RNA molecule at specific polynucleotide sequences, further referred to as "target sequences" or "target sites". Endonucleases can be classified as rare-cutting endonucleases when having typically a polynucleotide recognition site greater than 12 base pairs (bp) in length, more preferably of 14-55 bp. Rare-cutting endonucleases significantly increase HR by inducing DNA double-strand breaks (DSBs) at a defined locus (Perrin, Buckle et al. 1993; Rouet, Smih et al. 1994; Choulika, Perrin et al. 1995; Pingoud and Silva 2007). Rare-cutting endonucleases can for example be a homing endonuclease (Paques and Duchateau 2007), a chimeric Zinc-Finger nuclease (ZFN) resulting from the fusion of engineered zinc-finger domains with the catalytic domain of a restriction enzyme such as FokI (Porteus and Carroll 2005), a Cas9 endonuclease from CRISPR system (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) or a chemical endonuclease (Eisenschmidt, Lanio et al. 2005; Arimondo, Thomas et al. 2006). In chemical endonucleases, a chemical or peptidic cleaver is conjugated either to a polymer of nucleic acids or to another DNA recognizing a specific target sequence, thereby targeting the cleavage activity to a specific sequence. Chemical endonucleases also encompass synthetic nucleases like conjugates of orthophenanthroline, a DNA cleaving molecule, and triplex-forming oligonucleotides (TFOs), known to bind specific DNA sequences (Kalish and Glazer 2005). Such chemical endonucleases are comprised in the term "endonuclease" according to the present invention.

[0530] By a "TALE-nuclease" (TALEN) is intended a fusion protein consisting of a nucleic acid-binding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, like for instance I-TevI, ColE7, NucA and Fok-I. In a particular embodiment, the TALE domain can be fused to a meganuclease like for instance I-CreI and I-OnuI or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE-Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927. Transcription Activator like Effector (TALE) are proteins from the bacterial species *Xanthomonas* comprise a plurality of repeated sequences, each repeat comprising di-residues in position 12 and 13 (RVD) that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered by the applicant in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALE-nuclease have been already described and used to stimulate gene targeting and gene modifications (Boch, Scholze et al. 2009; Moscou and Bogdanove 2009; Christian, Cermak et al. 2010; Li, Huang et al. 2011). Engineered TAL-nucleases are commercially available under the trade name TALEN™ (Cellectis, 8 rue de la Croix Jarry, 75013 Paris, France).

[0531] The rare-cutting endonuclease according to the present invention can also be a Cas9 endonuclease. Recently, a new genome engineering tool has been developed based on the RNA-guided Cas9 nuclease (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) from the type II prokaryotic CRISPR (Clustered Regularly Interspaced Short palindromic Repeats) adaptive immune system (see for review (Sorek, Lawrence et al. 2013)). The CRISPR Associated (Cas) system was first discovered in bacteria and functions as a defense against foreign DNA, either viral or plasmid. CRISPR-mediated genome engineering first proceeds by the selection of target sequence often flanked by a short sequence motif, referred as the proto-spacer adjacent motif (PAM). Following target sequence selection, a specific crRNA, complementary to this target sequence is engineered. Trans-activating crRNA (tracrRNA) required in the CRISPR type II systems paired to the crRNA and bound to the provided Cas9 protein. Cas9 acts as a molecular anchor facilitating the base pairing of tracRNA with cRNA (Deltcheva, Chylinski et al. 2011). In this ternary complex, the dual tracrRNA:crRNA structure acts as guide RNA that directs the endonuclease Cas9 to the cognate target sequence. Target recognition by the Cas9-tracrRNA:crRNA complex is initiated by scanning the target sequence for homology between the target sequence and the crRNA. In addition to the target sequence-crRNA complementarity, DNA targeting requires the presence of a short motif adjacent to the protospacer (protospacer adjacent motif—PAM). Following pairing between the dual-RNA and the target sequence, Cas9 subsequently introduces a blunt double strand break 3 bases upstream of the PAM motif (Garneau, Dupuis et al. 2010).

[0532] Rare-cutting endonuclease can be a homing endonuclease, also known under the name of meganuclease. Such homing endonucleases are well-known to the art (Stoddard 2005). Homing endonucleases recognize a DNA target sequence and generate a single- or double-strand break. Homing endonucleases are highly specific, recognizing DNA target sites ranging from 12 to 45 base pairs (bp) in length, usually ranging from 14 to 40 bp in length. The homing endonuclease according to the invention may for example correspond to a LAGLIDADG endonuclease, to a

HNH endonuclease, or to a GIY-YIG endonuclease. Preferred homing endonuclease according to the present invention can be an I-Crel variant.

[0533] By "delivery vector" or "delivery vectors" is intended any delivery vector which can be used in the present invention to put into cell contact (i.e "contacting") or deliver inside cells or subcellular compartments (i.e "introducing") agents/chemicals and molecules (proteins or nucleic acids) needed in the present invention. It includes, but is not limited to liposomal delivery vectors, viral delivery vectors, drug delivery vectors, chemical carriers, polymeric carriers, lipoplexes, polyplexes, dendrimers, microbubbles (ultrasound contrast agents), nanoparticles, emulsions or other appropriate transfer vectors. These delivery vectors allow delivery of molecules, chemicals, macromolecules (genes, proteins), or other vectors such as plasmids, peptides developed by Diatos. In these cases, delivery vectors are molecule carriers. By "delivery vector" or "delivery vectors" is also intended delivery methods to perform transfection.

[0534] The terms "vector" or "vectors" refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A "vector" in the present invention includes, but is not limited to, a viral vector, a plasmid, a RNA vector or a linear or circular DNA or RNA molecule which may consists of a chromosomal, non chromosomal, semi-synthetic or synthetic nucleic acids. Preferred vectors are those capable of autonomous replication (episomal vector) and/or expression of nucleic acids to which they are linked (expression vectors). Large numbers of suitable vectors are known to those of skill in the art and commercially available.

[0535] Viral vectors include retrovirus, adenovirus, parvovirus (e. g. adenoassociated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e. g., influenza virus), rhabdovirus (e. g., rabies and vesicular stomatitis virus), paramyxovirus (e. g. measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus, herpesvirus (e. g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e. g., vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, In Fundamental Virology, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

[0536] By "lentiviral vector" is meant HIV-Based lentiviral vectors that are very promising for gene delivery because of their relatively large packaging capacity, reduced immunogenicity and their ability to stably transduce with high efficiency a large range of different cell types. Lentiviral vectors are usually generated following transient transfection of three (packaging, envelope and transfer) or more plasmids into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface glycoproteins with receptors on the cell surface. On entry, the viral RNA undergoes reverse transcription, which is mediated by the viral reverse transcriptase complex. The product of reverse transcription is a double-stranded linear viral DNA, which is the substrate for viral integration in the DNA of infected cells. By "integrative lentiviral vectors (or LV)", is meant such vectors as non limiting example, that are able to integrate the genome of a target cell. At the opposite by "non-integrative lentiviral vectors (or NILV)" is meant efficient gene delivery vectors that do not integrate the genome of a target cell through the action of the virus integrase.

[0537] Delivery vectors and vectors can be associated or combined with any cellular permeabilization techniques such as sonoporation or electroporation or derivatives of these techniques.

[0538] By cell or cells is intended any eukaryotic living cells, primary cells and cell lines derived from these organisms for in vitro cultures.

[0539] By "primary cell" or "primary cells" are intended cells taken directly from living tissue (i.e. biopsy material) and established for growth in vitro, that have undergone very few population doublings and are therefore more representative of the main functional components and characteristics of tissues from which they are derived from, in comparison to continuous tumorigenic or artificially immortalized cell lines.

[0540] As non-limiting examples cell lines can be selected from the group consisting of CHO-K1 cells; HEK293 cells; Caco2 cells; U2-OS cells; NIH 3T3 cells; NSO cells; SP2 cells; CHO-S cells; DG44 cells; K-562 cells, U-937 cells; MRC5 cells; IMR90 cells; Jurkat cells; HepG2 cells; HeLa cells; HT-1080 cells; HCT-116 cells; Hu-h7 cells; Huvec cells; Molt 4 cells.

[0541] All these cell lines can be modified by the method of the present invention to provide cell line models to produce, express, quantify, detect, study a gene or a protein of interest; these models can also be used to screen biologically active molecules of interest in research and production and various fields such as chemical, biofuels, therapeutics and agronomy as non-limiting examples.

[0542] by "mutation" is intended the substitution, deletion, insertion of up to one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty five, thirty, forty, fifty, or more nucleotides/amino acids in a polynucleotide (cDNA, gene) or a polypeptide sequence. The mutation can affect the coding sequence of a gene or its regulatory sequence. It may also affect the structure of the genomic sequence or the structure/stability of the encoded mRNA.

[0543] by "variant(s)", it is intended a repeat variant, a variant, a DNA binding variant, a TALE-nuclease variant, a polypeptide variant obtained by mutation or replacement of at least one residue in the amino acid sequence of the parent molecule.

[0544] by "functional variant" is intended a catalytically active mutant of a protein or a protein domain; such mutant may have the same activity compared to its parent protein or protein domain or additional properties, or higher or lower activity.

[0545] "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.

[0546] "similarity" describes the relationship between the amino acid sequences of two or more polypeptides. BLASTP may also be used to identify an amino acid sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to a reference amino acid sequence using a similarity matrix such as BLOSUM45, BLOSUM62 or BLOSUM80. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent 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. For example, a functional variant of pTalpha can have 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to the amino acid sequence of SEQ ID NO: 107. A polynucleotide encoding such a functional variant would be produced by reverse translating its amino acid sequence using the genetic code.

[0547] "signal-transducing domain" or "co-stimulatory ligand" refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-IBB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[0548] 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.

[0549] A "co-stimulatory signal" as used herein refers to a signal, which in combination with primary signal, such as TCR/CD3 ligation, leads to T cell proliferation and/or upregulation or downregulation of key molecules.

[0550] The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus examples of cell surface markers that may act as ligands include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

[0551] The term "subject" or "patient" as used herein includes all members of the animal kingdom including non-human primates and humans.

[0552] The term "relapsed" refers to a situation where a subject or a mammal, who has had a remission of cancer after therapy has a return of cancer cells.

[0553] 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.

[0554] The term "drug resistance" refers to the condition when a disease does not respond to the treatment of a drug or drugs. Drug resistance can be either intrinsic (or primary resistance), which means the disease has never been responsive to the drug or drugs, or it can be acquired, which means the disease ceases responding to a drug or drugs that the disease had previously responded to (secondary resistance). In certain embodiments, drug resistance is intrinsic. In certain embodiments, the drug resistance is acquired.

[0555] 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, for instance, myelodysplasia, leukemia, lymphomas, such as cutaneous Lymphomas, non-Hodgkin's lymphoma, Hodgkin's disease (also called Hodgkin's lymphoma), and myeloma, such as acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), acute promyelocytic leukemia (APL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), acute undifferentiated leukemia (AUL), anaplastic large-cell lymphoma (ALCL), prolymphocytic leukemia (PML), juvenile myelomonocytic leukemia (JMML), adult T-cell ALL, AML with trilineage myelodysplasia (AML/TMDS), mixed lineage leukemia (MLL), myelodysplastic syndromes (MDSs), myeloproliferative disorders (MPD), and multiple myeloma (MM).

[0556] The term "leukemia" refers to malignant neoplasms of the blood-forming tissues, including, but not limited to, chronic lymphocytic leukemia or chronic lymphoid leukemia, chronic myelocytic leukemia, or chronic myelogenous leukemia, acute lymphoblastic leukemia, acute myeloid leukemia or acute myelogenous leukemia (AML) and acute myeloblastic leukemia.

[0557] In general, a primary cell is a cell isolated from a blood sample or a biopsy and then optionally further cultured in vitro. A cell line is a cellular culture of a transformed ie cancerous cell, preferably a homogenous cellular culture of a transformed ie cancerous cell (wherein a marker is represented by a Gaussian curve).

[0558] The above written description of the invention provides a manner and process of making and using it such that any person skilled in this art is enabled to make and use the same, this enablement being provided in particular for the subject matter of the appended claims, which make up a part of the original description.

[0559] Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and subranges within a numerical limit or range are specifically included as if explicitly written out.

[0560] The above description is presented to enable a person skilled in the art to make and use the invention, and is provided in the context of a particular application and its requirements. Various modifications to the preferred embodiments will be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the invention. Thus, this invention is not intended to be limited to the embodiments shown, but is to be accorded the widest scope consistent with the principles and features disclosed herein.

[0561] Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

General Method

[0562] In general, the CD123 CAR T cells of the invention were prepared using T cells purified from Buffy coat samples from different donors. The process and products satisfies the requirement of the Good Manufacturing Practices (FDA 21 CFR and EU GMP).

[0563] The clinical essay was conducted under Good Clinical Practices (UK GCP or USA GCP)

[0564] *Blood* 2014; 124(21):4689

Preclinical Study

[0565] Primary T-Cell Cultures

[0566] T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium. The PBMC layer was recovered and T cells were purified using a commercially available T-cell enrichment kit. Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20 ng/mL Human IL-2, 5% Human, and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies).

[0567] CAR mRNA Transfection

[0568] Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15 μg of mRNA encoding the different CAR constructs. CAR mRNAs were produced using T7 mRNA polymerase transfections done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4 cm gap cuvettes in a final volume of 200 μl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media and incubated at 37° C. with 5% CO_2. IL-2 was added 2 h after electroporation at 20 ng/mL.

T-Cell Transduction

[0569] Transduction of T-cells with recombinant lentiviral vectors expression the CAR was carried out three days after T-cell purification/activation. Lentiviral vectors were produced by Vectalys 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 10^6 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 CD123 protein together with a murine IgG1 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.

[0570] Degranulation Assay (CD107a Mobilization)

[0571] T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing various levels of the CD123 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 6 hours at 37° C. with 5% CO_2. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody at the beginning of the co-culture, together with 1 μg/ml of anti-CD49d, 1 μg/ml of anti-CD28, and 1× Monensin solution. After the 6 h incubation period, cells were stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24 h after mRNA transfection.

[0572] IFN Gamma Release Assay

[0573] T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing various levels of the CD123 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 24 hours at 37° C. with 5% CO_2. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFN gamma detection in the cell culture supernatants was done by ELISA assay. The IFN gamma release assays were carried by starting the cell co-cultures 24 h after mRNA transfection.

[0574] Cytotoxicity Assay

[0575] T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing CD123) and 10,000 control (CD123neg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet) before co-culturing them with CAR+ T-cells. The co-cultures were incubated for 4 hours at 37'C with 5% CO_2. After this incubation period, cells were labelled with a fixable viability dye and analyzed by flow cytometry. Viability of each cellular population (target cells or CD123neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48 h after mRNA transfection.

[0576] T-Cell Transduction

[0577] Transduction of T-cells with recombinant lentiviral vectors expression the CAR was carried out three days after T-cell purification/activation. 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 CD123 protein, together with a murine IgG1 Fc fragment. Binding of this protein to the CAR molecule was detected with a fluorochrome-conjugated secondary antibody targeting the mouse Fc portion of the protein, and analyzed by flow cytometry.

[0578] Anti-Tumor Mouse Model

[0579] Immuno-deficient NOG mice were intravenously (iv) injected with (CD123 expressing_MOLM13-Luciferase cells as an AML xenograft mouse model. Optionally, mice received an anti-cancer treatment that is PNA or FLAG. Mice were then iv injected (either 2 or 7 days after injection of the tumor cell line) with different doses of CAR+ T-cells to be tested, or with T-cells that were not transduced with the CAR lentiviral vector. Bioluminescent signals were determined at the day of T-cell injection (DO), at D7, 14, 21, 28 and 40 after T-cell injection in order to follow tumoral progression on the different animals.

Clinical Study

[0580] A Phase I dose escalation trial is designed to evaluate the safety and the biologic efficacy of allogeneic TCR KO specific cytotoxic T-lymphocytes (CTL) genetically modified to express artificial T-cell receptors (CAR) targeting the CD123 molecule (CD123CAR) in patients who have relapsed/refractory Acute myeloid Leukemia (AML), blastic plasmacytoid dendritic cell neoplasm, bridge to transplant.

[0581] Each patient will receive at least one dose of donor derived, genetically modified CTL and will be monitored for toxicity and detection of transduced CTL as well as disease specific markers.

[0582] Any of the following may vary individually upon medical indication.

Condition	Intervention	Phase
Acute myeloid Leukemia Blastic plasmacytoid dendritic cell neoplasm Bridge to transplant	Biological: Biological/Genetically Modified T cells	Phase 1

[0583] Study Type: Interventional

[0584] Study Design: Endpoint Classification: Safety/Efficacy Study

[0585] Intervention Model: Single Group Assignment

[0586] Masking: Open Label

[0587] Primary Purpose: Treatment

[0588] Official Title: A Phase I Dose Escalation Trial Using In Vitro Expanded Allogeneic Cytotoxic T-Lymphocytes (CTLs) Genetically Targeted to the B-Cell Specific Antigen CD123 Positive Residual Or Relapsed Acute myeloid Leukemia, Blastic plasmacytoid dendritic cell neoplasm, during Bridge to transplant.

Key words Acute myeloid Leukemia, Blastic plasmacytoid dendritic cell neoplasm, Bridge to transplant

Primary Outcome Measures:

[0589] Evaluate the safety/persistence of escalating doses of allogeneic specific CTL modified to express artificial T cell receptors targeting CD123 molecule given for persistence or relapse of AML, for Blastic plasmacytoid dendritic cell neoplasm or in a Bridge to transplant.

Secondary Outcome Measures:

[0590] To assess the effects of the adoptively transferred CD123 specific T-cells on the progression of AML.

[0591] To quantitate the number of CD123 chimeric antigen receptor (CD123 CAR) positive T-cells in the blood at defined intervals post infusion in order to determine their survival and proliferation in the host

[0592] To quantitate the number of CD123 chimeric antigen receptor (CD123 CAR) positive T-cells in the blood at defined intervals post infusion of Rituximab (375 mg/m2)

Arms	Assigned interventions
Experimental: Biological/ Genetically Modified T cells Patients with persistent minimal residual disease (+MRD) or relapsed AML will receive a conditioning chemotherapy regimen followed by intravenous infusion of allogeneic specific cytotoxic T-cells (CTLs) genetically modified ex vivo to express the CD123-specific chimeric artificial T-cell receptor.	Biological: Biological/Genetically Modified T cells Following completion of the chemotherapy, genetically modified T cells will be given intravenously at one of 3 dose levels (10^5, 10^6 and 10^7). After the infusion patients will be monitored clinically and with serial blood and marrow evaluations to assess toxicity, therapeutic effects, and the in-vivo survival of the genetically modified T-cells.

Eligibility

Genders Eligible for Study: Both

Accepts Healthy Volunteers: No

Criteria

Inclusion Criteria:

[0593] History of CD123+ leukemia with evidence of bone marrow relapse or persistent.

[0594] Persistent minimal residual disease must be demonstrated by morphology, FISH, flow cytometry or RT-PCR with at least 2 sequential testings separated by at least 1 week.

[0595] No age restriction for patients

[0596] KPS or Lansky score> or = to 40

[0597] Renal function (measured prior to conditioning chemotherapy)

[0598] Hepatic function (measured prior to conditioning chemotherapy):

[0599] AST≤5× the institutional ULN Elevation secondary to leukemic involvement is not an exclusion criterion. Leukemic involvement will be determined by the presence of progressive relapse defined by escalating bone marrow or peripheral blood leukemia blasts within the previous month and the absence of initiation of know hepatotoxic medication (e.g. azoles).

[0600] Total bilirubin≤2.5× the institutional ULN

[0601] Adequate cardiac function (e.g. LVEF≥40%) as assessed by ECHO or MUGA or other similar cardia imaging performed within 1 month of enrollment.

[0602] Pulmonary function (measured prior to conditioning chemotherapy):

[0603] Oxygen saturation≥90% on room air

Donor Eligibility:

[0604] The donor, including a third party donor, must consent to a leukapheresis or whole blood donation(s) obtained at one or more phlebotomies which, in aggregate, will total approximately 250 ml for adults and no more than 5 ml/kg per draw from pediatric donors.

[0605] Related donors<18 years of age requiring placement of a leukapheresis catheter will donate peripheral blood collected by phlebotomy (including a unit of blood if weight permits) and shall not undergo catheter placement for leukapheresis as this is considered above minimal risk to the donor.

[0606] There is no upper age limit for a donor. However, the minimum age for a related donor is 7 years as this is the youngest age a person can be considered capable of giving assent to participate in a research study.

[0607] Donor's high resolution HLA typing must be available for review

[0608] CBC within one week of donation. Results of tests must be within a range that would not preclude donating blood or undergoing leukapheresis.

[0609] Serologic testing for transmissible diseases will be performed as per institutional guidelines adopted from extant NMDP and FACT guidelines. Donors should be considered eligible to donate leukapheresis or blood based on these guidelines (i.e. blood donation guidelines)

Exclusion Criteria:

[0610] Patients with active HIV, hepatitis B or hepatitis C infection.

[0611] Patients with any concurrent active malignancies as defined by malignancies requiring any therapy other than expectant observation.

[0612] Females who are pregnant.

[0613] Patients will be excluded if they have isolated extra-medullary relapse of ALL.

[0614] Patients with active (grade 2-4) acute graft versus host disease (GVHD), chronic GVHD or an overt autoimmune disease (e.g. hemolytic anemia) requiring glucocorticosteroid treatment (>0.5 mg/kg/day prednisone or its equivalent) as treatment

[0615] Active central nervous system (CNS) leukemia, as defined by unequivocal morphologic evidence of lymphoblasts in the cerebrospinal fluid (CSF) or symptomatic CNS leukemia (i.e. cranial nerve palsies or other significant neurologic dysfunction) within 28 days of treatment. Prophylactic intrathecal medication is not a reason for exclusion.

[0616] Adult patients (≥18 years old) with the following cardiac conditions will be excluded:

[0617] New York Heart Association (NYHA) stage III or IV congestive heart failure

[0618] Myocardial infarction≤6 months prior to enrollment

[0619] History of clinically significant ventricular arrhythmia or unexplained syncope, not believed to be vasovagal in nature or due to dehydration.

[0620] History of severe non-ischemic cardiomyopathy with EF≤20%

[0621] The first data demonstrate that the engineered T cell of the invention can be infused iv at a dose of 10^7 cells/kg to relapse refractory AML patients and selectively clear CD123-expressing cancerous cells for at least 11 months.

EXAMPLES

Example 1: Proliferation of TCRalpha Inactivated Cells Expressing a CD123-CAR

[0622] Heterodimeric TALE-nuclease targeting two 17-bp long sequences (called half targets) separated by an 15-bp spacer within T-cell receptor alpha constant chain region (TRAC) gene were designed and produced. Each half target is recognized by repeats of the half TALE-nucleases listed in Table 10.

TABLE 10

TAL-nucleases targeting TCRalpha gene

Target	Target sequence	Repeat sequence	Half TALE-nuclease
TRAC_T01	TTGTCCCACAGATATCC Agaaccctgaccctg (SEQ ID NO: 13)	Repeat TRAC_T01-L (SEQ ID NO: 14)	TRAC_T01-L TALEN (SEQ ID NO: 16)
	CCGTGTACCAGCTGAGA (SEQ ID NO: 13)	Repeat TRAC_T01-R (SEQ ID NO: 15)	TRAC_T01-R TALEN (SEQ ID NO: 17)

[0623] 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.

[0624] 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 CD-123 CAR previously described (SEQ ID NO: 23 to 48). 2 days post-transduction, $CD3_{NEG}$ cells were purified using anti-CD3 magnetic beads and 5 days post-transduction cells were reactivated with soluble anti-CD28 (5 μg/ml).

[0625] 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 CD-123 CARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

[0626] To investigate whether the human T cells expressing the CD123-CAR 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 CD-123 CAR assayed for CD25

expression at their surface in order to assess their activation in comparison with the non-transduced cells. Increased CD25 expression is expected both in CD28 reactivation or no reactivation conditions.

Example 2

[0627] Construction of CD123 CAR Using Anti-CD123 scFv Antibody Fragments Derived from Klon43, Functional Analysis in TCR KO and dCK KO TCR CD123 Expressing Cells

[0628] An scFv from Klon 43 or humanized scFv was prepared using a combination of a VH (SEQ ID NO 12, a L (SEQ ID NO 10), a VL SEQ ID NO 11, or a VH selected from SEQ ID NO 24 to SEQ ID NO. 30 a linker L and a VL selected from SEQ ID NO. 18 to SEQ ID NO. 23, respectively was used to generate CD123 Chimeric Antigen Receptors (CD123 CARs of the invention) and to screen them to select performant (with anti-tumoral activity with the less side effects).

[0629] Architectures V1 or V3, preferably V3 were used (FIG. **2** and FIG. **3**) and activity was determined upon expression in human T-cells (FIG. **4**, FIG. **5**, FIG. **6** and FIG. **7**).

[0630] The results illustrated in FIG. **4** shows degranulation activity of different a scFv according to the invention for one architecture (v3: CD8-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD123 expressing cells (RPM18226), or with cells that do not express CD123 (K562). White bars correspond to degranulation signals observed in T-cells that were cultured alone, black bars represent the signals observed when T-cells were co-cultured with RPM18226 cells, and gray bars show degranulation signals of T-cells co-cultured with K562 cells.

[0631] FIG. **5** shows the amount of IFN gamma released by T-cells when co-cultured for 24 h with cells expressing different levels of CD123 (KG1a or RPM18226), or with cells that do not express CD123 (K562). IFN gamma release from T-cells cultured alone, in the same conditions that the co-cultures, is also shown. The experiments were done for three independent donors, and results from a representative donor are shown here.

[0632] FIGS. **6** and **7** show a dose-response of the specific cytolytic activity of CAR-T cells in vivo in mice treated with PNA (20 mg/kg) ip.

[0633] Immunodefficient mice were injected with MOLM13-Luciferase cells 2 days before injection of non-transduced human T-cells, or with different doses of anti-CD123 CAR+ T-cells. The results represent the bioluminescent signal observed at different time points after T-cell injection.

Example 3: Clinical Study

[0634] Clinical study provided data demonstrating the feasibility of the clinical study described herein.

[0635] At least one patient suffering refractory/relapsed AML received a treatment according to the invention that significantly increased her life expectancy by more than 16 months and reduced the cancerous cells below detection level for at least 6 months.

[0636] Engineered immune cells expressing the CD123 were detected at least 3 months after infusion.

Administration of Rituximab (Rituxan®).

[0637] The total dose of Rituximab administered by intravenous route during 4 day after an initial rituximab dose of 375 mg/m2 was 2,250 mg/m2.

[0638] Premedication was performed before each infusion with acetaminophen and an antihistamine.

[0639] In another embodiment, the first Infusion was initiated at a rate of 50 mg/hr. In the absence of infusion toxicity, infusion rate was 50 mg/hr incremented every 30 minutes, to a maximum of 400 mg/hr.

[0640] For the subsequent Infusions, it was performed at a rate of 100 mg/hr. In the absence of infusion toxicity, increased to a maximum of 400 mg/hr to reach 2,250 mg/m2.

[0641] The results show that the level of CD123 CAR cells in the blood of patients was below detection at 24 h after the last infusion.

Klo43-1

(SEQ ID NO. 31)

MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGGSLSLSCAASG

FTFTDYYMSWVRQPPGKALEWLALIRSKADGYTTEYSASVKGRFTLS

RDDSQSILYLQMNALRPEDSATYYCARDAAYYSYYSPEGAMDYWGQG

TSVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSHKFMSTSVGDRVN

ITCKASQNVDSAVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGRGS

GTDFTLTISSVQAEDLAVYYCQQYYSTPWTFGGGTKLEIKRGLAVST

ISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP

FMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQL

YNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMA

EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0642] In one embodiment Klon43-1 is

EVKLVESGGGLVQPGGSLSLSCAASGFTFTDYYMSWVRQPPGKALEW

LALIRSKADGYTTEYSASVKGRFTLSRDDSQSILYLQMNALRPEDS

ATYYCARDAAYYSYYSPEGAMDYWGQGTSVTVSSGGGGSGGGGSG

GGGSMADYKDIVMTQSHKFMSTSVGDRVNITCKASQNVDSAVAWYQ

QKPGQSPKALIYSASYRYSGVPDRFTGRGSGTDFTLTISSVQAEDL

AVYYCQQYYSTPWTFGGGTKLEIKRGLAVSTISSFFPPGYQIYIWA

PLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS

CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL

DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG

KGHDGLYQGLSTATKDTYDALHMQALPPR

Klo43-3

(SEQ ID NO. 32)

MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGGSLSLSCAASGFT

FTDYYMSWVRQPPGKALEWLALIRSKADGYTTEYSASVKGRFTLSRDDS

-continued

QSILYLQMNALRPEDSATYYCARDAAYYSYYSPEGAMDYWGQGTSVTV

SSGGGGSGGGGSGGGGSMADYKDIVMTQSHKFMSTSVGDRVNITCKASQ

NVDSAVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGRGSGTDFTLTIS

SVQAEDLAVYYCQQYYSTPWTFGGGTKLEIKRTTTPAPRPPTPAPTIAS

QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI

TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV

KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR

RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK

DTYDALHMQALPPR

[0643] In one embodiment Klon43-3 is

EVKLVESGGGLVQPGGSLSLSCAASGFTFTDYYMSWVRQPPGKALE

WLALIRSKADGYTTEYSASVKGRFTLSRDDSQSILYLQMNALRPED

SATYYCARDAAYYSYYSPEGAMDYWGQGTSVTVSSGGGGSGGGGSG

GGGSMADYKDIVMTQSHKFMSTSVGDRVNITCKASQNVDSAVAWYQ

QKPGQSPKALIYSASYRYSGVPDRFTGRGSGTDFTLTISSVQAEDL

AWVYYCQQYYSTPTFGGGTKLEIKRTTTPAPRPPTPAPTIASQPLS

LRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYC

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSR

SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP

QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD

ALHMQALPPR

Klo43-5

(SEQ ID NO. 33)

MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGGSLSLSCAASGFT

FTDYYMSWVRQPPGKALEWLALIRSKADGYTTEYSASVKGRFTLSRDDS

QSILYLQMNALRPEDSATYYCARDAAYYSYYSPEGAMDYWGQGTSVTVS

SGGGGSGGGGSGGGGSMADYKDIVMTQSHKFMSTSVGDRVNITCKASQ

-continued

NVDSAVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGRGSGTDFTLTIS

SVQAEDLAVYYCQQYYSTPWTFGGGTKLEIKREPKSPDKTHTCPPCPAPP

VAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGV

EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA

PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA

VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS

VMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRG

RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD

APAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG

LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR

[0644] In one embodiment Klon43-5 is

EVKLVESGGGLVQPGGSLSLSCAASGFTFTDYYMSWVRQPPGKALEW

LALIRSKADGYTTEYSASVKGRFTLSRDDSQSILYLQMNALRPEDS

ATYYCARDAAYYSYYSPEGAMDYWGQGTSVTVSSGGGGSGGGGSGG

GGSMADYKDIVMTQSHKFMSTSVGDRVNITCKASQNVDSAVAWYQQ

KPGQSPKALIYSASYRYSGVPDRFTGRGSGTDFTLTISSVQAEDLA

VYYCQQYYSTPWTFGGGTKLEIKREPKSPDKTHTCPPCPAPPVAGP

SVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA

PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD

IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV

FSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVIT

LYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR

VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG

KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL

STATKDTYDALHMQALPPR

[0645] The Humanized CD123 CAR of the invention comprise one of the following sequences:

Version 1 VH1
VL1
MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED

GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR

RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

-continued

VL2

MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED

GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR

RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

VL3

MALPVTALLLPLALLLHAARPEVKLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYSASVKGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED

GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR

RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

VL4

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Version 1 VH2
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VL2
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VL4
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VL5
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VL6
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Version 1 VH3
VL1
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VL2
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VL3
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VL4
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VL5
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VL6
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Version 1 VH4
VL1
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VL2
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VL3
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VL4
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VL5
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VL6
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Version 1 VH5
VL1
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VL2
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VL3
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VL4
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VL5
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VL6
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Version 1 VH6
VL1
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VL2
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DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

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VL3
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VL4
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VL5
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DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

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VL6
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Version 1 VH7
VL1
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VL2
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VL3
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YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

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VL4
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YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

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VL5
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YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

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VL6
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YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE

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Version 3 VH1
VL1
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DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDS

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VL2
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VL3
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GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL4
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VL5
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GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL6
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Version 3 VH2
VL1
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DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDS

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VL2
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VL3
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GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL4
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NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR

GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL5
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NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR

GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL6
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Version 3 VH3
VL1
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VL2
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NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR

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VL3
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GKGHDGLYQGLSTATKDTYDALHMQALPPR

VL4
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VL5
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VL6
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Version 3 VH4
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VL3
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VL4
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VL5
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VL6
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VH5
Version 3 VH5
VL1
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VL2
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VL4
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VL5
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VL6
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Version 3 VH6
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VL2
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VL3
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DGLYQGLSTATKDTYDALHMQALPPR

VL4
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VL5
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VL6
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VH7
Version 3 VH7
VL1
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VL2
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VL3
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VL4
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Version 5 VH1

VL1

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VL2

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VL3
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VL5
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VL6
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Version 5 VH2
VL1
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VL2

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VL3

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VL4

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VL6
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Version 5 VH3
VL1
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VL2
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VL3
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VL4
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Version 5 VH4

VL1

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VL2

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VL4
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VL5
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VL6
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AWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VH5
Version 5 VH5
VL1
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

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GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL2

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL3

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL4

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL5

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

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VL6
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

Version 5 VH6
VL1
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL2
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL3
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL4
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

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TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL5
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL6
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGLIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAM

DYWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSA

VAWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQ

GTKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW

YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE

PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK

SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VH7
Version 5 VH7
VL1
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIVMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ

VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL2
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKALIYSASYRYSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ

VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

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VL3
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMDYWGQGTL

VTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKA

PKALIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTH

TCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY

NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV

KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK

SLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL4
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGF

IRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMDYWGQGTL

VTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKA

PKLLIYSASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHT

CPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK

GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS

LSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL5
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKLLIYSASYRQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGT

KVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ

VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR

WQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

VL6
MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLE

WVGFIRSKADGYTTEYAASVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCTRDAAYYSYYSPEGAMD

YWGQGTLVTVSSGGGGSGGGGSGGGGSMADYKDIQMTQSPSSVSASVGDRVTITCRASQNVDSAV

AWYQQKPGKAPKLLIYSASYGQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQG

TKVEIKREPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP

QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYC

[0646] The following sequences are contemplated as preferred CAR of the invention

(SEQ ID NO 172)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYS

GVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIAS

QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR

PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR.

(SEQ ID NO 173)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKLLIYSASYRYS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIAS

QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR

PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR.

(SEQ ID NO 174)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIA

SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG

RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL

HMQALPPR.

(SEQ ID NO 175)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIA

SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG

RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL

HMQALPPR.

(SEQ ID NO 176)
EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

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SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIA

SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG

RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL

HMQALPPR.

(SEQ ID NO 177)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPDRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIA

SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG

RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL

HMQALPPR.

(SEQ ID NO 178)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIA

SQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG

RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL

HMQALPPR.

(SEQ ID NO 179)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYS

GVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKRTTTPAPRPPTPAPTIAS

QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMR

PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR

DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR.

(SEQ ID NO 180)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYS

GVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCPA

PPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC

-continued

RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 181)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKLLIYSASYRYS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCPA

PPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC

RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 182)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCP

APPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

LHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS

CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 183)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSS

GGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIY

SASYRYSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHT

CPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR

EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK

NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS

VMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE

EDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG

KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 184)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCP

APPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

-continued

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

LHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS

CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 185)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYAASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCKASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPDRFSGSGSGTDFTLTISSLQPEDLATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCP

APPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

LHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS

CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 186)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSQSIAYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIQMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRY

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCP

APPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL

TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA

LHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS

CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK

NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

(SEQ ID NO 187)

EVQLVESGGGLVQPGRSLRLSCTASGFTFTDYYMSWVRQAPGKGLEWVGLIRSKADGYTTEYSASV

KGRFTISRDDSKSILYLQMNSLKTEDTAVYYCARDAAYYSYYSPEGAMDYWGQGTLVTVSSGGGGS

GGGGSGGGGSDIVMTQSPSSVSASVGDRVTITCRASQNVDSAVAWYQQKPGKAPKALIYSASYRYS

GVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQYYSTPWTFGQGTKVEIKREPKSPDKTHTCPPCPA

PPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT

CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL

HNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC

RFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN

PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR.

[0647] According to the present invention these anti-CD123 CAR can include at least one mimotope.

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SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20180051089A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. The CD123 specific chimeric antigen receptor (CAR) comprising

an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH optionally humanized, a linker, a VL optionally humanized, and a hinge,

a transmembrane domain,

a cytoplasmic domain, and

at least one epitope specific for a monoclonal antibody (mimotope).

2. The CD123 CAR according to claim **1** comprising

an extracellular domain comprising an extra cellular ligand binding-domain comprising successively, a VH of SEQ ID NO 12, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29 or SEQ ID NO 30, optionally humanized, a linker, a VL of SEQ ID NO 11, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22 or SEQ ID NO 23, optionally humanized, and a hinge,

a transmembrane domain from CD8 alpha, and

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

3. The CD123 CAR according to claim **1**, wherein the CAR lacks a sequence having identity to human CD28 NP_006130.1.

4. The CD123 CAR according to claim **1** comprising a sequence of SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174, SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186 and SEQ ID NO 187, optionally further comprising at least one SEQ ID NO 161.

5. The CD123 CAR according to claim **1**, wherein said extracellular ligand binding-domain comprises at least one epitope specific for a monoclonal antibody (mimotope) of SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169 or SEQ ID NO 170, preferably of SEQ ID NO 161 and of SEQ ID NO 169.

6. The CD123 CAR according to claim **1** comprising a sequence of SEQ ID NO 160, SEQ ID NO 171, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 195, SEQ ID NO 196, or SEQ ID NO 197.

7. A polynucleotide encoding the CD123 specific CAR according to claim **1**.

8. An expression vector comprising the polynucleotide according to claim **7**.

9. An expression vector comprising a backbone and at least one sequence encoding the CAR according to claim **6**.

10. An expression vector comprising a backbone, an EF1 promotor, an RQR8 open reading frame (RQR8 ORF), a sequence coding any one of the CD123 CAR of claim **6**.

11. An engineered immune cell comprising a T Cell Receptor (TCR) knock-out (KO) or TCR and human deoxycytidine kinase (dCK) KO and a CD123 specific CAR according to claim **1**, wherein the CAR is expressed on the cell surface of the immune cell.

12. An engineered immune cell comprising a polynucleotide coding a CD123 specific CAR according to claim **7**.

13. The engineered immune cell according to claim **11** further expressing a suicide domain at the cell surface.

14. The engineered immune cell according to claim **11**, wherein the cell suppresses expression of at least one MHC protein.

15. A method of treating a disease or condition associated with CD123 comprising administering to a subject in need thereof the engineered immune cell according to claim **11**.

16. The method of treating according to claim **15**, wherein the disease or condition is acute myelogenous leukemia (AML), BPDNL, or during or before bone marrow transplantation.

17. The method according to claim **15**, wherein the disease or condition is a lymphoproliferative disorder, acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, myelodysplastic syndrome, or BPDNL.

18. The CD123 specific CAR according to claim **1**, wherein the linker is a linker of sequence (GGGGS)n, wherein "n" is 1-4.

19. The CD123 specific CAR according to claim **18**, wherein "n" is 3.

20. The CD123 specific CAR according to claim **5**, wherein the epitope specific for a monoclonal antibody is SEQ ID NO 161 or 169.

21. The method of treating according to claim **16**, wherein the AML is refractory AML or relapsed AML.

22. The method of treating according to claim **17**, wherein the lymphoproliferative disorder is leukemia or lymphoma.

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