(19) DANMARK

(10) **DK/EP 3126390 T3**

Patent- og Varemærkestyrelsen

(12) Oversættelse af europæisk patentskrift

(51) Int.Cl.: *C 07 K 16/28 (2006.01)* *A 61 K 39/395 (2006.01)* *A 61 P 35/02 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2020-01-20**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2019-10-16**

(86) Europæisk ansøgning nr.: **15713513.8**

(86) Europæisk indleveringsdag: **2015-04-02**

(87) Den europæiske ansøgnings publiceringsdag: **2017-02-08**

(86) International ansøgning nr.: **EP2015057331**

(87) Internationalt publikationsnr.: **WO2015150526**

(30) Prioritet: **2014-04-03 DK 201470171**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **CD33-SPECIFIKKE KIMÆRE ANTIGENRECEPTORER TIL CANCERIMMUNTERAPI**

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DESCRIPTION

Field of the invention

[0001] The present invention relates to Chimeric Antigen Receptors (CAR) that are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward CD33, a cell surface glycoprotein found on most myeloid cells and used to diagnose acute myeloid Leukemia (AML) in patients. The CARs according to the invention are particularly useful to treat malignant cells bearing CD33, when expressed in T-cells or NK cells. The resulting engineered immune cells display high level of specificity toward malignant cells, conferring safety and efficiency for immunotherapy.

Background of the invention

[0002] Adoptive immunotherapy, which involves the transfer of autologous antigen-specific T cells generated *ex vivo,* is a promising strategy to treat viral infections and cancer. The T cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T cells or redirection of T cells through genetic engineering (Park, Rosenberg et al. 2011). Transfer of viral antigen specific T cells is a well-established procedure used for the treatment of transplant associated viral infections and rare viral-related malignancies. Similarly, isolation and transfer of tumor specific T cells has been shown to be successful in treating melanoma.

[0003] Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (CARs) (Jena, Dotti et al. 2010). CARs are synthetic receptors consisting of a targeting moiety that is associated with one or more signaling domains in a single fusion molecule. In general, the binding moiety of a CAR consists of an antigen-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, as well as transmembrane and hinge domains have been added to form CARs of second and third generations, leading to some successful therapeutic trials in humans, where T-cells could be redirected against malignant cells expressing CD19 (June et al., 2011). However, the particular combination of signaling domains, transmembrane and co-stimulatory domains used with respect to CD19 ScFv, was rather antigen-specific and cannot be expanded to any antigen markers.

[0004] Acute myeloid leukaemia (AML) is the second most common acute leukaemia with approximately 13,300 new cases per year in the United States and 8,800 annual deaths. The commonly applied therapy of leukaemic diseases includes irradiation and/or chemotherapy. Furthermore, under certain circumstances, the additional possibility of bone marrow transplantation is regarded suitable. However, these therapies are relatively toxic to the patient and very often do not lead to a complete cure from the disease. Thus, although a complete remission can be achieved for 65-80% of patients receiving chemotherapy, most of these patients relapse (Cros et al., 2004) because the cells that survived the chemotherapy are enriched in AML leukaemia stem cells (AML-LSCs), and constitute a particularly dangerous reservoir of cells capable of re-expanding and causing a relapse. Leukaemia stem cells have been particularly well characterized for acute myeloid leukaemia. AML-LSCs express a characteristic set of cell-surface antigens including among others CD33. Patients older than 60 years have a poor prognosis with only 10% to 15% of 4-year disease-free survival (Gardin et al., 2007). This high relapse rate for AML patients and the poor prognosis for older patients highlight the urgent need for novel therapeutics preferentially targeting $CD33^+$ cells.

[0005] CD33 (Sialic acid-binding Ig-like lectin 3) or SIGLEC3, referred to as P20138 under the UniProtKB/Swiss-

Prot protein database, is a transmembrane receptor expressed on cells of myeloid lineage. It is usually considered myeloid-specific, but it can also be found on some lymphoid cells. It binds sialic acids, therefore is a member of the SIGLEC family of lectins.

[0006] In the past, different approaches have been used to develop unconjugated monoclonal antibodies with antitumor activity against CD33. However these attempts failed to address malignant cells specifically.

[0007] In 2000 Gemtuzumabozogamicin (Mylotarg™, GO), a calicheamicinconjugated humanized anti-CD33 monoclonal antibody, was approved by the American Food and Drug Administration (FDA) for treating patients older than 60 years with refractory or relapsed AML. However this was withdrawn from the market on June 21, 2010; GO consisted of a humanized anti-CD33 IgG-antibody, chemically coupled to the cytotoxic agent calicheamicin. Post-approval study (SWOG S0106) raised concerns about the product's safety, while other clinical trials (British MRC AML-15 and the HOVON-43 trials) failed to demonstrate any clinical benefit (Maniecki et al., 2011). Side effects were found to include hepatic veno-occlusive disease, pulmonary toxicity and severe hypersensitivity reactions, whereas in vitro studies revealed antigen-independent cytotoxicities towards CD33 negative cell lines (Schwemmlein ef al, 2006).

[0008] More recently, tri-specific polypeptide molecules combining immunoglobulin domains from CD123, CD16 and CD33 antibodies were proposed (WO2011/070109) to obviate the specificity issues previously encountered with therapeutics targeting CD33.

[0009] As an alternative to the previous strategies, the present invention provides with CD33 specific CARs, which can be expressed in immune cells to target CD33+ malignant cells with significant clinical advantage.

Summary of the invention

[0010] The inventors have generated CD33 specific CAR having different structure and comprising different scFV derived from different CD33 specific antibodies. CAR polypeptides as described comprise an amino acid sequence selected from SEQ ID NO.19 to 42. More preferred CAR polypeptides of the invention comprise an amino acid sequence of SEQ ID NO. 68, or with at least 80% identity with SEQ ID NO. 68. Following non-specific activation in vitro (e.g. with anti CD3/CD28 coated beads and recombinant IL2), T-cells from donors have been transformed with polynucleotides expressing these CARs using viral transduction. In certain instances, the T-cells were further engineered to create non-alloreactive T-cells, more especially by disruption of a component of TCR (αβ -T-Cell receptors) to prevent Graft versus host reaction.

[0011] The resulting engineered T-cells displayed reactivity in-vitro against CD33 positive cells to various extend, showing that the CARs of the present invention contribute to antigen dependent activation, and also proliferation, of the T-cells, making them useful for immunotherapy.

[0012] The polypeptides and polynucleotide sequences encoding the CARs of the present invention are detailed in the present specification.

[0013] The engineered immune cells of the present invention are particularly useful for therapeutic applications such as B-cell lymphoma or leukemia treatments.

Brief description of the figures

[0014]

Figure 1: Schematic representation of an engineered immune cell according as described. The engineered

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

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

Figure 3: Schematic representation of different CAR with a V1, V3 or V5 structure.

Figure 4: Degranulation activity (% CD8/CD107a+ cells and mean fluorescence intensity (MFI) CD107a in CD8+ cells) of the M195 scFv with the three architectures (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD33 expressing cells (U937), or with cells that do not express CD33 (Jeko). The data is presented as a percentage (%) of degranulation and mean fluorescence intensity (MFI).

Figure 5: Degranulation activity (% CD8/CD107a+ cells and MFI CD107a in CD8+ cells) of the m2H12 and My9.6 scFvs with the three architectures (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD33 expressing cells (U937), or with cells that do not express CD33 (Jeko). The data is presented as a percentage (%) of degranulation and mean fluorescence intensity (MFI).

Figure 6: Degranulation activity (% CD8/CD107a+ cells) of 7 constructions : M195-V1, M195-V3, M195-V5, m2H12-V1, m2H12-V3 and My9.6-V1 and My9.6-V3 (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with cells expressing high or intermediate levels of CD33 (U937 and K562, respectively), or with cells that do not express CD33 (Jeko-1). The data is presented as a percentage (%) of degranulation.

Figure 7: Amount of IFN gamma released by anti-CD33 CAR expressing T-cells when co-cultured for 24h with cells expressing different levels of CD33 (U937 and K562), or with cells that do not express CD33 (Jeko-1). IFN gamma release from T-cells cultured alone, in the same conditions that the cocultures, is also shown. The experiments were done using three independent donors, and results from one representative donor are shown here.

Figure 8: Specific cytolytic activity of anti-CD33 CAR expressing-T cells. Assays were done 48h after CAR mRNA transfection. T-cells were co-cultured with U937+Jeko or K562+Jeko cells for 4 hours. Cellular viability for each of the cell lines was determined at the end of the co-cultured and a specific cell lysis percentage was calculated.

Table 1: Sequence of the different CAR components

Functional domains	SEQ ID #	Raw 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	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACD
IgG1 hinge	SEQ ID NO.5	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDT LMIARTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Functional domains	SEQ ID #	Raw amino acid sequence
CD8α transmembrane domain	SEQ ID NO.6	IYIWAPLAGTCGVLLLSLVITLYC
41BB transmembrane domain	SEQ ID NO.7	IISFFLALTSTALLFLLFFLTLRFSVV
41BB intracellular domain	SEQ ID NO.8	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL
CD3ζ intracellular domain	SEQ ID NO.9	RVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
Linker	SEQ ID NO.10	GGGGSGGGGSGGGGS

Table 2: Sequence of the different CAR components

ScFv sequences	SEQ ID #	Raw amino acid sequence
M195 heavy chain variable region	SEQ ID NO.11	EVQLQQSGPELVKPGASVKISCKASGYTFTDYN MHWVKQSHGKSLEWIGYIYPYNGGTGYNQKF KSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCA RGRPAMDYWGQGTSVTVS
M195 light chain variable region	SEQ ID NO.12	DIVLTQSPASLAVSLGQRATISCRASESVDNYGI SFMNWFQQKPGQPPKLLIYAASNQGSGVPAR FSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKE VPWTFGGGTKLEIK
M2H12 heavy chain variable region	SEQ ID NO.13	QVQLQQSGPELVRPGTFVKISCKASGYTFTNYD INWVNQRPGQGLEWIGWIYPGDGSTKYNEKF
		KAKATLTADKSSSTAYLQLNNLTSENSAVYFCAS GYEDAMDYWGQGTSVTVSS
M2H12 light chain variable region	SEQ ID NO.14	DIKMTQSPSSMYASLGERVIINCKASQDINSYLS WFQQKPGKSPKTLIYRANRLVDGVPSRFSGSG SGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGA GTKLELKR
DRB2heavy chain variable region	SEQ ID NO.15	EVKLQESGPELVKPGASVKMSCKASGYKFTDYV VHWLKQKPGQGLEWIGYINPYNDGTKYNEKF KGKATLTSDKSSSTAYMEVSSLTSEDSAVYYCA RDYRYEVYGMDYWGQGTSVTVSS
DRB2 light chain variable region	SEQ ID NO.16	DIVLTQSPTIMSASPGERVTMTCTASSSVNYIH WYQQKSGDSPLRWIFDTSKVASGVPARFSGSG SGTSYSLTISTMEAEDAATYYCQQWRSYPLTFG DGTRLELKRADAAPTVS
My9-6 heavy chain variable region	SEQ ID NO.17	QVQLQQPGAEVVKPGASVKMSCKASGYTFTS YYIHWIKQTPGQGLEWVGVIYPGNDDISYNQK FKGKATLTADKSSTTAYMQLSSLTSEDSAVYYC AREVRLRYFDVWGAGTTVTVSS

ScFv sequences	SEQ ID #	Raw amino acid sequence
My9-6 light chain variable region	SEQ ID NO.18	NIMLTQSPSSLAVSAGEKVTMSCKSSQSVFFSS SQKNYLAWYQQIPGQSPKLLIYWASTRESGVP DRFTGSGSGTDFTLTISSVQSEDLAIYYCHQYLS SRTFGGGTKLEIKR

Table 3: CAR of structure V-1

CAR Designation	CAR Structure						
V-1	signal peptide (optional)	VH	VL	FcγRIIIα hinge	CD8α TM	41BB - IC	CD3ζ CD
M195-1 (SEQ ID NO.19)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
M2H12-1 (SEQ ID NO.25)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
DRB2-1 (SEQ ID NO.31)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
My9-6-1 (SEQ ID NO.37)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.3	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 4: CAR of structure V-2

CAR Designation	CAR Structure						
V-2	signal peptide (optional)	VH	VL	FcγRIIIα hinge	41BB-TM	41BB - IC	CD3ζ CD
M195-2 (SEQ ID NO.20)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
M2H12-2 (SEQ ID NO.26)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
DRB2-2 (SEQ ID NO.32)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
My9-6-2 (SEQ ID NO.38)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.3	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Table 5: CAR of structure V-3

CAR Designation	CAR Structure						
V-3	signal peptide (optional)	VH	VL	CD8α hinge	CD8α TM	41BB - IC	CD3ζCD
M195-3 (SEQ ID NO.21)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
M2H12-3 (SEQ ID NO.27)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
DRB2-3 (SEQ ID NO.33)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
My9-6-3 (SEQ ID NO. 39)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.4	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 6: CAR of structure V-4

CAR Designation	CAR Structure						
V-4	signal peptide (optional)	VH	VL	CD8α hinge	41BB-TM	41BB - IC	CD3ζ CD
M195-4 (SEQ ID NO.22)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
M2H12-4 (SEQ ID NO.28)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

CAR Designation	CAR Structure						
V-4	signal peptide (optional)	VH	VL	CD8α hinge	41BB-TM	41BB - IC	CD3ζ CD
DRB2-4 (SEQ ID NO.34)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
My9-6-5 (SEQ ID NO.40)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.4	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Table 7: CAR of structure V-5

CAR Designation	CAR Structure						
V-5	signal peptide (optional)	VH	VL	IgG1 hinge	CD8 TM	41BB - IC	CD3 CD
M195-5 (SEQ ID NO.23)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
M2H12-5 (SEQ ID NO.29)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
DRB2-5 (SEQ ID NO.35)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9
My9-6-5 (SEQ ID NO.41)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.5	SEQ ID NO.6	SEQ ID NO.8	SEQ ID NO.9

Table 8: CAR of structure V-6

CAR Designation	CAR Structure						
V-6	signal peptide (optional)	VH	VL	IgG1 hinge	41BB-TM	41BB -IC	CD3ζ CD
M195-6 (SEQ ID NO.24)	SEQ ID NO.1	SEQ ID NO.11	SEQ ID NO.12	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
M2H12-6 (SEQ ID NO.30)	SEQ ID NO.1	SEQ ID NO.13	SEQ ID NO.14	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
DRB2-6 (SEQ ID NO.36)	SEQ ID NO.1	SEQ ID NO.15	SEQ ID NO.16	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9
My9-6-6 (SEQ ID NO.42)	SEQ ID NO.1	SEQ ID NO.17	SEQ ID NO.18	SEQ ID NO.5	SEQ ID NO.7	SEQ ID NO.8	SEQ ID NO.9

Detailed description of the invention

[0015] Unless specifically defined herein, all technical and scientific terms used have the same meaning as commonly understood by a skilled artisan in the fields of gene therapy, biochemistry, genetics, and molecular biology.

[0016] The materials, methods, and examples are illustrative only and are not intended to be limiting, unless otherwise specified.

[0017] 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.

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[0018] The present invention provides a CD33 specific chimeric antigen receptor (CAR) having a polypeptide structure selected from V1 and V3 as illustrated in Figure 2, wherein said structure V1 comprises:

1. (a) an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody,
2. (b) a FcRIIIα hinge,
3. (c) a CD8α transmembrane domain and
4. (d) a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB; and

wherein said structure V3 comprises:

1. (a) an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody,
2. (b) a CD8α hinge,
3. (c) a CD8α transmembrane domain and
4. (d) a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB .

[0019] In a preferred embodiment, the present invention provides a CD33 specific CAR as above wherein said structure V3 comprises a CD8α hinge and a CD8α transmembrane domain.

[0020] The present invention provides a CD33 specific CAR as above, wherein said CD8α hinge has at least 80 % identity with SEQ ID NO.4.

[0021] The present invention provides a CD33 specific CAR as above, which comprises a polypeptide sequence having at least 80 % identity with SEQ ID NO. 27.

[0022] In an embodiment, the present invention provides a CD33 specific CAR as above wherein said structure V1 comprises a FcγRIIIα hinge and a CD8α transmembrane domain.

[0023] The present invention provides a CD33 specific CAR as above, wherein said FcγRIIIα hinge has at least 80 % identity with SEQ ID NO.3.

[0024] The present invention provides a CD33 specific CAR as above, which comprises a polypeptide sequence having at least 80 % identity with SEQ ID NO. 19 or SEQ ID NO.21.

[0025] The present invention provides a CD33 specific CAR as above, wherein said VH has at least 80 % identity with a polypeptide sequence selected from SEQ ID NO. 11, SEQ ID NO. 13, SEQ ID NO. 15 and SEQ ID NO. 17 and said VL has at least 80 % identity with a polypeptide sequence selected from SEQ ID NO. 12, SEQ ID NO. 14, SEQ ID NO. 16 and SEQ ID NO. 18.

[0026] The present invention provides a CD33 specific CAR as above, wherein co-stimulatory domain from 4-1BB has at least 80 % identity with SEQ ID NO.8.

[0027] The present invention provides a CD33 specific CAR as above, wherein said CD3 zeta signaling domain has at least 80 % identity with SEQ ID NO.9.

[0028] The present invention provides a CD33 specific CAR as above, wherein said CD8α transmembrane domain has at least 80 % identity with SEQ ID NO.6.

[0029] The present invention provides a CD33 specific CAR as above, further comprising a signal peptide.

[0030] The present invention provides a CD33 specific CAR as above, wherein said signal peptide has at least 80% sequence identity with SEQ ID NO.1 or SEQ ID NO.2.

[0031] In one embodiment the present invention provides a CD33 specific CAR as above, which comprises a polypeptide sequence having at least 80 % identity with SEQ ID NO. 48, SEQ ID NO. 50, SEQ ID NO. 54, SEQ ID NO. 56, SEQ ID NO. 66 or SEQ ID NO. 68, preferably at least 80 % identity with SEQ ID NO. 66 or SEQ ID NO. 68.

[0032] The present invention provides a CD33 specific CAR as above further comprising another extracellular ligand binding domain which is not specific for CD33.

[0033] In one aspect the present invention provides a polynucleotide encoding a CAR according to any one of the embodiments above.

[0034] In one aspect the present invention provides an expression vector comprising a polynucleotide as above.

[0035] In one aspect, the present invention provides an engineered immune cell expressing at the cell surface membrane a CD33 specific CAR according to any one of the above.

[0036] The present invention provides an engineered immune cell as above, derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. The present invention provides an engineered immune cell as above, wherein expression of TCR is suppressed.

[0037] The present invention provides an engineered immune cell as above, wherein said engineered immune cell is modified to be resistant to at least one immune suppressive or chemotherapy drug.

[0038] The present invention provides an engineered immune cell as above, for use in therapy.

[0039] The present invention provides an engineered immune cell for use in therapy as above, wherein the patient is a human.

[0040] The present invention provides an engineered immune cell for use in therapy as above, wherein the condition is a pre-malignant or malignant cancer condition characterized by CD33-expressing cells.

[0041] The present invention provides an engineered immune cell for use in therapy as above, wherein the condition is a condition which is characterized by an overabundance of CD33-expressing cells.

[0042] The present invention provides an engineered immune cell for use in therapy as above wherein the condition is a hematological cancer condition.

[0043] The present invention provides an engineered immune cell for use in therapy as above, wherein the haematological cancer condition is leukemia.

[0044] The present invention provides an engineered immune cell for use in therapy as above, wherein said leukemia is selected from the group consisting of acute myelogenous leukemia (AML), chronic myelogenous

leukemia, melodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

[0045] The present invention provides an engineered immune cell for use in therapy as above, wherein said leukemia is acute myelogenous leukemia (AML).

[0046] The present invention provides an engineered immune cell for use in therapy as above, wherein said hematological cancer is a malignant lymphoproliferative disorder.

[0047] The present invention provides an engineered immune cell for use in therapy as above, wherein said malignant lymphoproliferative disorder is a lymphoma.

[0048] The present invention provides 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).

[0049] In one aspect, the present invention provides a method of impairing a hematologic cancer cell comprising contacting said hematologic cancer cell with an engineered immune cell as above in an amount effective to cause impairment of said cancer cell.

[0050] The present disclosure provides a method of engineering an immune cell comprising:

1. (a) Providing an immune cell,
2. (b) Expressing at the surface of said cell at least one CD33 specific chimeric antigen receptor as described above.

[0051] The present disclosure provides a method of engineering an immune cell as above comprising:

1. (a) Providing an immune cell,
2. (b) Introducing into said cell at least one polynucleotide encoding said CD33 specific chimeric antigen receptor, as above
3. (c) Expressing said polynucleotide into said cell, optionally expressing said CD33 specific chimeric antigen receptor.
   The present invention provides a method of engineering an immune cell as above further comprising:
4. (d) Inhibiting TRC expression and /or CD33 expression.

[0052] In one embodiment, the immune cell provided for the step (a) of the method described above is an immune cell wherein the expression of TRC and/or CD33 at the cell surface is inhibited, and optionally resistant to at least one drug used to treat a cancer, in particular AML.

[0053] The present disclosure provides a method of engineering an immune cell as above further comprising:

1. (a) Introducing at least one other chimeric antigen receptor which is not specific for CD33.

[0054] The present invention also provides a method of treating a subject in need thereof comprising:

1. (a) Providing an immune cell expressing at the surface a CD33 specific Chimeric Antigen Receptor according to any one of the above;
2. (b) Administrating said immune cells to said patient.

[0055] The present disclosure provides a method as above, wherein said immune cell is provided from a donor.

[0056] The present invention provides a method of treating a subject in need thereof as above wherein said immune cell (to be engineered according to the invention) is provided from the patient himself.

CD33 specific Chimeric Antigen Receptors

[0057] The present invention relates to new designs of anti-CD33 chimeric antigen receptor (CAR or CD33 CAR or CD33 specific CAR or anti-CD33 CAR) comprising an extracellular ligand-binding domain, a transmembrane domain and a signaling transducing domain.

[0058] More precisely, the present invention relates to new CD33 specific CAR comprising an extra cellular ligand binding-domain comprising a VH and a VL from a monoclonal anti-CD33 antibody, a hinge selected from FcRIIIα hinge and CD8alpha hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0059] The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising the light (V_L) and the heavy (V_H) variable fragment of a target antigen specific monoclonal anti CD-33 antibody joined by a flexible linker. Said V_L and V_H are preferably selected from the antibodies referred to as M195, m2H12, DRB2 and My9-6 as indicated in Table 2.

[0060] In an even more preferred embodiment said V_L and V_H comprises SEQ ID NO 17 and 18, optionally humanized.

[0061] They are preferably linked together by a flexible linker comprising for instance the sequence SEQ ID NO.10. In other words, said CARs preferentially comprise an extracellular ligand-binding domain comprising a polypeptide sequence displaying at least 90 %, 95 %,97 % or 99 %identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 11 to SEQ ID NO: 18.

[0062] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide of SEQ ID NO: 11 and SEQ ID NO: 12.

[0063] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide of SEQ ID NO: 13 and SEQ ID NO: 14.

[0064] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide of SEQ ID NO: 15 and SEQ ID NO: 16.

[0065] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide of SEQ ID NO: 17 and SEQ ID NO: 18.

[0066] 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.

[0067] As used herein, the term "conservative sequence modifications" or "humanization" is intended to refer to amino acid modifications that do not significantly affect or alter the characteristics of the CAR (as compared to that of a CAR constructed using the original anti-CD33) and/or that do not significantly affect the activity of the CAR containing the modified amino acid sequence and reduce or abolish a human antimouse antibody (HAMA) response. 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. Accordingly, the present invention provides a (humanized) CD33 CAR, wherein VH has at least 80% identity with SEQ ID NO 11, SEQ ID NO13, SEQ ID NO15, or SEQ ID NO17 and VL has at least 80% identity with SEQ ID NO 12, SEQ ID NO14, SEQ ID NO16, or SEQ ID NO18.

[0068] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide sequence 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 an amino acid sequence of SEQ ID NO: 11 and SEQ ID NO 12.

[0069] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide sequence 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 an amino acid sequence of SEQ ID NO 13 and SEQ ID NO 14.

[0070] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide sequence 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 an amino acid sequence of SEQ ID NO 15 and SEQ ID NO 16.

[0071] In one embodiment, said CAR of the invention preferentially comprises an extracellular ligand-biding domain comprising a polypeptide sequence 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 an amino acid sequence of SEQ ID NO 17 and SEQ ID NO 18.

[0072] 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 33 using the functional assays described herein.

[0073] 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.

[0074] 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 description can include as non limiting examples those derived from TCRzeta, FcRgamma, FcRbeta, FcRepsilon, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d. According to the invention, 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 % - 99 % or 100 % sequence identity with amino acid sequence selected from the group consisting of (SEQ ID NO: 9).

[0075] 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, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[0076] A "co-stimulatory molecule" refers to the cognate binding partner on a T-cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

[0077] The signal transduction domain of the CAR as described comprises a part of co-stimulatory signal molecule selected from the group consisting of fragment of 4-1BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In particular the signal transduction domain of the CAR of the present invention comprises amino acid sequence which comprises at least 70%, preferably at least 80%, more preferably at least 90 %, 95 % 97 % 699 % or 100% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8.

[0078] A CAR according to the present invention is expressed on the surface membrane of the cell. Thus, such CAR further comprises a CDα transmembrane domain. In a preferred embodiment said transmembrane domain is derived from the human CD8 alpha chain (e.g. NP_001139345.1) The transmembrane domain further 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. In a preferred embodiment said hinge

domain comprises a part of human FcγRIIIα receptor or CD8 alpha chain respectively referred to in this specification as SEQ ID NO. 3 and SEQ ID NO. 4, or hinge polypeptides which display preferably at least 80%, more preferably at least 90 %, 95 % 97 % 99 % or 100% sequence identity with these polypeptides.

[0079] A CAR according to the invention generally comprises a transmembrane domain (TM) from CD8α showing 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide of SEQ ID NO. 6. In a preferred embodiment, a CAR according to the invention generally further comprises a transmembrane domain (TM) from CD8α showing 100% identity with the polypeptide of SEQ ID NO. 6.

[0080] 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 CD33 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. The present invention also relates to an isolated immune cell which comprises a population of CARs each one comprising different extracellular ligand binding domains.

[0081] The present invention provides a CD33 specific CAR having one of the polypeptide structure selected from V1 and V3 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, a hinge selected from FcRIIIα hinge and CD8α hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0082] The present invention provides a CD33 CAR comprising a polypeptide sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 66 and SEQ ID NO: 68, preferably said CAR comprises a polypeptide sequence selected from SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68 and more preferably, said CAR preferentially comprises a polypeptide sequence selected from SEQ ID NO: 66 and SEQ ID NO: 68, and even more preferably, said CAR preferentially comprises a polypeptide of SEQ ID NO: 68.

[0083] The present invention also provides :

- A CD33 specific chimeric antigen receptor (CAR) comprising a polypeptide sequence 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 an amino acid sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 66 and SEQ ID NO: 68,

more preferably, a CAR comprising a polypeptide sequence displaying 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 an amino acid sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56,

SEQ ID NO: 66 and SEQ ID NO: 68,
even more preferably, a CARs comprising a polypeptide sequence displaying 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 an amino acid sequence selected from the group consisting of SEQ ID NO: 66 and SEQ ID NO: 68.

[0084] Preferably, the present invention provides a CD33 specific CAR having a structure V1 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, a FcRIIIα hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0085] Preferably, the present invention provides a CD33 specific CAR having the polypeptide structure V1 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody selected from M195, m2h12, and My9.6, optionally humanized, a FcRIIIα hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0086] More preferably, the present invention provides a CD33 specific chimeric antigen receptor (CAR) having the polypeptide structure V1 as illustrated in Figure 2, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 54, SEQ ID NO: 60, and SEQ ID NO: 66, more preferably said CAR preferentially comprises a polypeptide sequence comprising and amino acid sequences consisting of SEQ ID NO: 48, SEQ ID NO: 54, and SEQ ID NO: 66.

[0087] Preferably, the present invention provides a CD33 specific chimeric antigen receptor (CAR) having a structure V3 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, CD8α hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0088] Preferably, the present invention provides a CD33 specific CAR having a structure V3 as illustrated in Figure 2, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody selected from M195, m2h12, DRB2, and My9.6, optionally humanized, a FcRIIIα hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0089] More preferably, the present invention provides a CD33 specific CAR having a structure V3 as illustrated in Figure 2, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 50, SEQ ID NO: 56, SEQ ID NO: 62, and SEQ ID NO: 68, more preferably said CAR preferentially comprises a polypeptide sequence comprising and amino acid sequences consisting of SEQ ID NO: 50, SEQ ID NO: 56, and SEQ ID NO: 68.

[0090] In one embodiment, the present invention provides a CD33 specific chimeric antigen receptor comprising:

- a optional signal peptide having an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO. 1 or 2; Preferably the optional signal peptide has an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO 1. Preferably, the signal peptide is present.
- a VH domain separated to a VL domain by a linker, said VH and VL contributing to the binding to CD33;
- a Hinge derived from Fcgamma (γ) RIIIalpha (α) having an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO. 3;
- a transmembrane domain derived from CD8alpha(α) having an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide of SEQ ID NO. 6;
- a co-stimulatory signal molecule derived from 4-1BB having an amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90 %, 95 % 97 %, 99 % or 100% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8;

- an intracellular signaling domain comprising the CD3zeta signaling domain having an amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97%, 99 % or 100 % sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 9;

[0091] In one embodiment, the present invention provides a CD33 specific chimeric antigen receptor comprising:

- a optional signal peptide having an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO. 1 or 2; Preferably the optional signal peptide has an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO 1. Preferably, the signal peptide is present.
- a VH domain separated to a VL domain by a linker, said VH and VL contributing to the binding to CD33;
- a Hinge derived from human CD8 alpha chain having an amino acid sequence with at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with the polypeptide of SEQ ID NO. 4;
- a transmembrane domain derived from CD8alpha(α) having an amino acid sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide of SEQ ID NO. 6;
- a co-stimulatory signal molecule derived from 4-1BB having an amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90 %, 95 % 97 %, 99 % or 100% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8;
- an intracellular signaling domain comprising the CD3zeta signaling domain having an amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97%, 99 % or 100 % sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 9;

[0092] In one embodiment, the present invention provides at least one of the following CD33 specific CAR having the following sequences - or 80% of the following sequences:

M195-1

SEQ ID NO 48:

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQSHGKSLEWIGY
IYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRPAMDYWGQGTSVTVSSGG
GGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAA
SNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKGLAVSTISSFFPPGY
QIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
HDGLYQGLSTATKDTYDALHMQALPPR

M195-3

SEQ ID NO 50:

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQSHGKSLEWIGY
IYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRPAMDYWGQGTSVTVSSGG
GGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESVDNYGISFMNWFQQKPGQPPKLLIYAA
SNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIKTTTPAPRPPTPAPTI
ASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQE
EDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

m2H12-1

SEQ ID NO 54:

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRPGQGLEWIGW
IYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYEDAMDYWGQGTSVTVSSG
GGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVIINCKASQDINSYLSWFQQKPGKSPKTLIYRANRL
VDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELKRGLAVSTISSFFPPGYQIY
IWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ
QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD
GLYQGLSTATKDTYDALHMQALPPR

m2H12-3
SEQ ID NO 56:

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRPGQGLEWIGW
IYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYEDAMDYWGQGTSVTVSSG
GGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVIINCKASQDINSYLSWFQQKPGKSPKTLIYRANRL
VDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELKRTTTPAPRPPTPAPTIAS
QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED
GCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY
NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

DRB2-1
SEQ ID NO 60:

MALPVTALLLPLALLLHAARPEVKLQESGPELVKPGASVKMSCKASGYKFTDYVVHWLKQKPGQGLEWIGY
INPYNDGTKYNEKFKGKATLTSDKSSSTAYMEVSSLTSEDSAVYYCARDYRYEVYGMDYWGQGTSVTV
SSGGGGSGGGGSGGGGSDIVLTQSPTIMSASPGERVTMTCTASSSVNYIHWYQQKSGDSPLRWIFDTS
KVASGVPARFSGSGSGTSYSLTISTMEAEDAATYYCQQWRSYPLTFGDGTRLELKRADAAPTVSGLAVS
TISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

DRB2-3
SEQ ID NO 62:

MALPVTALLLPLALLLHAARPEVKLQESGPELVKPGASVKMSCKASGYKFTDYVVHWLKQKPGQGLEWIGY
INPYNDGTKYNEKFKGKATLTSDKSSSTAYMEVSSLTSEDSAVYYCARDYRYEVYGMDYWGQGTSVTV
SSGGGGSGGGGSGGGGSDIVLTQSPTIMSASPGERVTMTCTASSSVNYIHWYQQKSGDSPLRWIFDTS
KVASGVPARFSGSGSGTSYSLTISTMEAEDAATYYCQQWRSYPLTFGDGTRLELKRADAAPTVSTTTPA
PRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPF
MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

My9.6-1
SEQ ID NO 66:

MALPVTALLLPLALLLHAARPQVQLQQPGAEVVKPGASVKMSCKASGYTFTSYYIHWIKQTPGQGLEWVGV
IYPGNDDISYNQKFKGKATLTADKSSTTAYMQLSSLTSEDSAVYYCAREVRLRYFDVWGAGTTVTVSS
GGGGSGGGGSGGGGSNIMLTQSPSSLAVSAGEKVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQSPKLL
IYWASTRESGVPDRFTGSGSGTDFTLTISSVQSEDLAIYYCHQYLSSRTFGGGTKLEIKRGLAVSTISSF
FPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
RRGKGHDGLYQGLSTATKDTYDALHMQALPPR

My9.6-3
SEQ ID NO 68:

MALPVTALLLPLALLLHAARPQVQLQQPGAEVVKPGASVKMSCKASGYTFTSYYIHWIKQTPGQGLEWVGV
IYPGNDDISYNQKFKGKATLTADKSSTTAYMQLSSLTSEDSAVYYCAREVRLRYFDVWGAGTTVTVSS
GGGGSGGGGSGGGGSNIMLTQSPSSLAVSAGEKVTMSCKSSQSVFFSSSQKNYLAWYQQIPGQSPKLL
IYWASTRESGVPDRFTGSGSGTDFTLTISSVQSEDLAIYYCHQYLSSRTFGGGTKLEIKRTTTPAPRPPT
PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQ
TTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

[0093] In one embodiment, the present invention provides primary T cells endowed with at least one of these CD33 specific CAR.

[0094] In a preferred embodiment, the present invention provides a CD33 specific chimeric antigen receptor (CAR) comprising a polypeptide sequence displaying 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 the amino acid sequence of SEQ ID NO:68.

[0095] In this embodiment, said CAR preferentially comprises a polypeptide sequence displaying 81% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 82% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 83% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 84% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 85% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 86% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 87% identity with the amino acid sequence of SEQ ID NO:68,
said CARs preferentially comprises a polypeptide sequence displaying 88% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 89% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 90% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 91% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 92% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 93% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 94% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 95% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 96% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 97% identity with the amino acid sequence of SEQ ID NO:68
said CAR preferentially comprises a polypeptide sequence displaying 98% identity with the amino acid sequence of SEQ ID NO:68,
said CAR preferentially comprises a polypeptide sequence displaying 99% identity with the amino acid sequence of SEQ ID NO:68,
In one embodiment, said CAR consists of SEQ ID NO:68.

[0096] The present invention provides a CD33 specific CAR comprising

1. (a) an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, optionally humanized
2. (b) a CD8α hinge,
3. (c) a CD8α transmembrane domain and
4. (d) a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0097] The present invention provides a CD33 specific CAR comprising

1. (a) an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, optionally humanized

2. (b) a FcγRIIIα hinge,
3. (c) a CD8α transmembrane domain and
4. (d) a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB

Polynucleotides, vectors:

[0098] Described are polynucleotides, vectors encoding any one of the above described CARs according to the invention.

[0099] The present invention also relates to a polynucleotide encoding an anti-CD33 CAR according to the invention. Also described is a vector, preferably to a lentiviral vector, comprising at least one of said polynucleotide encoding an anti-CD33 CAR according to the invention.

[0100] 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).

[0101] 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.

[0102] 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. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830). In a preferred embodiment the signal peptide comprises the amino acid sequence SEQ ID NO: 1 and 2.

[0103] 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.

Methods of engineering immune cells endowed with CARs:

[0104] The present disclosure encompasses the method of preparing immune cells for immunotherapy

comprising introducing *ex-vivo* into said immune cells the polynucleotides or vectors encoding one of the CD33 CAR as previously described.

[0105] In a preferred embodiment, said polynucleotides are included in lentiviral vectors in view of being stably expressed in the immune cells.

[0106] According to further embodiments, said method further comprises the step of genetically modifying said cell to make them more suitable for allogeneic transplantation.

[0107] 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.

[0108] According to another aspect of the description, at least one gene allowing the expression of one or more component of CD33 is inactivated in the CD33 specific CAR immune cells. Inhibiting CD33 surface expression in cells expressing an anti-CD33 CAR is part of a method for preparing the anti-CD33 CAR expressing immune cells as described. A general method is described in WO 2013/176915. Inactivation of CD33 gene can be combined with the activation/or inactivation of a gene encoding or regulating CD33 expression such as sialic acid binding Ig-like lectins (Cao H, Crocker PR. Evolution of CD33-related siglecs: regulating host immune functions and escaping pathogen exploitation? Immunology. 2011 Jan;132(1):18-26) so that cell surface expression of CD33 the anti-CD33 CAR expressing immune cells is inhibited and consequently do not alter the survival or inhibit the activity the anti-CD33 CAR expressing neighboring cells.

[0109] According to another aspect, the immune cells can be further genetically engineered to improve their resistance to immunosuppressive drugs or chemotherapy treatments, which are used as standard care for treating CD33 positive malignant cells. For instance, CD52 and glucocorticoid receptors (GR), which are drug targets of Campath (alemtuzumab) and glucocorticoids treatments, can be inactivated to make the cells resistant to these treatments and give them a competitive advantage over patient's own T-cells not endowed with specific CD33 CARs. Expression of CD3 gene can also be suppressed or reduced to confer resistance to Teplizumab, which is another immune suppressive drug. Expression of HPRT can also be suppressed or reduced according to the invention to confer resistance to 6- thioguanine, a cytostatic agent commonly used in chemotherapy especially for the treatment of acute lymphoblasic leukemia. Expression of the "GLI1" gene may be reduced.

[0110] According to further aspect of the invention, the immune cells can be further manipulated to make them more active or limit exhaustion, by inactivating genes encoding proteins that act as "immune checkpoints" that act as regulators of T-cells activation, such as PDCD1 or CTLA-4. Examples of genes, which expression could be reduced or suppressed are indicated in Table 9.

Table 9: List of genes encoding immune checkpoint proteins.

Pathway		Genes that can be inactivated In the pathway
Co-inhibitory receptors	CTLA4 (CD152)	CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22
	PDCD1 (PD-1, CD279)	PDCD1
	CD223 (lag3)	LAG3
	HAVCR2 (tim3)	HAVCR2
	BTLA(cd272)	BTLA
	CD160(by55)	CD160
	IgSF family	TIGIT
		CD96
		CRTAM
	LAIR1(cd305)	LAIR1

Pathway		Genes that can be inactivated In the pathway
	SIGLECs	SIGLEC7
		SIGLEC3
		SIGLEC9
	CD244(2b4)	CD244
Death receptors	TRAIL	TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7
	FAS	FADD, FAS
Cytokine signalling	TGF-beta signaling	TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1
	IL10 signalling	IL10RA, IL10RB, HMOX2
	IL6 signalling	IL6R, IL6ST
Prevention of TCR signalling		CSK, PAG1
		SIT1
Induced Treg	induced Treg	FOXP3
Transcription factors controlling exhaustion	transcription factors controlling exhaustion	PRDM1 (=blimp1, heterozygotes mice control chronic viral infection better than wt or conditional KO)
		BATF
Hypoxia mediated tolerance	iNOS induced guanylated cyclase	GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3

[0111] In a preferred embodiment said method of further engineering the immune cells involves introducing into said T cells polynucleotides, in particular mRNAs, encoding specific rare-cutting endonuclease to selectively inactivate the genes, as those mentioned above, by DNA cleavage. In a more preferred embodiment said rare-cutting endonucleases are TALE-nucleases or Cas9 endonuclease. TAL-nucleases have so far proven higher specificity and cleavage efficiency over the other types of rare-cutting endonucleases, making them the endonucleases of choice for producing of the engineered immune cells on a large scale with a constant turn-over.

Delivery methods

[0112] The different methods described above involve introducing CAR 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.

[0113] 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.

Engineered immune cells

[0114] 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.

[0115] 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.

[0116] As a preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a CD33 CAR as described above, that do not express functional TCR and that are reactive towards CD33 positive cells, for their allogeneic transplantation into patients.

[0117] As an even more preferred embodiment, the present invention provides the engineered immune cells according to the invention comprising T-cells or a population of T-cells endowed with a CD33 CAR, said CD33 CAR comprising a polypeptide structure selected from V1 and V3, said polypeptide structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD33 antibody, a hinge selected from a FcRIIIα hinge and CD8α hinge, a CD8α transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

[0118] In one embodiment, the engineered immune cells according to the invention comprise a specific CD33 CAR comprising a monoclonal anti-CD33 antibody selected from M195, m2h12, DRB2, and My9.6, or from M195, m2h12, and My9.6, and optionally humanized.

[0119] In a more preferred embodiment, said CD33 CAR comprises a polypeptide selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 66 and SEQ ID NO: 68, or comprise a polypeptide selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68.

Activation and expansion of T cells

[0120] Whether prior to or after genetic modification of the T cells, even if the genetically modified immune cells of the present invention are activated and proliferate independently of antigen binding mechanisms, the immune cells, particularly T-cells of the present invention can be further activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded *in vitro.*

[0121] 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.

[0122] 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% C02). T cells that have been exposed to varied stimulation times may exhibit different characteristics

[0123] 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.

Therapeutic applications

[0124] In another embodiment, isolated cell obtained by the different methods or cell line derived from said isolated cell as previously described can be used as a medicament. In another embodiment, said medicament can be used for treating cancer, particularly for the treatment of B-cell lymphomas and leukemia in a patient in need thereof. In another embodiment, said isolated cell according to the invention or cell line derived from said isolated cell can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

[0125] 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.

[0126] In one embodiment, an anti-CD33 CAR expressing T cell according to the invention is provided for its use as a medicament.

[0127] In a particular embodiment, said anti-CD33 CAR expressing T cell according to the invention provided for its use as a medicament, comprises a polypeptide selected from SEQ ID NO 19, SEQ ID NO 21, SEQ ID NO 27, and SEQ ID NO 37.

[0128] Even more preferably, said anti-CD33 CAR expressing T cell according to the invention provided for its use as a medicament, comprises a polypeptide selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 66 and SEQ ID NO: 68, or comprise a polypeptide selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68; any of these CAR may be humanized.

[0129] In one embodiment, an anti-CD33 CAR expressing T cell according to the invention provided for its use as a medicament, comprises a polypeptide of SEQ ID NO: 68 or a polypeptide having at least 80% identity with a polypeptide of SEQ ID NO: 68, optionally humanized.

[0130] In other words, the invention is related to an anti-CD33 CAR expressing T cell according to the invention comprising a polypeptide having at least 80% identity with any one of the polypeptide selected from SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68, optionally humanized for its use as a medicament.

[0131] Engineered immune cells that can be for use as a medicament. Preferably, engineered immune cells may comprise primary T cells, wherein a CD33 expression in altered, a TCR expression is altered, optionally resistant to at least one drug used to treat an hematological cancer.

[0132] In general, said medicament can be for use in treating a CD33-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a CD33-expressing cell, ie a condition linked to the detrimental activity of CD33-expressing cells.

[0133] Said medicament can be for use in treating patients diagnosed wherein a pre-malignant or malignant cancer condition characterized by CD33-expressing T cells, especially by an overabundance of CD33-expressing T cells. Such conditions are found in hematologic cancers, such as leukemia or malignant lymphoproliferative disorders such as B-cell lymphoproliferative disorders.

[0134] Another example of CD33-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a CD33-expressing cell that can be treated with an anti-CD33 CAR expressing T cell as described is Alzheimer disease.

[0135] A Lymphoproliferative disorder can be lymphoma, in particular multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

[0136] Any one of the CD33-mediating or CD33-involving disease in particular malignant lymphoproliferative disorder or leukemia may be treated and the health condition of the patient suffering said pathological condition may be improved, with the anti-CD33 CAR-expressing T cells of the present invention.

[0137] In a preferred embodiment, the cancer that may be treated using the anti-CD33 CAR - expressing T cells of the present invention is leukemia, a disease associated to leukemia or a complication thereof, in particular AML, an AML subtype, AML-related complication, and AML-related conditions.

[0138] A leukemia that can also be prevented or treated using the anti-CD33 CAR -expressing T cells of the present invention can be acute myelogenous leukemia (AML), chronic myelogenous leukemia, melodysplastic syndrome, acute lymphoid leukemia, acute lymphoblastic leukemia, chronic lymphoid leukemia, and

myelodysplastic syndrome.

[0139] AML or AML subtypes that may be treated using the anti-CD33 CAR-expressing T 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 leukemia, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b), acute megakaryoblastic leukemia (M7), acute basophilic leukemia, acute panmyelosis with myelofibrosis, whether involving CD33-positive cells.

[0140] Subtypes of AML that may be treated using the anti-CD33 CAR-expressing T cells of the present invention also include, AML with t (8;21) (q22;q22), (*AML1/ETO*), AML with inv (16) (p13;q22) or t (16;16) (p13;q22), (CBFβ/*MYH11*), AML with t (15;17) (q22;q12), (*PML/PARα*) and variants, AML with t(9;11)(p22;q23), (*MLLT3/MLL*), AML with t(6;9)(p23;q34) (*DEK/NUP214*), AML with inv(3)(q21q26) or t(3;3)(q21;q26), (*RPN1/EVI1*), AML with t(1;22)(p13;q13) (RBM15/MKL1) (megakaryocytic), AML with myelodysplasia-related changes including AML arising from prior MDS or MDS/MPN, AML with an MDS-related cytogenetic abnormality, and AML with multilineage dysplasia, alkylating agent/radiation related AML, AML, minimally differentiated (also known as AML-M0), AML without maturation (also known as AML-M1), AML with maturation (also known as AML-M2), Acute myelomonocytic leukemia (also known as AML-M4), Acute monoblastic/monocytic leukemia (also known as AML-M5), Acute erythroid leukemia (also known as AML-M6) and pure erythroid leukemia.

[0141] Accordingly, AML classified as AML with specific genetic abnormalities are conditions that may be treated using the anti-CD33 CAR-expressing T cells of the present invention. Classification is based on the ability of karyotype to predict response to induction therapy, relapse risk, survival.

[0142] Accordingly, AML that may be treated using the anti-CD33 CAR-expressing T cells of the present invention may be AML with a translocation between chromosomes 8 and 21, AML with a translocation or inversion in chromosome 16, AML with a translocation between chromosomes 9 and 11, APL (M3) with a translocation between chromosomes 15 and 17, AML with a translocation between chromosomes 6 and 9, AML with a translocation or inversion in chromosome 3, AML (megakaryoblastic) with a translocation between chromosomes 1 and 22 .

[0143] The present invention is particularly useful for the treatment of AML associated with these particular cytogenetic markers.

[0144] The present invention also provides an anti-CD33 CAR expressing T cell for use in 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) 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.

[0145] Preferably, the present invention provides an anti-CD33 CAR expressing T cell for use in 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.

GROUP OF PATIENTS

[0146] In a preferred embodiment, the invention provides a medication for leukemia in particular for AML in patients over 60 years, in patients of less than 20 years, in particular in children.

[0147] In a more preferred embodiment, the present invention provides a pediatric treatment, in particular a

pediatric treatment against AML, or AML-related diseases or complications.

[0148] In still another preferred embodiment, the present invention is used as a treatment in AML patients with low, poor or unfavorable status that is to say with a predicted survival of less than 5 years survival rate. In this group, patients suffering AML with the following cytogenetic characteristics : -5; 5q; -7; 7q-;11q23; non t(9;11); inv(3); t(3;3); t(6;9); t(9;22) is associated with poor-risk status (Byrd J.C. et al., December 15, 2002; Blood: 100 (13) and is especially contemplated to be treated according to the present invention or with an object of the present invention.

[0149] In one embodiment, the anti-CD33 CAR expressing T cell of the present invention may be used as a treatment in case of AML relapse, or in case of refractory or resistant AML. Preferably, T cells comprising at least one humanized anti-CD33 CAR of the invention comprising or consisting of SEQ ID NO. 1 and of a polypeptide selected from a polypeptide having at least 80% to 100% identity with SEQ ID NO 19, SEQ ID NO 21, SEQ ID NO 27 and SEQ ID NO 37, or a combination thereof, 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.

[0150] In another preferred embodiment, said least one anti-CD33 CAR T cell of the invention, is used for preventing cancer cells development occurring in particular after anti-cancer treatment, during bone marrow depletion or before bone marrow transplantation, after bone marrow destruction.

AML COMPLICATIONS

[0151] In one particular embodiment the invention provides a medicament that improves the health condition of a patient, in particular a patient undergoing a complication related to AML. More preferably, said engineered anti-CD33 CAR expressing T cell of the invention is expressing at least one anti-CD33 CAR comprising a SEQ ID NO 1 and a polypeptide having at least 80% identity with SEQ ID NO 19, SEQ ID NO 21, SEQ ID NO 27 and SEQ ID NO 37, or a combination thereof, and is for use as a medicament for the treatment of a complication related to AML.

[0152] In one particular embodiment the invention provides a medicament that improves the health condition of a patient suffering from AML, in particular a patient undergoing a complication related to AML, said medicament comprising an anti-CD33 CAR expressing T cell of the invention comprising a polypeptide having at least 80% identity with a polypeptide selected from SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68.

[0153] 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

[0154] In one embodiment, the present invention also provides an anti-CD33 CAR expressing T cell for use in the treatment of a pathological condition related to AML. Preferably, the present invention provides a cell expressing at least one anti-CD33 CAR comprising a polypeptide having at least 80% identity with a polypeptide selected from SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 66 and SEQ ID NO: 68 for use in the treatment of a pathological condition related to AML.

[0155] The present invention provides a medicament 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.

[0156] According to another aspect, the present invention provides a composition for use in the treatment of AML associated diseases, in particular hematologic malignancy related to AML.

[0157] Hematologic malignancy related to AML conditions include myelodysplasia syndromes (MDS, formerly known as "preleukemia") which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

[0158] In another embodiment, the invention provides a medicament according to the invention that improves the health state of a patient suffering multiple myeloma.

[0159] 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).

[0160] Also described is an anti-CD33 CAR expressing T cell for use in the treatment of Alzheimer's disease.

[0161] The present invention provides anti-CD33 CAR expressing T cells for use in the treatment of patients diagnosed with a pre-malignant or malignant cancer condition characterized by CD33-expressing cells, especially by an overabundance of CD33-expressing cells. Such conditions are found in hematologic cancers, such as leukemia or malignant lymphoproliferative disorders.

[0162] Leukemia can be acute myelogenous leukemia, chronic myelogenous leukemia, melodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

[0163] Lymphoproliferative disorder can be lymphoma, in particular multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

[0164] Cancers that may be treated may comprise nonsolid tumors (such as hematological tumors, including but not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma and the like. Types of cancers to be treated with the CARs of the invention include, but are not limited leukemia or lymphoid malignancies. Adult tumors/cancers and pediatric tumors/cancers are also included.

[0165] 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.

[0166] 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.

[0167] The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

[0168] The administration of the cells or population of cells can consist of the administration of 10^4-10^9 cells per kg body weight, preferably 10^5 to 10^6 cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage 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.

[0169] 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.

[0170] 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. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery.

Other definitions

[0171]

- Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.- Amino acid residues in a polypeptide sequence are designated herein according to the one-letter code, in which, for example, Q means Gln or Glutamine residue, R means Arg or Arginine residue and D means Asp or Aspartic acid residue.
- Amino acid substitution means the replacement of one amino acid residue with another, for instance the replacement of an Arginine residue with a Glutamine residue in a peptide sequence is an amino acid substitution.
- Nucleotides are designated as follows: one-letter code is used for designating the base of a nucleoside: a is adenine, t is thymine, c is cytosine, and g is guanine. For the degenerated nucleotides, r represents g or a

(purine nucleotides), k represents g or t, s represents g or c, w represents a or t, m represents a or c, y represents t or c (pyrimidine nucleotides), d represents g, a or t, v represents g, a or c, b represents g, t or c, h represents a, t or c, and n represents g, a, t or c.

- "As used herein, "nucleic acid" or "polynucleotides" refers to nucleotides and/or polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Nucleic acids can be either single stranded or double stranded.
- By chimeric antigen receptor (CAR) is intended molecules that combine a binding domain against a component present on the target cell, for example an antibody-based specificity for a desired antigen (e.g., tumor antigen) with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific anti-target cellular immune activity. Generally, CAR consists of an extracellular single chain antibody (scFvFc) fused to the intracellular signaling domain of the T cell antigen receptor complex zeta chain (scFvFc:ζ) and have the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity.
- By V1 structure is intended molecules that combine
  - a CD8alpha signal peptide,
  - a VH domain separated to a VL domain by a linker, said VH and VL contributing to the binding to CD33,
  - a Hinge from Fcgamma (γ) RIIIalpha (α)
  - a transmembrane domain derived from CD8alpha(α)
  - a cytoplasmic domain derived from 41BB and CD3 zeta (ζ)
- By V2 structure is intended molecules with a V1 structure and wherein the transmembrane domain derived from 41BB

  By V3 structure is intended molecules that combine
  - a CD8alpha signal peptide,
  - a VH domain separated to a VL domain by a linker, said VH and VL contributing to the binding to CD33,
  - a Hinge from CD8alpha (α)
  - a transmembrane domain derived from CD8alpha(α)
  - a cytoplasmic domain derived from 41BB and CD3 zeta (ζ)
- By V4 structure is intended molecules with a V3 structure and wherein the transmembrane domain derived from 41BB.
- By V5 structure is intended molecules that combine
  - a CD8alpha signal peptide,
  - a VH domain separated to a VL domain by a linker, said VH and VL contributing to the binding to CD33,
  - a Hinge from IgG1 (α)
  - a transmembrane domain derived from CD8alpha(α)
  - a cytoplasmic domain derived from 41BB and CD3 zeta (ζ).
- By V6 structure is intended molecules with a V5 structure and wherein the transmembrane domain derived from 41BB.

The CAR structures as described are illustrated in Figure 2, preferably in Figure 3.

[0172] The term "chemotherapy" refers to any therapy using a chemical, in particular those used against cancer. - 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".

- 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).

[0173] The rare-cutting endonuclease 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).

[0174] 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 may for example correspond to a LAGLIDADG endonuclease, to a HNH endonuclease, or to a GIY-YIG endonuclease. Preferred homing endonuclease can be an I-*CreI* variant.

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

[0175] 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).

- 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 nonlimiting example, that are able to integrate the genome of a target cell. At the opposite by "non-integrative lentiviral vectors (or NILV)" is meant efficient gene delivery vectors that do not integrate the genome of a target cell through the action of the virus integrase.

- Delivery vectors and vectors can be associated or combined with any cellular permeabilization techniques such as sonoporation or electroporation or derivatives of these techniques.
- By cell or cells is intended any eukaryotic living cells, primary cells and cell lines derived from these organisms for *in vitro* cultures.
- By "primary cell" or "primary cells" are intended cells taken directly from living tissue (i.e. biopsy material) and established for growth in vitro, that have undergone very few population 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.

[0176] 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.

[0177] All these cell lines can be modified by the method of the present invention to provide cell line models to produce, express, quantify, detect, study a gene or a protein of interest; these models can also be used to screen biologically active molecules of interest in research and production and various fields such as chemical, biofuels, therapeutics and agronomy as non-limiting examples.

- by "mutation" is intended the substitution, deletion, insertion of up to one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty five, thirty, forty, fifty, or more nucleotides/amino acids in a polynucleotide (cDNA, gene) or a polypeptide sequence. The mutation can affect the coding sequence of a gene or its regulatory sequence. It may also affect the structure of the genomic sequence or the structure/stability of the encoded mRNA.
- by "variant(s)", it is intended a repeat variant, a variant, a DNA binding variant, a TALE-nuclease variant, a polypeptide variant obtained by mutation or replacement of at least one residue in the amino acid sequence of the parent molecule.
- by "functional variant" is intended a catalytically active mutant of a protein or a protein domain; such mutant may have the same activity compared to its parent protein or protein domain or additional properties, or higher or lower activity.
- "identity" refers to sequence identity between two nucleic acid molecules or polypeptides. Identity can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base, then the molecules are identical at that position. A degree of similarity or identity between nucleic acid or amino acid sequences is a function of the number of identical or matching nucleotides at positions shared by the nucleic acid sequences. Various alignment algorithms and/or programs may be used to calculate the identity between two sequences, including FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default setting. For example, polypeptides having at least 70%, 85%, 90%, 95%, 98% or 99% identity to specific polypeptides described herein and preferably exhibiting substantially the same functions, as well as polynucleotide encoding such polypeptides, are contemplated. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the

percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity of similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. The polynucleotide sequences of similar polypeptides are deduced using the genetic code and may be obtained by conventional means, in particular by reverse translating its amino acid sequence using the genetic code.

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

[0178] A "co-stimulatory molecule" refers to the cognate binding partner on a Tcell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor.

[0179] 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.

[0180] 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.

[0181] The term "subject" or "patient" as used herein includes all members of the animal kingdom including non-human primates and humans.

[0182] The terms "drug used to treat a cancer, in particular AML" refers to medicament used for the treatment of cancer, in particular AML and are described for example in WO2015/140268.

[0183] 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.

[0184] 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.

GENERAL METHODS:

Inactivation of specific gene(s) in primary T cells

[0185] Inactivation of specific gene(s) in primary T cells may be performed before or after CAR introduction into T cells.

[0186] At least one gene, one gene or two genes may be inactivated in one step or in successive steps. In a preferred embodiment two genes may be inactivated at once, preferably TCRalpha gene and CD33 gene.

[0187] In general, heterodimeric nuclease, in particular TALE-Nuclease targeting two long sequences (called half targets) separated by a spacer within a target gene is designed and produced.

[0188] Each TALE-nuclease construct may be cloned in an appropriate mammalian expression vector. mRNA encoding TALE-nuclease cleaving a targeted genomic sequence may be synthesized from plasmid carrying the coding sequence downstream a promoter.

[0189] Cells are purified T cells preactivated with anti-CD3/CD28 coated beads. Cells are transfected with each of the 2 mRNAs encoding both half TALE-nucleases, in particular both half TALE-nucleases and spacer.

[0190] Cells may be reactivated with soluble anti-CD28 to measure cell proliferation and the activation marker CD25 detected to assess the activation state of the cells.

Chimeric Antigen Receptors

NUCLEIC ACIDS- VECTORS

[0191] An acid nucleic (mRNA or lentiviral vector) encoding an anti-CD33 CAR as described is constructed according to the architecture designed in Figure 2 or Figure 3.

[0192] Anti-CD33 CAR lentiviral vectors may be prepared for example as previously described in WO2013176915, WO2013176916, or in WO2014130635. Lentiviral vectors are produced by Vectalys SA (Toulouse, France).

[0193] CAR mRNAs may be produced using T7 mRNA polymerase and transfections done using Cytopulse technology.

Screening and selection of anti-CD33 CAR

- ***Primary T-cell cultures***

[0194] T cells are purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium. The PBMC layer is recovered and T cells purified using a commercially available T-cell enrichment kit. Purified T cells are activated in X-Vivo™-15 medium (Lonza) using Human IL-2 and Dynabeads Human T activator CD3/CD28.

CAR mRNA transfection

[0195] Transfections of *CAR mRNAs* encoding the different CAR constructs are performed at Day 4 or Day 11 after T-cell purification and activation.

[0196] T-cell transduction with recombinant lentiviral vectors allowing the expression of *CAR* Transduction of T-cells with recombinant lentiviral vectors are carried out three days after T-cell purification/activation. Lentiviral vectors are produced by Vectalys SA (Toulouse, France), by transfecting genomic and helper plasmids in HEK-293 cells. Transductions may be carried out at various multiplicity of infection (MOI), in particular at a MOI of 5. CAR detection at the surface of T-cells is performed using a recombinant protein consisting on the extracellular domain of the human CD33 protein fused together with a murine IgG1 Fc fragment (produced by LakePharma).

[0197] Binding of this protein to the CAR molecule is detected with a PE-conjugated secondary antibody (Jackson Immunoresearch) targeting the mouse Fc portion of the protein, and

- ***Degranulation assay (CD107a mobilization)***

[0198] T-cells are incubated together with an equal amount of cells expressing various levels of the CD33 protein. Co-cultures are maintained for at least 6 hours. CD107a staining is performed during cell stimulation, by the addition of a fluorescent anti-CD107a antibody at the beginning of the co-culture. After the 6h incubation period, cells are stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and analyzed by flow cytometry. The degranulation activity is determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells.

[0199] Degranulation assays are carried out 24h after mRNA transfection.

- ***IFN gamma release assay***

[0200] 24h after mRNA transfection, anti-CD33 CAR expressing T-cells are incubated together with cell lines expressing various levels of the CD33 protein for 24 hours at 37°C. The supernatants are recovered and IFN gamma detection in the cell culture supernatants is done by ELISA assay.

- ***Cytotoxicity assay***

[0201] CD33 CAR expressing T-cells are incubated together with target cells (expressing various levels of CD33) or (CD33neg) cells in the same well. Target CD33+ and control CD33-target cells are labelled with fluorescent intracellular dyes (eg. CFSE or Cell Trace Violet), before co-culture with for 4 hours at 37°C. After this incubation period, cells are labelled with a fixable viability dye and analyzed by flow cytometry. Viability of each cellular population (target cells or CD33neg control cells) is determined and the % of specific cell lysis is calculated. Cytotoxicity assays are carried out 48h after mRNA transfection.

- ***Anti-tumor mouse model***

[0202] Immuno deficient NOG mice are intravenously (iv) injected with CD33 expressing -Luciferase cells. Optionally, mice receive an anti-cancer treatment before injection with anti-CD33 CAR+ T-cells. Mice are then iv injected (eg either 2 or 7 days after injection of the tumor cell line) with different doses of anti-CD33 CAR+ T-cells to be tested, or with T-cells that were not transduced with the CAR lentiviral vector. Bioluminescent signals are determined at the day of T-cell injection (D0), at D7, 14, 21, 28 and 40 after T-cell injection in order to follow

tumoral progression in the different animals.

[0203] Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

Examples

Construction of CD33 CAR using various anti-CD33 antibody fragments

[0204] Different architectures of CAR were designed (Figure 3) namely V1 to V6. As a first step, 4 different scFv's from M195, DRB2, m2H12 and My9.6 antibody were constructed to generate Chimeric Antigen Receptors (CARs) (SEQ ID NO 48 to SEQ ID NO 71) in T cells.

Example 1: Proliferation of TCRalpha inactivated cells expressing a CD33-CAR.

- ***Primary T-cell cultures***

[0205] T cells were purified from various 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 (Stem Cell Technologies). Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2, 5% Human Serum, and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies). After activation cells were grown and maintained in X-Vivo™-15 medium (Lonza) supplemented with 20ng/mL Human IL-2 and 5% Human Serum.

- ***CAR mRNA transfection***

[0206] Transfections of each CD33 CAR generated using various anti-CD33 antibody fragments were done at Day 4 or Day 11 after T-cell purification and activation. 5 million cells were transfected with 15µg of mRNA encoding the different CAR constructs. CAR mRNAs were produced using the mMESSAGE mMACHINE T7 Kit (Life Technologies) and purified using RNeasy Mini Spin Columns (Qiagen). Transfections were done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4cm gap cuvettes in a final volume of 200µl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media (Lonza) and incubated at 37°C with 5% CO_2. IL-2 was added 2h after electroporation at 20ng/mL.

Primary T cells transduction

- ***T-cell transduction***

[0207] Transduction of T-cells with recombinant lentiviral vectors expression the antiCD33 CAR were carried out three days after T-cell purification/activation. Lentiviral vectors produced by Vectalys SA (Toulouse, France) were used. Anti-CD33 CAR detection at the surface of T-cells is performed using a recombinant protein consisting on

the extracellular domain of the human CD33 protein fused together with a murine IgG1 Fc fragment (produced by LakePharma). Binding of this protein to the anti-CD33 CAR molecule is detected with a PE-conjugated secondary antibody (Jackson Immunoresearch) targeting the mouse Fc portion of the protein, and analyzed by flow cytometry.

[0208] Heterodimeric TALE-nuclease targeting two 17-bp long sequences (called half targets) separated by an 15-bp spacer within T-cell receptor alpha constant chain region (TRAC) gene were designed and produced. Each half target is recognized by repeats of the half TALE-nucleases listed in Table 10.

Table 10: TAL-nucleases targeting TCRalpha gene

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

[0209] 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.

[0210] Purified T cells preactivated during 72 hours with anti-CD3/CD28 coated beads were transfected with each of the 2 mRNAs encoding both half TRAC_T01 TALE-nucleases. 48 hours post-transfection, different groups of T cells from the same donor were respectively transduced with a lentiviral vector encoding one of the CD33 CAR previously described (SEQ ID NO: 19 to 42). 2 days post-transduction, $CD3_{NEG}$ cells were purified using anti-CD3 magnetic beads and 5 days post-transduction cells were reactivated with soluble anti-CD28 (5 µg/ml).

[0211] 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 CD33 CARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

[0212] To investigate whether the human T cells expressing the CD33CAR 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 CD33 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.

[0213] The present invention provided a CD33 specific CAR T cell wherein the level of TCR at the cell surface was below detected.

Example 2:

Inactivation of the CD33 gene

[0214] To inactivate the CD33 gene, heterodimeric TALE-nuclease targeting two sequences (called **Sequence bound by TALEN Left and Sequence bound by TALEN Right, see table 11)** separated by a 10 or 15-bp spacer within CD33 gene were designed and produced as described below. Each half target is recognized by repeats of the half TALE-nucleases listed in Table 11. The constructs were then introduced into T cells together with and at the same time that those designed to inactivate the TCR alpha gene.

Table 11: TAL-nucleases targeting TCRalpha gene

Target	Target sequences
TALEN1_CD33_Exon3	TGCATCCCCTCTTTCTCCTCACTAGACTTGACCCACAGGCCCAA (SEQ ID NO:72)
TALEN2_CD33_Exon3	TTCTCCTCACTAGACTTGACCCACAGGCCCAAAATCCTCATCCCTGGCA (SEQ ID NO:73)
TALEN1_CD33_Exon4	TCCTCTCCTAGATGTTCCACAGAACCCAACAACTGGTATCTTTCCAGGA (SEQ ID NO:74)
TALEN2_CD33_Exon4	TCCTAGATGTTCCACAGAACCCAACAACTGGTATCTTTCCAGGA (SEQ ID NO:75)

Sequence bound by TALEN Left	Spacer	Sequence bound by TALEN Right
TGCATCCCCTCTTTCTC (SEQ ID NO:76)	CTCACTAGAC (SEQ ID NO:80)	TTGACCCACAGGCCCAA (SEQ ID NO:84)
TTCTCCTCACTAGACTT(SEQ ID NO:77)	GACCCACAGGCCCAA (SEQ ID NO:81)	AATCCTCATCCCTGGCA (SEQ ID NO:85)
TCCTCTCCTAGATGTTC (SEQ ID NO:78)	CACAGAACCCAACAA (SEQ ID NO:82)	CTGGTATCTTTCCAGGA (SEQ ID NO:86)
TCCTAGATGTTCCACAG (SEQ ID NO:79)	AACCCAACAA (SEQ ID NO:83)	CTGGTATCTTTCCAGGA (SEQ ID NO:87)

[0215] In the resulting cells, the extracellular domain of CD33 is truncated. Double staining of the resulting cells and analysis by flow cytometry indicated a drop in cell surface expression of CD33 and of TCR as compared to control (mock-transfected) cells.

[0216] Alternatively, purified TCR KO T cells preactivated during 72 hours with anti-CD3/CD28 coated beads may be used for CD33 inactivation.

[0217] To investigate the activated state of the human TCR KO and CD33 KO T cells expressing the CD33 CAR, the expression of the activation marker CD25 are analyzed by FACS 7 days post transduction.

[0218] Anti-CD33 CARs T cells and anti-CD33 CARs T cells wherein TCR alpha gene and/or CD33 gene is inactivated and wherein CAR is corresponding to architectures V1, V3 and V5, can be produced.

[0219] Cells are designated as "T cells" or "CD33 CAR", for convenience.

Example 3

Degranulation activity of CD33 CAR generated using various anti-CD33 antibody fragments

[0220] The activity of the constructs V1, V3 and V5 of M195, m2H12 and My9.6 as described above was first determined upon transient expression in human primary T-cells.

- ***Degranulation assay (CD107a mobilization)***

[0221] T-cells were then incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing various levels of the CD33 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 6 hours at 37°C with 5% 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 1µg/ml of anti-CD49d, 1µg/ml of anti-CD28, and 1x Monensin solution. After the 6h incubation period, cells were stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24h after mRNA transfection.

[0222] Among the CAR molecules generated as illustrated in Figure 3, 9 were tested for their degranulation activity (Figure 4 to Figure 6) and 7 of them were selected for further activity tests : interferon gamma release and cell lysis (Figure 7 and Figure 8).

[0223] Degranulation activity of each of the CAR comprising a M195, m2H12 or My9.6 scFv within one of the three architectures (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, or v5: IgG1-hinge/CD8-transmembrane), was measured when CAR+ T-cells were co-cultured for 6 hours with CD33 expressing cells (U937), or with cells expressing undetectable level CD33 (Jeko or Jeko-1). The results are illustrated in **Figure 4 to Figure 6.**

[0224] **Figure 4** shows degranulation activity (% CD8/CD107a+ cells and MFI CD107a in CD8+ cells) of the M195 scFv with the three architectures (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD33 expressing cells (U937), or with cells that do not express CD33 (Jeko). The data is presented as a percentage (%) of degranulation and mean fluorescence activity (MFI).

[0225] **Figure 5** shows degranulation activity (% CD8/CD107a+ cells and MFI CD107a in CD8+ cells) of the m2H12 and My9.6 scFvs with the three architectures (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with CD33 expressing cells (U937), or with cells that do not express CD33 (Jeko). The data is presented as a percentage (%) of degranulation and mean fluorescence activity (MFI).

[0226] **Figure 6** shows degranulation activity (% CD8/CD107a+ cells) of 7 constructions : M195-V1, M195-V3, M195-V5, m2H12-V1, m2H12-V3 and My9.6-V1 and My9.6-V3 (v1: FcgRIII-hinge/CD8-transmembrane, v3: CD8-hinge/CD8-transmembrane, v5: IgG1-hinge/CD8-transmembrane), when CAR+ T-cells were co-cultured for 6 hours with cells expressing high or intermediate levels of CD33 (U937 and K562, respectively), or with cells that do not express CD33 (Jeko-1). The data is presented as a percentage (%) of degranulation and mean fluorescence activity (MFI).

Interferon gamma release and cell lysis induced by T cells expressing CD33 CAR generated using anti-CD33 scFv antibody fragments derived from M195, M2H12, and My9.6

[0227] For this, T-cells from various donors were isolated from buffy-coat samples and activated using CD3/CD28 beads. Cells were transiently transfected with mRNAs encoding the different candidates at D11 after activation.

[0228] CAR activity was assessed by measuring the IFN gamma release, and the cytotoxic activity when co-cultured with cells expressing various levels of CD33 as follows.

- ***IFN gamma release assay***

[0229] Anti-CD33 CAR-expressing T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing various levels of the CD33 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 24 hours at 37°C with 5% 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 24h after mRNA transfection.

[0230] Figure 7 shows the amount of IFN gamma released by CD33 car T-cells (T cells) when co-cultured for 24h with cells expressing different levels of CD33 (U937 and K562), or with cells that do not express CD33 (Jeko-1). 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.

[0231] T cells expressing CD33 CAR generated using anti-CD33 scFv antibody fragments derived from M195, M2H12, and My9.6 were then tested for their capacity to lyse CD33+ expressing target cancer cells (U937: Human leukemic monocyte lymphoma cell and K562 human leukemic cell derived from of a patient with chronic myelogenous leukemia (CML) as follows.

- ***Cytotoxicity assay***

[0232] Anti-CD33 CAR-expressing T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing CD33) and 10,000 control (CD33neg) 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 CD33neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48h after mRNA transfection.

[0233] Figure 8 shows the specific cytolytic activity of CAR-T cells. Assays were done 48h after CAR mRNA transfection. T-cells were co-cultured with U937+Jeko or K562+Jeko cells for 4 hours. Cellular viability for each of the cell lines was determined at the end of the co-cultured and a specific cell lysis percentage was calculated.

[0234] All constructions were active, with My9.6 V3 (My9.6-3, SEQ ID NO 68) CAR exhibiting the higher overall activity as compared to control than other CARS.

[0235] The above examples demonstrate that the exemplified CAR structures (V1, V3 and V5) comprising a VH-Linker-VL sequence binding to CD33 are active in all assay.

[0236] CARs having the structures V1 and V3 were found particularly active in the degranulation assay, INFy release assay and cytotoxic assay see figures 4 to 8.

[0237] The present invention provides therefore an anti-CD33 CAR expressing T cell active against cancer cells.

Examples of CAR polypeptide sequences:

[0238]

M195-1 (SEQ ID NO.1 + SEQ ID NO.19)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV

DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCK
RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ
LYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKG
ERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

M195-2 (SEQ ID N0.1 + SEQ ID NO.20)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV
DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVV
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN
QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

M195-3 (SEQ ID NO.1 + SEQ ID NO.21)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV
DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP
EEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPR
RKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PR

M195-4 (SEQ ID NO.1 + SEQ ID NO.22)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV
DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRG
LDFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR

FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ
ALPPR

M195-5 (SEQ ID N0.1 + SEQ ID NO.23)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV
DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCS
CRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMG
GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH
MQALPPR

M195-6 (SEQ ID N0.1 + SEQ ID NO.24)

MALPVTALLLPLALLLHAARPEVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQS
HGKSLEWIGYIYPYNGGTGYNQKFKSKATLTVDNSSSTAYMDVRSLTSEDSAVYYCARGRP
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIVLTQSPASLAVSLGQRATISCRASESV
DNYGISFMNWFQQKPGQPPKLLIYAASNQGSGVPARFSGSGSGTDFSLNIHPMEEDDTAM
YFCQQSKEVPWTFGGGTKLEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIAR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDG
CSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE

MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

m2H12-1 (SEQ ID NO.1 + SEQ ID NO.25)

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRP
GQGLEWIGWIYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYED
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVIINCKASQDI
NSYLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQ
YDEFPLTFGAGTKLELKRGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKK
LLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG
KGHDGLYQGLSTATKDTYDALHMQALPPR

m2H12-2 (SEQ ID NO.1 + SEQ ID NO.26)

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRP
GQGLEWIGWIYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYED
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVIINCKASQDI
NSYLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQ
YDEFPLTFGAGTKLELKRGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVKRGR
KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYN
ELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR
RGKGHDGLYQGLSTATKDTYDALHMQALPPR

m2H12-3 (SEQ ID N0.1 + SEQ ID NO.27)

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRP
GQGLEWIGWIYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYED
AMDYWGQGTSVTVSSGGGGSGGGGSGGGGSDIKMTQSPSSMYASLGERVIINCKASQDI
NSYLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQ
YDEFPLTFGAGTKLELKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFAC
DIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG
GCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

m2H12-4 (SEQ ID NO.1 + SEQ ID NO.28)

MALPVTALLLPLALLLHAARPQVQLQQSGPELVRPGTFVKISCKASGYTFTNYDINWVNQRP
GQGLEWIGWIYPGDGSTKYNEKFKAKATLTADKSSSTAYLQLNNLTSENSAVYFCASGYED
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m2H12-5 (SEQ ID NO.1 + SEQ ID NO.29)

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KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
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SLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFP
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m2H12-6 (SEQ ID N0.1 + SEQ ID NO.30)

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DRB2-1 (SEQ ID NO.1 + SEQ ID NO.31)

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DRB2-3 (SEQ ID NO.1 + SEQ ID NO.33

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DRB2-4 (SEQ ID NO.1 + SEQ ID NO.34)

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DRB2-5 (SEQ ID NO.1 + SEQ ID NO.35)

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DRB2-6 (SEQ ID NO.1 + SEQ ID NO.36)

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My9.6-1 (SEQ ID N0.1 + SEQ ID NO.37)

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My9.6-3 (SEQ ID NO.1 + SEQ ID NO.39)

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My9.6-4 (SEQ ID N0.1 + SEQ ID NO.40)

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My9.6-5 (SEQ ID NO.1 + SEQ ID NO.41)

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My9.6-6 (SEQ ID NO.1 + SEQ ID NO.42)

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[0239]

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225 230

<210> 6

<211> 24

<212> PRT

<213> homo sapiens

<220>

<223> CD8alpha transmembrane domain

<400> 6

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
1 5 10 15

Ser Leu Val Ile Thr Leu Tyr Cys
20

<210> 7

<211> 27

<212> PRT

<213> homo sapiens

<220>

<223> 41BB transmembrane domain

<400> 7

Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu
1 5 10 15

Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val
20 25

<210> 8

<211> 42

<212> PRT

<213> homo sapiens

<220>

<223> Fragment of 4-1BB (residues 214-255)

<400> 8

```
Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1               5                   10                  15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
            20                  25                  30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
        35                  40
```

<210> 9

<211> 112

<212> PRT

<213> homo sapiens

<220>

<223> fragment of T-cell surface glycoprotein CD3 zeta chain

<400> 9

```
Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
1               5                   10                  15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            20                  25                  30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        35                  40                  45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    50                  55                  60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
65                  70                  75                  80

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                85                  90                  95

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            100                 105                 110
```

<210> 10

<211> 15

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> linker

<400> 10

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1               5                   10                  15
```

<210> 11

<211> 115

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195 heavy chain variable region

<400> 11

```
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
            100                 105                 110

Thr Val Ser
        115
```

<210> 12

<211> 111

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195 light chain variable region

<400> 12

```
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr
            20                  25                  30

Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
        35                  40                  45

Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
    50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
65                  70                  75                  80

Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
                85                  90                  95

Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> 13
<211> 117
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> m2H12 heavy chain variable region

<400> 13
```
Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1               5                   10                  15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
                85                  90                  95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
            100                 105                 110

Val Thr Val Ser Ser
        115
```

<210> 14
<211> 108
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> m2H12 light chain variable region

<400> 14
```
Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
1               5                   10                  15

Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
            20                  25                  30

Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
        35                  40                  45

Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Tyr
65                  70                  75                  80
```

Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp Glu Phe Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
100 105

<210> 15

<211> 120
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> DRB2 heavy chain variable region

<400> 15

Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
20 25 30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 16

<211> 115
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> DRB2 light chain variable region

<400> 16
Asp Ile Val Leu Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile
20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe
35 40 45

Asp Thr Ser Lys Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr
85 90 95

Phe Gly Asp Gly Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro
100 105 110

Thr Val Ser
115

<210> 17
<211> 118
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> My9-6 heavy chain variable region

<400> 17
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe

50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

<210> 18
<211> 113
<212> PRT
<213> artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> My9-6 light chain variable region

<400> 18
Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Val Phe Phe Ser
20 25 30

Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln
85 90 95

Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg

<210> 19

<211> 436

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-1 polypeptide CAR sequence

<400> 19

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val
145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly
180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu

195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly
245 250 255

Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu
260 265 270

Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys
275 280 285

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
290 295 300

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
305 310 315 320

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
325 330 335

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
340 345 350

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
355 360 365

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
370 375 380

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
385 390 395 400

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
405 410 415

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
420 425 430

Leu Pro Pro Arg
435

<210> 20

<211> 439

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-2 polypeptide CAR sequence

<400> 20

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val
145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly
180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu
195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly
245 250 255

Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu
260 265 270

Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly
275 280 285

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
290 295 300

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
305 310 315 320

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
325 330 335

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
340 345 350

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
355 360 365

Asp Pro Glu Met Glu Glu Lys Pro Arg Arg Lys Asn Pro Gln Glu Glu

Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
370 375 380

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
385 390 395 400

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
405 410 415

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
420 425 430

Met Gln Ala Leu Pro Pro Arg
435

<210> 21

<211> 465

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-3 polypeptide CAR sequence

<400> 21

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val
145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly
180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu
195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
245 250 255

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
260 265 270

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
275 280 285

Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser
290 295 300

Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr
305 310 315 320

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
325 330 335

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
340 345 350

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
355 360 365

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
370 375 380

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
385 390 395 400

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
405 410 415

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
420 425 430

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
435 440 445

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
450 455 460

Arg
465

<210> 22

<211> 468

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-4 polypeptide CAR sequence

<400> 22

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr

20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val
145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly
180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu
195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr
245 250 255

Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala
260 265 270

Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile
275 280 285

Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu
290 295 300

Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys
305 310 315 320

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
325 330 335

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
340 345 350

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
355 360 365

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
370 375 380

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
385 390 395 400

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
405 410 415

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
420 425 430

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
435 440 445

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
450 455 460

Leu Pro Pro Arg
465

<210> 23

<211> 651

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-5 polypeptide CAR sequence

<400> 23

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val

145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly

180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu
195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys
245 250 255

Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
260 265 270

Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val
275 280 285

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
290 295 300

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305 310 315 320

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
325 330 335

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
340 345 350

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
355 360 365

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
370 375 380

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
385 390 395 400

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
405 410 415

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
420 425 430

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
435 440 445

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
450 455 460

Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala Pro Leu
465 470 475 480

Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
485 490 495

Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
500 505 510

Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
515 520 525

Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
530 535 540

Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
545 550 555 560

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
565 570 575

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
580 585 590

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
595 600 605

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
610 615 620

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
625 630 635 640

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
645 650

<210> 24

<211> 654

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> M195-6 polypeptide CAR sequence

<400> 24

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly Tyr Asn Gln Lys Phe
50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
100 105 110

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val
130 135 140

Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val
145 150 155 160

Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly
165 170 175

Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly
180 185 190

Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu

195 200 205

Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln
210 215 220

Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys
245 250 255

Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
260 265 270

Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val
275 280 285

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
290 295 300

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
305 310 315 320

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
325 330 335

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
340 345 350

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
355 360 365

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
370 375 380

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
385 390 395 400

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
405 410 415

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
420 425 430

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
435 440 445

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
450 455 460

Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile Ser Phe Phe Leu Ala
465 470 475 480

Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg
485 490 495

Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys
500 505 510

Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys
515 520 525

Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val
530 535 540

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
545 550 555 560

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
565 570 575

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg
580 585 590

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
595 600 605

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg
610 615 620

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
625 630 635 640

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
645 650

<210> 25

<211> 434

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-1 polypeptide CAR sequence

<400> 25

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp
210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln

245 250 255

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
260 265 270

Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu
275 280 285

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
290 295 300

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
305 310 315 320

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
325 330 335

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
340 345 350

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
355 360 365

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
370 375 380

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
385 390 395 400

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
405 410 415

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
420 425 430

Pro Arg

<210> 26

<211> 437

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-2 polypeptide CAR sequence

<400> 26

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp
210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln
245 250 255

Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu
260 265 270

Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys
275 280 285

Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
290 295 300

Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
305 310 315 320

Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
325 330 335

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
340 345 350

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
355 360 365

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
370 375 380

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
385 390 395 400

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
405 410 415

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
420 425 430

Ala Leu Pro Pro Arg
435

<210> 27

<211> 463

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-3 polypeptide CAR sequence

<400> 27

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp
210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
245 250 255

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
260 265 270

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile
275 280 285

Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val
290 295 300

Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
305 310 315 320

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
325 330 335

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg
340 345 350

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
355 360 365

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
370 375 380

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
385 390 395 400

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
405 410 415

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
420 425 430

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
435 440 445

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455 460

<210> 28

<211> 466

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-4 polypeptide CAR sequence

<400> 28

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp
210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
245 250 255

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
260 265 270

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Ile Ser
275 280 285

Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe
290 295 300

Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu
305 310 315 320

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
325 330 335

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
340 345 350

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
355 360 365

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

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
370 375 380

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
385 390 395 400

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
405 410 415

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
420 425 430

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
435 440 445

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
450 455 460

Pro Arg
465

<210> 29

<211> 649

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-5 polypeptide CAR sequence

<400> 29

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp

210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
245 250 255

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
260 265 270

Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val
275 280 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
290 295 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305 310 315 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
325 330 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
340 345 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
355 360 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
370 375 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385 390 395 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
405 410 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
420 425 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
435 440 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
450 455 460

Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly
465 470 475 480

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
485 490 495

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
500 505 510

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
515 520 525

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
530 535 540

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
545 550 555 560

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
565 570 575

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
580 585 590

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
595 600 605

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
610 615 620

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
625 630 635 640

Leu His Met Gln Ala Leu Pro Pro Arg
645

<210> 30

<211> 652

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> m2H12-6 polypeptide CAR sequence

<400> 30

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Arg Pro Gly Thr
1 5 10 15

Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser Ala Val Tyr Phe Cys
85 90 95

Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
115 120 125

Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
130 135 140

Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys Lys Ala Ser Gln Asp
145 150 155 160

Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
165 170 175

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
180 185 190

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
195 200 205

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Tyr Asp
210 215 220

Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
225 230 235 240

Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
245 250 255

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys

260 265 270

Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val
275 280 285

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
290 295 300

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305 310 315 320

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
325 330 335

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
340 345 350

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
355 360 365

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
370 375 380

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385 390 395 400

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
405 410 415

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
420 425 430

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
435 440 445

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
450 455 460

Leu Ser Leu Ser Pro Gly Lys Ile Ile Ser Phe Phe Leu Ala Leu Thr
465 470 475 480

Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser
485 490 495

Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
500 505 510

Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys
515 520 525

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
530 535 540

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
545 550 555 560

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
565 570 575

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
580 585 590

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
595 600 605

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
610 615 620

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
625 630 635 640

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
645 650

<210> 31

<211> 444

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-1 polypeptide CAR sequence

<400> 31

Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
20 25 30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
130 135 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145 150 155 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
165 170 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
180 185 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
195 200 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225 230 235 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Gly Leu Ala Val Ser Thr
245 250 255

Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro
260 265 270

Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu
275 280 285

Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
290 295 300

Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys

305 310 315 320

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
325 330 335

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
340 345 350

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
355 360 365

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
370 375 380

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
385 390 395 400

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
405 410 415

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
420 425 430

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
435 440

<210> 32

<211> 447

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-2 polypeptide CAR sequence

<400> 32

```
Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
            20                  25                  30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
    130                 135                 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145                 150                 155                 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
                165                 170                 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
            180                 185                 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
        195                 200                 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
    210                 215                 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225                 230                 235                 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Gly Leu Ala Val Ser Thr
                245                 250                 255

Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu
            260                 265                 270

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
        275                 280                 285

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
    290                 295                 300

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
305                 310                 315                 320
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Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg
325 330 335

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
340 345 350

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
355 360 365

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
370 375 380

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
385 390 395 400

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
405 410 415

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
420 425 430

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
435 440 445

<210> 33

<211> 473

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-3 polypeptide CAR sequence

<400> 33

Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
20 25 30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
130 135 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145 150 155 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
165 170 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
180 185 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
195 200 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225 230 235 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Thr Thr Thr Pro Ala Pro
245 250 255

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu
260 265 270

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg
275 280 285

Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly
290 295 300

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
305 310 315 320

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
325 330 335

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
340 345 350

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
355 360 365

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
370 375 380

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
385 390 395 400

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
405 410 415

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
420 425 430

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
435 440 445

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
450 455 460

Leu His Met Gln Ala Leu Pro Pro Arg
465 470

<210> 34

<211> 476

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-4 polypeptide CAR sequence

<400> 34

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Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
            20                  25                  30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile

        35                  40                  45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
        115                 120                 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
    130                 135                 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145                 150                 155                 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
                165                 170                 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
            180                 185                 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
        195                 200                 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
    210                 215                 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225                 230                 235                 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Thr Thr Thr Pro Ala Pro
                245                 250                 255

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu
            260                 265                 270

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg
        275                 280                 285

Gly Leu Asp Phe Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr
    290                 295                 300

Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser
305                 310                 315                 320
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Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
325 330 335

Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys
340 345 350

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
355 360 365

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
370 375 380

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
385 390 395 400

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
405 410 415

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
420 425 430

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
435 440 445

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
450 455 460

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
465 470 475

<210> 35

<211> 659

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-5 polypeptide CAR sequence

<400> 35

Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
20 25 30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
130 135 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145 150 155 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
165 170 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
180 185 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
195 200 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225 230 235 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Glu Pro Lys Ser Pro Asp
245 250 255

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro

260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
290 295 300

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
340 345 350

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
485 490 495

Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu
500 505 510

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
515 520 525

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
530 535 540

Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
545 550 555 560

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
565 570 575

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
580 585 590

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
595 600 605

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
610 615 620

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
625 630 635 640

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
645 650 655

Pro Pro Arg

<210> 36

<211> 662

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> DRB2-6 polypeptide CAR sequence

<400> 36

Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Lys Phe Thr Asp Tyr
20 25 30

Val Val His Trp Leu Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Thr
130 135 140

Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr Met Thr Cys Thr Ala
145 150 155 160

Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Asp
165 170 175

Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys Val Ala Ser Gly Val
180 185 190

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr
195 200 205

Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln
210 215 220

Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly Thr Arg Leu Glu Leu
225 230 235 240

Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Glu Pro Lys Ser Pro Asp
245 250 255

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp

290 295 300

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
340 345 350

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
    450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

Lys Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe
                485 490 495

Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg
            500 505 510

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
        515 520 525

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
    530 535 540

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
545 550 555 560

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
                565 570 575

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
            580 585 590

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
        595 600 605

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
    610 615 620

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
625 630 635 640

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
                645 650 655

Gln Ala Leu Pro Pro Arg
            660

<210> 37

<211> 440

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-1 polypeptide CAR sequence

<400> 37

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
        35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe
    50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Gly Leu Ala Val Ser Thr Ile Ser Ser Phe
245 250 255

Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
260 265 270

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg
275 280 285

Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro
290 295 300

Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu
305 310 315 320

Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala

325 330 335

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
340 345 350

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
355 360 365

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
370 375 380

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
385 390 395 400

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
405 410 415

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
420 425 430

His Met Gln Ala Leu Pro Pro Arg
435 440

<210> 38

<211> 443

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-2 polypeptide CAR sequence

<400> 38

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Gly Leu Ala Val Ser Thr Ile Ser Ser Phe
245 250 255

Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser
260 265 270

Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val
275 280 285

Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
290 295 300

Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
305 310 315 320

Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
325 330 335

Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
340 345 350

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
355 360 365

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
370 375 380

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
385 390 395 400

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
405 410 415

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
420 425 430

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
435 440

<210> 39

<211> 469

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-3 polypeptide CAR sequence

<400> 39

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr
245 250 255

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala
260 265 270

Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe
275 280 285

Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val
290 295 300

Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys
305 310 315 320

Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
325 330 335

Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
340 345 350

Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
355 360 365

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
370 375 380

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
385 390 395 400

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
405 410 415

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
420 425 430

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
435 440 445

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
450 455 460

Ala Leu Pro Pro Arg
465

<210> 40

<211> 472

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-4 polypeptide CAR sequence

<400> 40

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe

50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr
245 250 255

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala
260 265 270

Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe
275 280 285

Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu
290 295 300

Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg
305 310 315 320

Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro
325 330 335

Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu
340 345 350

Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala
355 360 365

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
370 375 380

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
385 390 395 400

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
405 410 415

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
420 425 430

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
435 440 445

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
450 455 460

His Met Gln Ala Leu Pro Pro Arg
465 470

<210> 41

<211> 655

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-5 polypeptide CAR sequence

<400> 41

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Glu Pro Lys Ser Pro Asp Lys Thr His Thr
245 250 255

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
260 265 270

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu

275 280 285

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
290 295 300

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
325 330 335

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Glu

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile
465 470 475 480

Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val
485 490 495

Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
500 505 510

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
515 520 525

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg
530 535 540

Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln
545 550 555 560

Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp
565 570 575

Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro
580 585 590

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
595 600 605

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
610 615 620

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
625 630 635 640

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
645 650 655

<210> 42

<211> 658

<212> PRT

<213> artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> My9-6-6 polypeptide CAR sequence

<400> 42

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Val
35 40 45

Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Thr Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln Ser Pro Ser Ser Leu
130 135 140

Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln
145 150 155 160

Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr Leu Ala Trp Tyr Gln
165 170 175

Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser Glu Asp Leu Ala Ile
210 215 220

Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr Phe Gly Gly Gly Thr
225 230 235 240

Lys Leu Glu Ile Lys Arg Glu Pro Lys Ser Pro Asp Lys Thr His Thr
245 250 255

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
260 265 270

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu
275 280 285

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
290 295 300

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu

325 330 335

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile Ser
465 470 475 480

Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe
485 490 495

Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu
500 505 510

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
515 520 525

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
530 535 540

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
545 550 555 560

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
565 570 575

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
580 585 590

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
595 600 605

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
610 615 620

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
625 630 635 640

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
645 650 655

Pro Arg

<210> 43

<211> 49

<212> PRT

<213> Artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> Target TALEN TRAC_T01

<400> 43

Thr Thr Gly Thr Cys Cys Cys Ala Cys Ala Gly Ala Thr Ala Thr Cys
1 5 10 15

Cys Ala Gly Ala Ala Cys Cys Cys Thr Gly Ala Cys Cys Cys Thr Gly

20 25 30

Cys Cys Gly Thr Gly Thr Ala Cys Cys Ala Gly Cys Thr Gly Ala Gly
35 40 45

Ala

<210> 44
<211> 530
<212> PRT
<213> Artificial sequence

<220>
<223> description of artificial sequence: synthetic oligopeptide

<220>
<223> TAL binding domain TRAC_T01-L

<400> 44
Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys
1 5 10 15

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
20 25 30

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly
35 40 45

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
50 55 60

Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
65 70 75 80

Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
85 90 95

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
100 105 110

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
115 120 125

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
130 135 140

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
145 150 155 160

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
165 170 175

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
180 185 190

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
195 200 205

Gln Val Val Ala Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu
210 215 220

Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
225 230 235 240

Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala
245 250 255

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
260 265 270

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Ile Gly Gly Lys
275 280 285

Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala
290 295 300

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly
305 310 315 320

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
325 330 335

Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn
340 345 350

Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val
355 360 365

Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala
370 375 380

Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
385 390 395 400

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
405 410 415

Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala
420 425 430

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
435 440 445

Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val
450 455 460

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
465 470 475 480

Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu
485 490 495

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
500 505 510

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
515 520 525

Leu Glu
530

<210> 45

<211> 530

<212> PRT

<213> Artificial sequence

<220>

<223> description of artificial sequence: synthetic oligopeptide

<220>

<223> TAL binding domain TRAC_T01-R

<400> 45

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
1 5 10 15

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
20 25 30

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly
35 40 45

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
50 55 60

Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His
65 70 75 80

Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
85 90 95

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
100 105 110

Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu
115 120 125

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
130 135 140

Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
145 150 155 160

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
165 170 175

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
180 185 190

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
195 200 205

Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu
210 215 220

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
225 230 235 240

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala
245 250 255

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
260 265 270

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
275 280 285

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
290 295 300

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly
305 310 315 320

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
325 330 335

Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn
340 345 350

Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val

355 360 365

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
370 375 380

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
385 390 395 400

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
405 410 415

Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala
420 425 430

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
435 440 445

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
450 455 460

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
465 470 475 480

Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu
485 490 495

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
500 505 510

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
515 520 525

Leu Glu
530

<210> 46

<211> 2814

<212> DNA

<213> Artificial sequence

<220>

<223> description of artificial sequence: synthetic polynucleotide

<220>

<223> polynucleotide encoding TRAC_T01-L TALEN

<400> 46

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aaaccgaagg ttcgttcgac agtggcgcag caccacgagg cactggtcgg ccacgggttt    180
acacacgcgc acatcgttgc gttaagccaa cacccggcag cgttagggac cgtcgctgtc    240
aagtatcagg acatgatcgc agcgttgcca gaggcgacac acgaagcgat cgttggcgtc    300
ggcaaacagt ggtccggcgc acgcgctctg gaggccttgc tcacggtggc gggagagttg    360
agaggtccac cgttacagtt ggacacaggc caacttctca agattgcaaa acgtggcggc    420
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gtggccatcg ccagcaataa tggtggcaag caggcgctgg agacggtcca gcggctgttg    660
ccggtgctgt gccaggccca cggcttgacc ccccagcagg tggtggccat cgccagcaat    720
ggcggtggca agcaggcgct ggagacggtc cagcggctgt tgccggtgct gtgccaggcc    780

cacggcttga ccccggagca ggtggtggcc atcgccagcc acgatggcgg caagcaggcg    840
ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gaccccggag    900
caggtggtgg ccatcgccag ccacgatggc ggcaagcagg cgctggagac ggtccagcgg    960
ctgttgccgg tgctgtgcca ggcccacggc ttgaccccgg agcaggtggt ggccatcgcc   1020
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caggcccacg gcttgacccc ggagcaggtg gtggccatcg ccagcaatat tggtggcaag   1140
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ccggagcagg tggtggccat cgccagccac gatggcggca agcaggcgct ggagacggtc   1260
cagcggctgt tgccggtgct gtgccaggcc cacggcttga ccccggagca ggtggtggcc   1320
atcgccagca atattggtgg caagcaggcg ctggagacgg tccagcggct gttgccggtg   1380
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ctgtgccagg cccacggctt gacccccccag caggtggtgg ccatcgccag caataatggt   1440
ggcaagcagg cgctggagac ggtccagcgg ctgttgccgg tgctgtgcca ggcccacggc   1500
ttgaccccgg agcaggtggt ggccatcgcc agcaatattg gtggcaagca ggcgctggag   1560
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gtggccatcg ccagcaatgg cggtggcaag caggcgctgg agacggtcca gcggctgttg   1680
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<210> 47

<211> 2832

<212> DNA

<213> Artificial sequence

<220>

<223> description of artificial sequence: synthetic polynucleotide

<220>

<223> polynucleotide encoding TRAC_T01-R TALEN

<400> 47

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cagcaacagg agaagatcaa accgaaggtt cgttcgacag tggcgcagca ccacgaggca    180
ctggtcggcc acgggtttac acacgcgcac atcgttgcgt taagccaaca cccggcagcg    240
ttagggaccg tcgctgtcaa gtatcaggac atgatcgcag cgttgccaga ggcgacacac    300
gaagcgatcg ttggcgtcgg caaacagtgg tccggcgcac gcgctctgga ggccttgctc    360
acggtggcgg gagagttgag aggtccaccg ttacagttgg acacaggcca acttctcaag    420
attgcaaaac gtggcggcgt gaccgcagtg gaggcagtgc atgcatggcg caatgcactg    480
acgggtgccc cgctcaactt gaccccggag caggtggtgg ccatcgccag ccacgatggc    540
ggcaagcagg cgctggagac ggtccagcgg ctgttgccgg tgctgtgcca ggcccacggc    600
ttgacccccc agcaggtggt ggccatcgcc agcaatggcg gtggcaagca ggcgctggag    660
acggtccagc ggctgttgcc ggtgctgtgc caggcccacg gcttgacccc ggagcaggtg    720
gtggccatcg ccagccacga tggcggcaag caggcgctgg agacggtcca gcggctgttg    780
ccggtgctgt gccaggccca cggcttgacc ccggagcagg tggtggccat cgccagcaat    840

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cacggcttga ccccccagca ggtggtggcc atcgccagca ataatggtgg caagcaggcg    960
ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gaccccggag   1020
caggtggtgg ccatcgccag ccacgatggc ggcaagcagg cgctggagac ggtccagcgg   1080
ctgttgccgg tgctgtgcca ggcccacggc ttgacccccc agcaggtggt ggccatcgcc   1140
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cagttatctc gccctgatcc ggcgttggcc gcgttgacca acgaccacct cgtcgccttg   2160
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gaccgtatcc tggagatgaa ggtgatggag ttcttcatga aggtgtacgg ctacaggggc   2400
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caggccgacg aaatgcagag gtacgcggag gagaaccaga ccaggaacaa gcacaccaac   2580
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<210> 48

<211> 457

<212> PRT

<213> Artificial sequence

<220>

<223> M195-1

<400> 48

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            20                  25                  30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
        35                  40                  45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
    50                  55                  60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65                  70                  75                  80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
                85                  90                  95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
            100                 105                 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
    130                 135                 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145                 150                 155                 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
                165                 170                 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
            180                 185                 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
        195                 200                 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
    210                 215                 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225                 230                 235                 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
                245                 250                 255

Gly Thr Lys Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser
            260                 265                 270

Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly
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275 280 285

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
290 295 300

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
305 310 315 320

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro

325 330 335

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
340 345 350

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
355 360 365

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
370 375 380

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
385 390 395 400

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
405 410 415

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
420 425 430

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
435 440 445

Leu His Met Gln Ala Leu Pro Pro Arg
450 455

<210> 49

<211> 460

<212> PRT

<213> Artificial sequence

<220>

<223> M195-2

<400> 49

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
50 55 60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
85 90 95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145 150 155 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
165 170 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
180 185 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
195 200 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
210 215 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225 230 235 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
245 250 255

Gly Thr Lys Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser
260 265 270

Phe Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr
275 280 285

Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser
290 295 300

Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
305 310 315 320

Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys

325 330 335

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
340 345 350

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
355 360 365

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp
370 375 380

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
385 390 395 400

Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
405 410 415

Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys
420 425 430

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
435 440 445

Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455 460

<210> 50
<211> 486
<212> PRT
<213> Artificial sequence

<220>
<223> M195-3

<400> 50
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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
            20                  25                  30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
        35                  40                  45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
    50                  55                  60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65                  70                  75                  80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
                85                  90                  95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
            100                 105                 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
    130                 135                 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145                 150                 155                 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
                165                 170                 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
            180                 185                 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
        195                 200                 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
    210                 215                 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225                 230                 235                 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
                245                 250                 255

Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
    290                 295                 300
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Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305 310 315 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg

325 330 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
340 345 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
355 360 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
370 375 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385 390 395 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
405 410 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
420 425 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
435 440 445

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
450 455 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465 470 475 480

Gln Ala Leu Pro Pro Arg
485

<210> 51

<211> 489

<212> PRT

<213> Artificial sequence

<220>

<223> M195-4

<400> 51

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
50 55 60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
85 90 95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
100 105 110

100 105 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145 150 155 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
165 170 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
180 185 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
195 200 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
210 215 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225 230 235 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
245 250 255

Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro
260 265 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
275 280 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp

290 295 300

Phe Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala
305 310 315 320

Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys
325 330 335

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
340 345 350

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
355 360 365

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
370 375 380

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
385 390 395 400

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
405 410 415

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
420 425 430

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
435 440 445

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp

450 455 460

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
465 470 475 480

Leu His Met Gln Ala Leu Pro Pro Arg
485

<210> 52
<211> 672
<212> PRT
<213> Artificial sequence

<220>
<223> M195-5

<400> 52
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
50 55 60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
85 90 95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145 150 155 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
165 170 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
180 185 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
195 200 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
210 215 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225 230 235 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
245 250 255

Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr His

260 265 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
275 280 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
290 295 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305 310 315 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
325 330 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
340 345 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
355 360 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
370 375 380

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
385 390 395 400

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
405 410 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
420 425 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
435 440 445

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
450 455 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465 470 475 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr
485 490 495

Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
500 505 510

Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
515 520 525

Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
530 535 540

Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
545 550 555 560

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
565 570 575

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
580 585 590

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
595 600 605

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
610 615 620

610 615 620

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
625 630 635 640

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
645 650 655

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
660 665 670

<210> 53
<211> 675
<212> PRT
<213> Artificial sequence

<220>
<223> M195-6

<400> 53
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr

35 40 45

Thr Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys
50 55 60

Ser Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Tyr Asn Gly Gly Thr Gly
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser
85 90 95

Ser Ser Thr Ala Tyr Met Asp Val Arg Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Pro Ala Met Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro
145 150 155 160

Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg
165 170 175

Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Asn Trp Phe
180 185 190

Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser
195 200 205

Asn Gln Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
210 215 220

Thr Asp Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala
225 230 235 240

Met Tyr Phe Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly
245 250 255

Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr His
260 265 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
275 280 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
290 295 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305 310 315 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
325 330 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
340 345 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
355 360 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
370 375 380

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
385 390 395 400

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
405 410 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
420 425 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
435 440 445

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
450 455 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465 470 475 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile
485 490 495

Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe
500 505 510

Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu
515 520 525

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
530 535 540

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
545 550 555 560

Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
565 570 575

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
580 585 590

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
595 600 605

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
610 615 620

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
625 630 635 640

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
645 650 655

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
660 665 670

Pro Pro Arg
675

<210> 54

<211> 455

<212> PRT

<213> Artificial sequence

<220>

<223> m2H12-1

<400> 54

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe
260 265 270

Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
275 280 285

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly
290 295 300

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
305 310 315 320

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
325 330 335

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
340 345 350

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
355 360 365

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
370 375 380

Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
385 390 395 400

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
405 410 415

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
420 425 430

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
435 440 445

Met Gln Ala Leu Pro Pro Arg
450 455

<210> 55

<211> 458

<212> PRT

<213> Artificial sequence

<220>

<223> m2H12-2

<400> 55

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys

65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe
260 265 270

Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr
275 280 285

Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val
290 295 300

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
305 310 315 320

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
325 330 335

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
340 345 350

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
355 360 365

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
370 375 380

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
385 390 395 400

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
405 410 415

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
420 425 430

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
435 440 445

Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455

<210> 56
<211> 484
<212> PRT
<213> Artificial sequence

<220>
<223> m2H12-3

<400> 56
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro
260 265 270

Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys

275 280 285

Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala
290 295 300

Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu
305 310 315 320

Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys
325 330 335

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
340 345 350

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
355 360 365

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
370 375 380

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
385 390 395 400

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
405 410 415

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
420 425 430

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
435 440 445

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
450 455 460

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
465 470 475 480

Leu Pro Pro Arg

<210> 57
<211> 487
<212> PRT
<213> Artificial sequence

<220>
<223> m2H12-4

<400> 57
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro
260 265 270

Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys
275 280 285

Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala
290 295 300

Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu
305 310 315 320

Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly
325 330 335

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
340 345 350

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
355 360 365

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
370 375 380

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
385 390 395 400

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
405 410 415

Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
420 425 430

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
435 440 445

Ile Glu Met Lys Glu Glu Arg Arg Arg Glu Lys Glu His Asp Glu Leu

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
450 455 460

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
465 470 475 480

Met Gln Ala Leu Pro Pro Arg
485

<210> 58
<211> 670
<212> PRT
<213> Artificial sequence

<220>
<223> m2H12-5

<400> 58
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys
260 265 270

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp
485 490 495

Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile
500 505 510

Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys
515 520 525

Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys
530 535 540

Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val
545 550 555 560

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
565 570 575

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
580 585 590

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg
595 600 605

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
610 615 620

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

625 630 635 640

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
645 650 655

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
660 665 670

<210> 59
<211> 673
<212> PRT
<213> Artificial sequence

<220>
<223> m2H12-6

<400> 59
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
20 25 30

Val Arg Pro Gly Thr Phe Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Asn Tyr Asp Ile Asn Trp Val Asn Gln Arg Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Tyr Pro Gly Asp Gly Ser Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Ala Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Leu Gln Leu Asn Asn Leu Thr Ser Glu Asn Ser
100 105 110

Ala Val Tyr Phe Cys Ala Ser Gly Tyr Glu Asp Ala Met Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Lys Met Thr Gln Ser
145 150 155 160

Pro Ser Ser Met Tyr Ala Ser Leu Gly Glu Arg Val Ile Ile Asn Cys
165 170 175

Lys Ala Ser Gln Asp Ile Asn Ser Tyr Leu Ser Trp Phe Gln Gln Lys
180 185 190

Pro Gly Lys Ser Pro Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Val
195 200 205

Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr
210 215 220

Ser Leu Thr Ile Ser Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr
225 230 235 240

Cys Leu Gln Tyr Asp Glu Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys
245 250 255

Leu Glu Leu Lys Arg Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys
260 265 270

Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe
275 280 285

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val
290 295 300

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
305 310 315 320

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
325 330 335

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
340 345 350

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
355 360 365

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
370 375 380

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
385 390 395 400

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
405 410 415

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
420 425 430

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
435 440 445

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
450 455 460

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
465 470 475 480

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile Ser Phe
485 490 495

Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu
500 505 510

Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr
515 520 525

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
530 535 540

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu

545 550 555 560

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
565 570 575

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
580 585 590

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
595 600 605

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
610 615 620

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
625 630 635 640

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
645 650 655

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
660 665 670

Arg

<210> 60

<211> 465

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-1

<400> 60

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Gly
260 265 270

Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile
275 280 285

Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser
290 295 300

Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr
305 310 315 320

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu

325 330 335

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
340 345 350

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
355 360 365

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
370 375 380

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
385 390 395 400

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
405 410 415

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
420 425 430

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
435 440 445

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
450 455 460

Arg
465

<210> 61

<211> 468

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-2

<400> 61

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

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Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Gly
260 265 270

Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile
275 280 285

Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu
290 295 300

Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys

305 310 315 320

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
325 330 335

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
340 345 350

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
355 360 365

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
370 375 380

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
385 390 395 400

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
405 410 415

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
420 425 430

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
435 440 445

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
450 455 460

Leu Pro Pro Arg
465

<210> 62

<211> 494

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-3

<400> 62

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Thr
260 265 270

Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser
275 280 285

Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly

290 295 300

Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp
305 310 315 320

Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile
325 330 335

Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys
340 345 350

Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys
355 360 365

Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val
370 375 380

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
385 390 395 400

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
405 410 415

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg
420 425 430

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
435 440 445

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg
450 455 460

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
465 470 475 480

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 63

<211> 497

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-4

<400> 63

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Glu Ala Ser Val Lys Met Ser Cys Lys Ala Ser Glu Tyr

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Thr

260 265 270

Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser
275 280 285

Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly
290 295 300

Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Ile Ser Phe
305 310 315 320

Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu
325 330 335

Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr
340 345 350

Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
355 360 365

Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
370 375 380

Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln
385 390 395 400

Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu
405 410 415

Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly
420 425 430

Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
435 440 445

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu
450 455 460

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
465 470 475 480

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
485 490 495

Arg

<210> 64

<211> 680

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-5

<400> 64

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr

210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Glu
260 265 270

Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
275 280 285

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
290 295 300

Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305 310 315 320

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
325 330 335

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
340 345 350

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
355 360 365

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
370 375 380

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385 390 395 400

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
405 410 415

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
420 425 430

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
435 440 445

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
450 455 460

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465 470 475 480

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
485 490 495

Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
500 505 510

Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg
515 520 525

Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro
530 535 540

Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu
545 550 555 560

Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala
565 570 575

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
580 585 590

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
595 600 605

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
610 615 620

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
625 630 635 640

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
645 650 655

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
660 665 670

His Met Gln Ala Leu Pro Pro Arg
675 680

<210> 65

<211> 683

<212> PRT

<213> Artificial sequence

<220>

<223> DRB2-6

<400> 65

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Glu Leu
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Lys Phe Thr Asp Tyr Val Val His Trp Leu Lys Gln Lys Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Tyr Asn Asp Gly Thr Lys
65 70 75 80

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ser Asp Lys Ser
85 90 95

Ser Ser Thr Ala Tyr Met Glu Val Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Arg Tyr Glu Val Tyr Gly Met
115 120 125

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Arg Val Thr
165 170 175

Met Thr Cys Thr Ala Ser Ser Ser Val Asn Tyr Ile His Trp Tyr Gln
180 185 190

Gln Lys Ser Gly Asp Ser Pro Leu Arg Trp Ile Phe Asp Thr Ser Lys
195 200 205

Val Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
210 215 220

Ser Tyr Ser Leu Thr Ile Ser Thr Met Glu Ala Glu Asp Ala Ala Thr
225 230 235 240

Tyr Tyr Cys Gln Gln Trp Arg Ser Tyr Pro Leu Thr Phe Gly Asp Gly
245 250 255

Thr Arg Leu Glu Leu Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Glu
260 265 270

Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
275 280 285

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
290 295 300

Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305 310 315 320

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
325 330 335

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
340 345 350

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
355 360 365

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
370 375 380

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385 390 395 400

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
405 410 415

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
420 425 430

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
435 440 445

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
450 455 460

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465 470 475 480

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
485 490 495

Ser Leu Ser Pro Gly Lys Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser
500 505 510

Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val
515 520 525

Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
530 535 540

Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
545 550 555 560

Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
565 570 575

Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
580 585 590

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
595 600 605

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
610 615 620

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
625 630 635 640

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
645 650 655

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
660 665 670

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
675 680

<210> 66

<211> 461

<212> PRT

<213> Artificial sequence

<220>

<223> My9.6-1

<400> 66

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
115 120 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145 150 155 160

Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
195 200 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225 230 235 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
245 250 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Leu Ala Val Ser
260 265 270

Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala
275 280 285

Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr
290 295 300

Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
305 310 315 320

Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
325 330 335

Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
340 345 350

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
355 360 365

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
370 375 380

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
385 390 395 400

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
405 410 415

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
420 425 430

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
435 440 445

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455 460

<210> 67

<211> 464

<212> PRT

<213> Artificial sequence

<220>

<223> My9.6-2

<400> 67

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
            20                  25                  30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
        35                  40                  45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
    50                  55                  60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65                  70                  75                  80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
                85                  90                  95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
            100                 105                 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
        115                 120                 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145                 150                 155                 160

Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
                165                 170                 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
            180                 185                 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
        195                 200                 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
    210                 215                 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225                 230                 235                 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
                245                 250                 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Gly Leu Ala Val Ser
            260                 265                 270

Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe
        275                 280                 285

Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr
    290                 295                 300

Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
305                 310                 315                 320

Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
                325                 330                 335
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Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
340 345 350

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
355 360 365

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
370 375 380

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
385 390 395 400

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
405 410 415

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
420 425 430

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
435 440 445

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455 460

<210> 68

<211> 490

<212> PRT

<213> Artificial sequence

<220>

<223> My9.6-3

<400> 68

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
115 120 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145 150 155 160

Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
195 200 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225 230 235 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
245 250 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala
260 265 270

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser
275 280 285

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr
290 295 300

Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala
305 310 315 320

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
325 330 335

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
340 345 350

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
355 360 365

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg
370 375 380

Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn
385 390 395 400

Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg
405 410 415

Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro
420 425 430

Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala
435 440 445

Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His
450 455 460

Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp
465 470 475 480

Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 69

<211> 493

<212> PRT

<213> Artificial sequence

<220>

<223> My9.6-4

<400> 69

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
            20                  25                  30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
        35                  40                  45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
    50                  55                  60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65                  70                  75                  80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
                85                  90                  95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
            100                 105                 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
        115                 120                 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145                 150                 155                 160

Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
                165                 170                 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
            180                 185                 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
        195                 200                 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
    210                 215                 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225                 230                 235                 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
                245                 250                 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala
            260                 265                 270

Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser
        275                 280                 285

Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr
    290                 295                 300

Arg Gly Leu Asp Phe Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu
305                 310                 315                 320

Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe
                325                 330                 335

Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
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340 345 350

Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
355 360 365

Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
370 375 380

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
385 390 395 400

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
405 410 415

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
420 425 430

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
435 440 445

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
450 455 460

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
465 470 475 480

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 70
<211> 676
<212> PRT
<213> Artificial sequence

<220>
<223> My9.6-5

<400> 70
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
115 120 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145 150 155 160

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Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
195 200 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225 230 235 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
245 250 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Glu Pro Lys Ser Pro
260 265 270

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
275 280 285

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
290 295 300

Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
305 310 315 320

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
325 330 335

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
340 345 350

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
355 360 365

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
370 375 380

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
385 390 395 400

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
405 410 415

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
420 425 430

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
435 440 445

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
450 455 460

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
465 470 475 480

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
485 490 495

Gly Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu
500 505 510

Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys

515 520 525

Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr
530 535 540

Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly
545 550 555 560

Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
565 570 575

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
580 585 590

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
595 600 605

Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
610 615 620

Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
625 630 635 640

Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
645 650 655

Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
660 665 670

Leu Pro Pro Arg
675

<210> 71

<211> 679

<212> PRT

<213> Artificial sequence

<220>

<223> My9.6-6

<400> 71

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Val
20 25 30

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Ile His Trp Ile Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Val Gly Val Ile Tyr Pro Gly Asn Asp Asp Ile Ser
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
85 90 95

Ser Thr Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Glu Val Arg Leu Arg Tyr Phe Asp Val
115 120 125

Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Met Leu Thr Gln
145 150 155 160

Ser Pro Ser Ser Leu Ala Val Ser Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Val Phe Phe Ser Ser Ser Gln Lys Asn Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Ile Pro Gly Gln Ser Pro Lys Leu Leu Ile
195 200 205

Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro Asp Arg Phe Thr Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ser
225 230 235 240

Glu Asp Leu Ala Ile Tyr Tyr Cys His Gln Tyr Leu Ser Ser Arg Thr
245 250 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Glu Pro Lys Ser Pro
260 265 270

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
275 280 285

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
290 295 300

Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
305 310 315 320

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
325 330 335

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
340 345 350

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
355 360 365

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
370 375 380

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
385 390 395 400

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
405 410 415

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
420 425 430

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
435 440 445

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
450 455 460

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His

465 470 475 480

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
485 490 495

Gly Lys Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu
500 505 510

Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly
515 520 525

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
530 535 540

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
545 550 555 560

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp
565 570 575

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
580 585 590

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
595 600 605

Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly
610 615 620

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
625 630 635 640

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
645 650 655

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
660 665 670

Met Gln Ala Leu Pro Pro Arg
675

<210> 72
<211> 44
<212> DNA
<213> Artificial sequence

<220>
<223> TALEN1_CD33_Exon3

<400> 72
tgcatcccct ctttctcctc actagacttg acccacaggc ccaa 44

<210> 73
<211> 49
<212> DNA
<213> Artificial sequence

<220>
<223> TALEN2_CD33_Exon3

<400> 73
ttctcctcac tagacttgac ccacaggccc aaaatcctca tccctggca 49

<210> 74
<211> 49

<212> DNA
<213> Artificial sequence

<220>
<223> TALEN1_CD33_Exon4

<400> 74
tcctctccta gatgttccac agaacccaac aactggtatc tttccagga 49

<210> 75
<211> 44
<212> DNA
<213> Artificial sequence

<220>
<223> TALEN2_CD33_Exon4

<400> 75
tcctagatgt tccacagaac ccaacaactg gtatctttcc agga 44

<210> 76
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN1 Left exon 3

<400> 76
tgcatcccct ctttctc 17

<210> 77
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN2 Left exon 3

<400> 77
ttctcctcac tagactt 17

<210> 78
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN1 Left exon 4

<400> 78
tcctctccta gatgttc 17

<210> 79
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN2 Left exon 4

<400> 79
tcctagatgt tccacag 17

<210> 80
<211> 10
<212> DNA
<213> Artificial sequence

<220>
<223> spacer 1 (Exon3)

<400> 80
ctcactagac 10

<210> 81
<211> 15
<212> DNA
<213> Artificial sequence

<220>
<223> spacer2 (Exon3)

<400> 81
gacccacagg cccaa 15

<210> 82
<211> 15
<212> DNA
<213> Artificial sequence

<220>
<223> spacer 1 (Exon4)

<400> 82
cacagaaccc aacaa 15

<210> 83
<211> 10
<212> DNA
<213> Artificial sequence

<220>
<223> spacer 2 (Exon4)

<400> 83
aacccaacaa 10

<210> 84
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN1 right exon 3

<400> 84
ttgacccaca ggcccaa 17

<210> 85
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN2 right exon 3

<400> 85
aatcctcatc cctggca 17

<210> 86
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN1 right exon 4

<400> 86
ctggtatctt tccagga 17

<210> 87
<211> 17
<212> DNA
<213> Artificial sequence

<220>
<223> Sequence bound by TALEN2 right exon 4

<400> 87
ctggtatctt tccagga

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. CD33-specifik kimær antigenreceptor (CAR) med en polypeptidstruktur udvalgt blandt V1 og V3 som vist i figur 2, hvor strukturen V1 omfatter (a) et ekstracellulært ligandbindingsdomæne omfattende VH og VL fra et monoklonalt anti-CD33-antistof, (b) et FcγRIIIα-hængsel, (c) et CD8α-transmembrandomæne og (d) et cytoplasmisk domæne indbefattende et CD3-zeta-signaleringsdomæne og et co-stimulerende domæne fra 4-1BB; og hvor strukturen V3 omfatter (a) et ekstracellulært ligandbindingsdomæne omfattende VH og VL fra et monoklonalt anti-CD33-antistof, (b) et CD8α-hængsel, (c) et CD8α-transmembrandomæne og (d) et cytoplasmisk domæne indbefattende et CD3-zeta-signaleringsdomæne og et co-stimulerende domæne fra 4-1BB.

2. CD33-specifik CAR ifølge krav 1, hvor VH'en har mindst 80 % identitet med SEQ ID NO. 11, og VL'en har mindst 80 % identitet med SEQ ID NO. 12, eller VH'en har mindst 80 % identitet med SEQ ID NO. 13, og VL'en har mindst 80 % identitet med SEQ ID NO. 14, eller VH'en har mindst 80 % identitet med SEQ ID NO. 15, og VL'en har mindst 80 % identitet med SEQ ID NO. 16, eller VH'en har mindst 80 % identitet med SEQ ID NO. 17, og VL'en har mindst 80 % identitet med SEQ ID NO. 18.

3. CD33-specifik CAR ifølge krav 1 eller 2, hvor CAR'en har struktur V3, som omfatter (a) et ekstracellulært ligandbindingsdomæne omfattende VH og VL fra et monoklonalt anti-CD33-antistof, (b) et CD8α-hængsel, (c) et CD8α-transmembrandomæne og (d) et cytoplasmisk domæne indbefattende et CD3-zeta-signaleringsdomæne og et co-stimulerende domæne fra 4-1BB.

4. CD33-specifik CAR med struktur V3 ifølge krav 3, som omfatter en polypeptidsekvens med mindst 80 % identitet med SEQ ID NO. 27.

5. CD33-specifik CAR ifølge et hvilket som helst af kravene 1 til 4, yderligere omfattende et signalpeptid.

6. Polynukleotid, der koder for en CAR ifølge et hvilket som helst af kravene 1 til 5.

7. Genmanipuleret immuncelle, der ved celleoverflademembranen eksprimerer en CD33-specifik CAR ifølge et hvilket som helst af kravene 1 til 5.

8. Genmanipuleret immuncelle ifølge krav 7, der stammer fra inflammatoriske T-lymfocytter, cytotoksiske T-lymfocytter, regulatoriske T-lymfocytter eller hjælper-T-lymfocytter.

9. Genmanipuleret immuncelle ifølge krav 8, hvor ekspressionen af TCR er undertrykt.

10. Genmanipuleret immuncelle ifølge et hvilket som helst af kravene 7 til 9, hvor den genmanipulerede immuncelle er modificeret til at være resistent over for mindst et immunundertrykkende eller kemoterapeutisk lægemiddel.

11. Genmanipuleret immuncelle ifølge et hvilket som helst af kravene 7 til 10 til anvendelse i behandling.

12. Genmanipuleret immuncelle ifølge et hvilket som helst af kravene 7 til 10 til anvendelse ved behandling af leukæmi, hvor leukæmien er udvalgt fra gruppen bestående af akut myeloid leukæmi (AML), kronisk myeloid leukæmi, akut lymfoid leukæmi, kronisk lymfoid leukæmi og myelodysplastisk syndrom.

13. Genmanipuleret immuncelle til anvendelse i behandling ifølge krav 12, hvor leukæmien er akut myeloid leukæmi (AML).

14. Genmanipuleret immuncelle ifølge et hvilket som helst af kravene 7 til 10 til anvendelse ved behandling af lymfom, hvor lymfomet er udvalgt fra gruppen bestående af myelomatose, non-Hodgkins lymfom, Burkitts lymfom og follikulært lymfom (småcellet og storcellet).

DRAWINGS

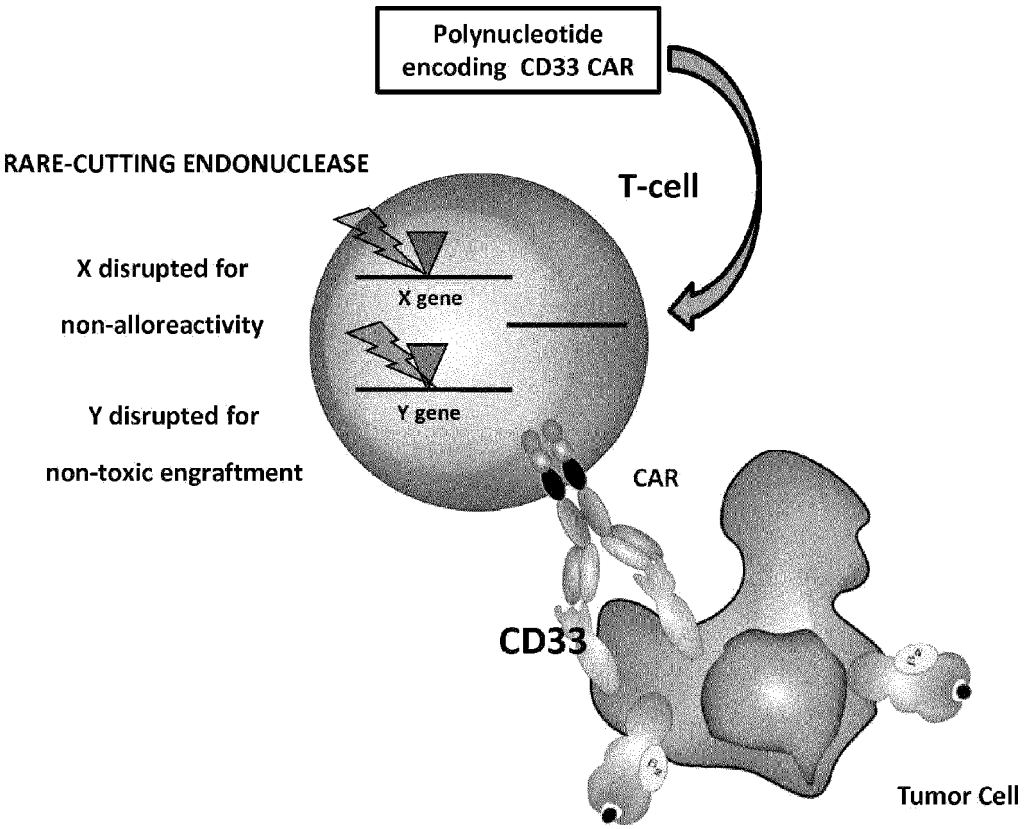

Figure 1

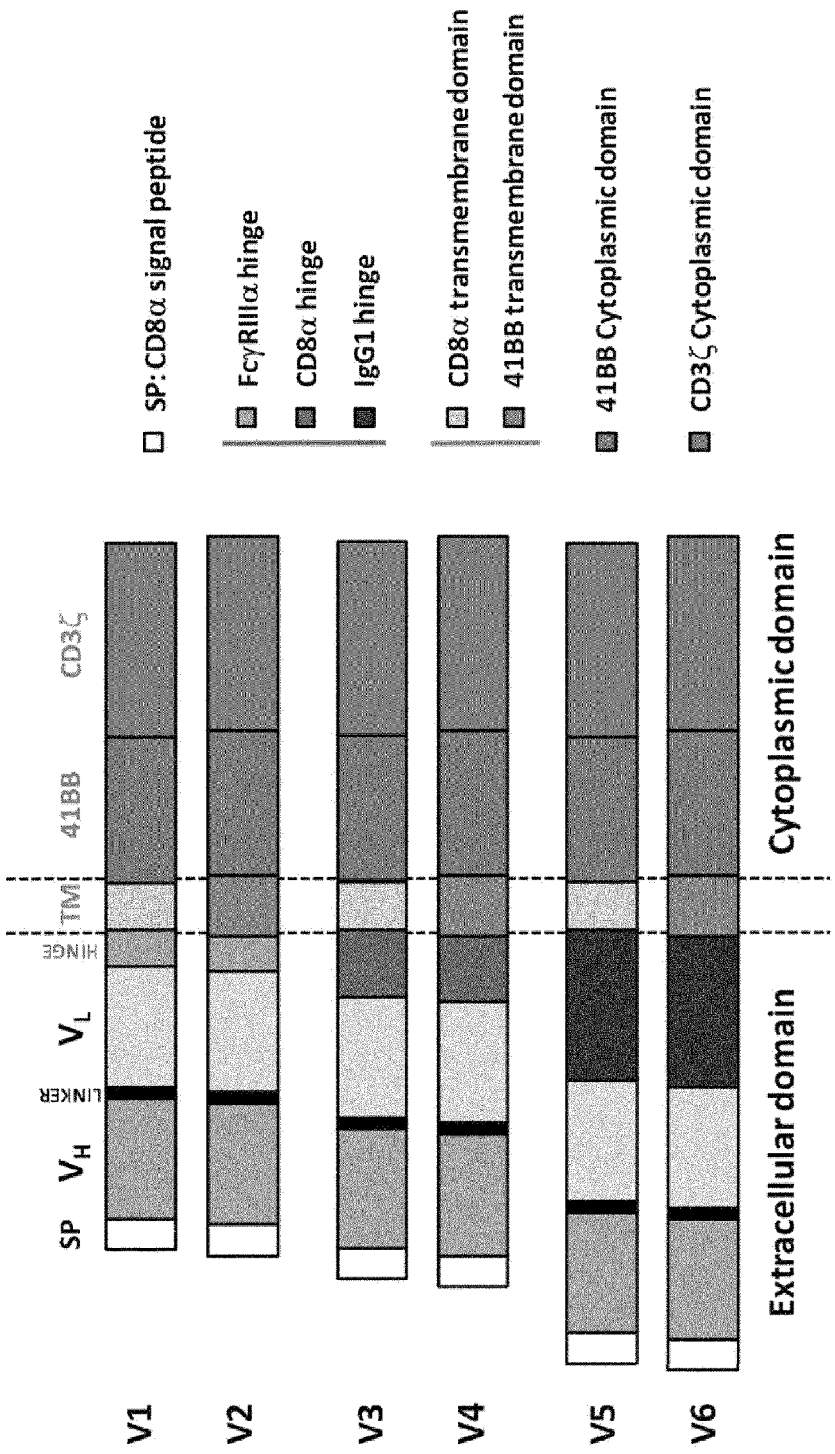
SP: CD8α signal peptide
FcγRIIIα hinge
CD8α hinge
IgG1 hinge
CD8α transmembrane domain
41BB transmembrane domain
41BB Cytoplasmic domain
CD3ζ Cytoplasmic domain
SP
V_H
LINKER
V_L
HINGE
TM
41BB
CD3ζ
Extracellular domain
Cytoplasmic domain
V1
V2
V3
V4
V5
V6

Figure 2

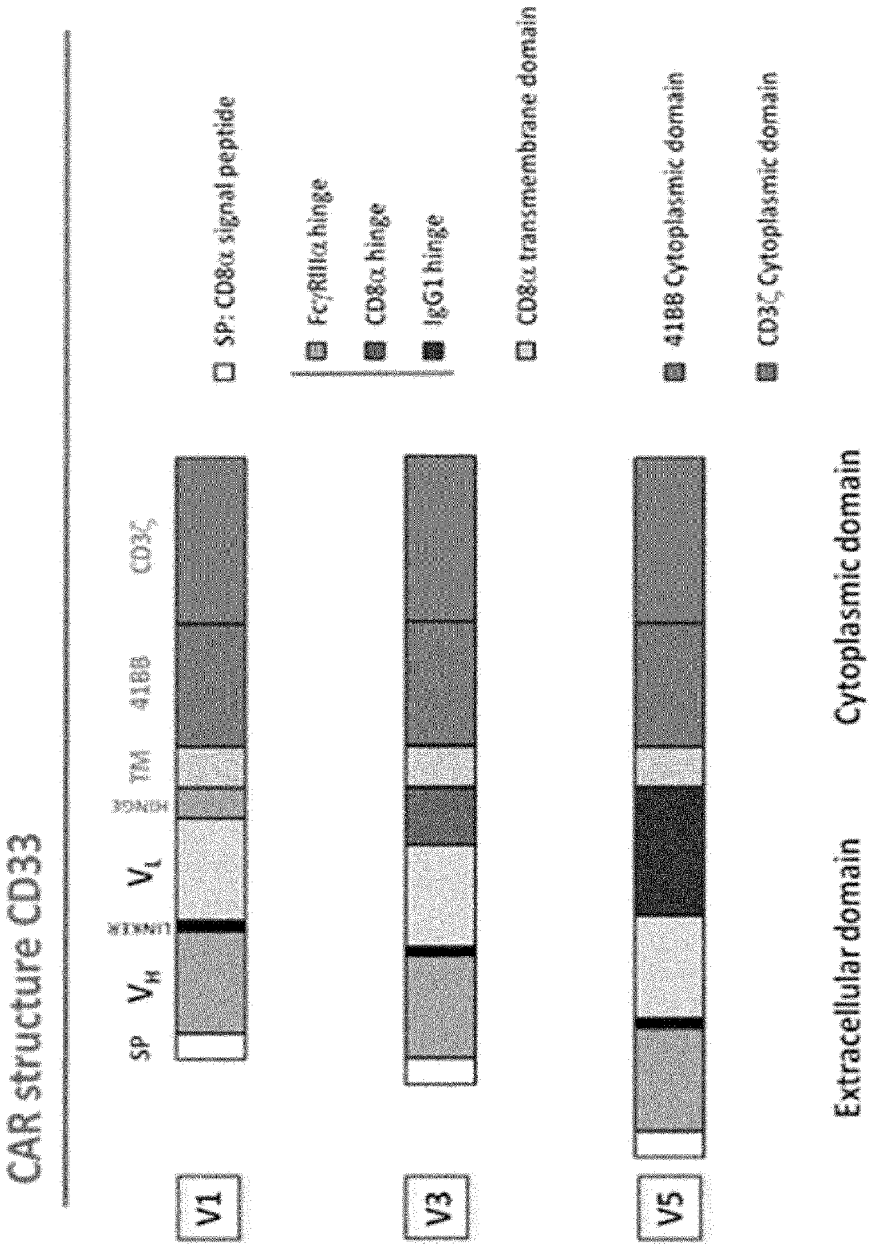
CAR structure CD33
V1
V3
V5
SP
V_H
LINKER
V_L
HINGE
TM
41BB
CD3ζ
SP: CD8α signal peptide
FcγRIIIα hinge
CD8α hinge
IgG1 hinge
CD8α transmembrane domain
41BB Cytoplasmic domain
CD3ζ Cytoplasmic domain
Extracellular domain
Cytoplasmic domain

Figure 3

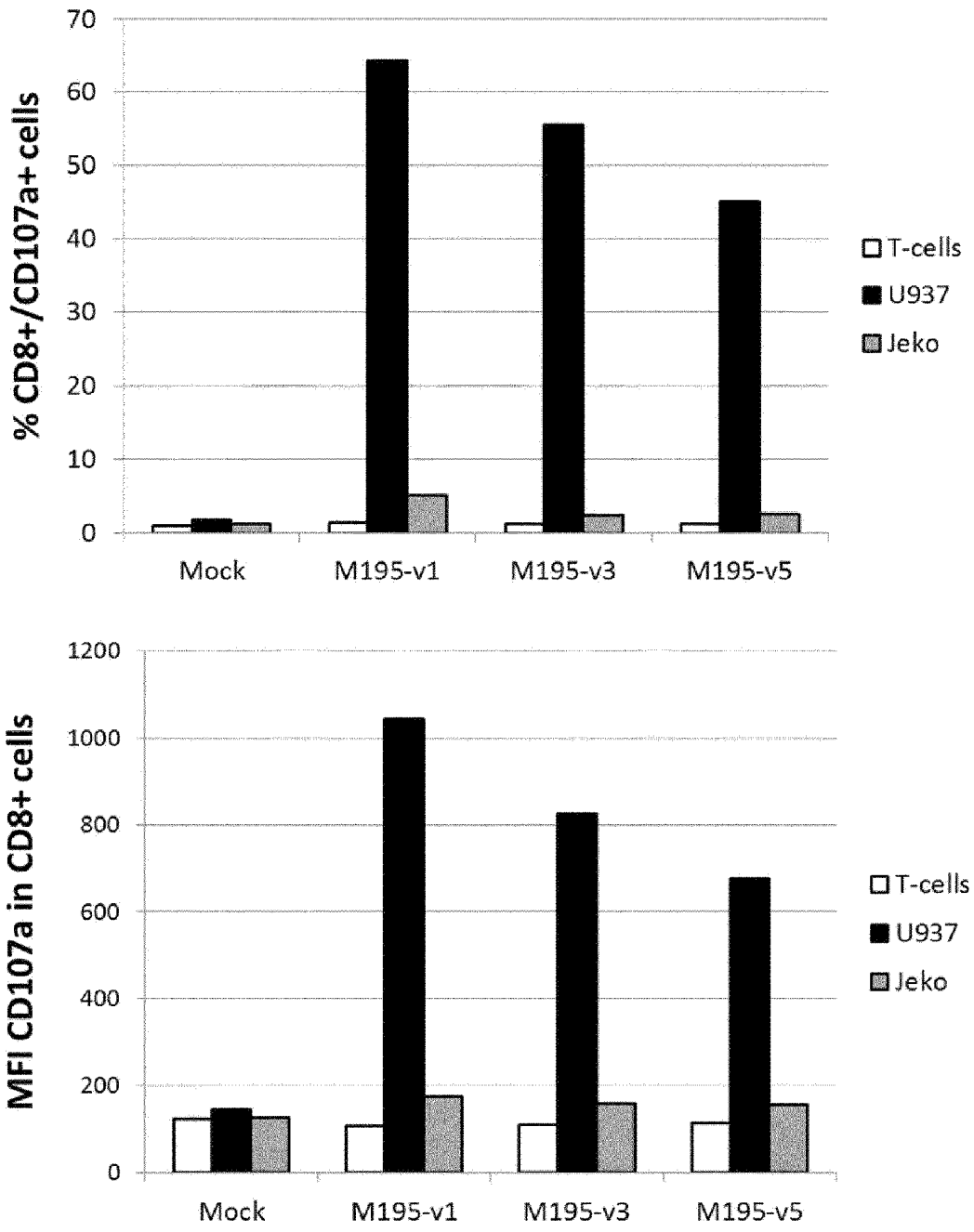
% CD8+/CD107a+ cells
70
60
50
40
30
20
10
0
Mock
M195-v1
M195-v3
M195-v5
T-cells
U937
Jeko
MFI CD107a in CD8+ cells
1200
1000
800
600
400
200
0
Mock
M195-v1
M195-v3
M195-v5
T-cells
U937
Jeko

Figure 4

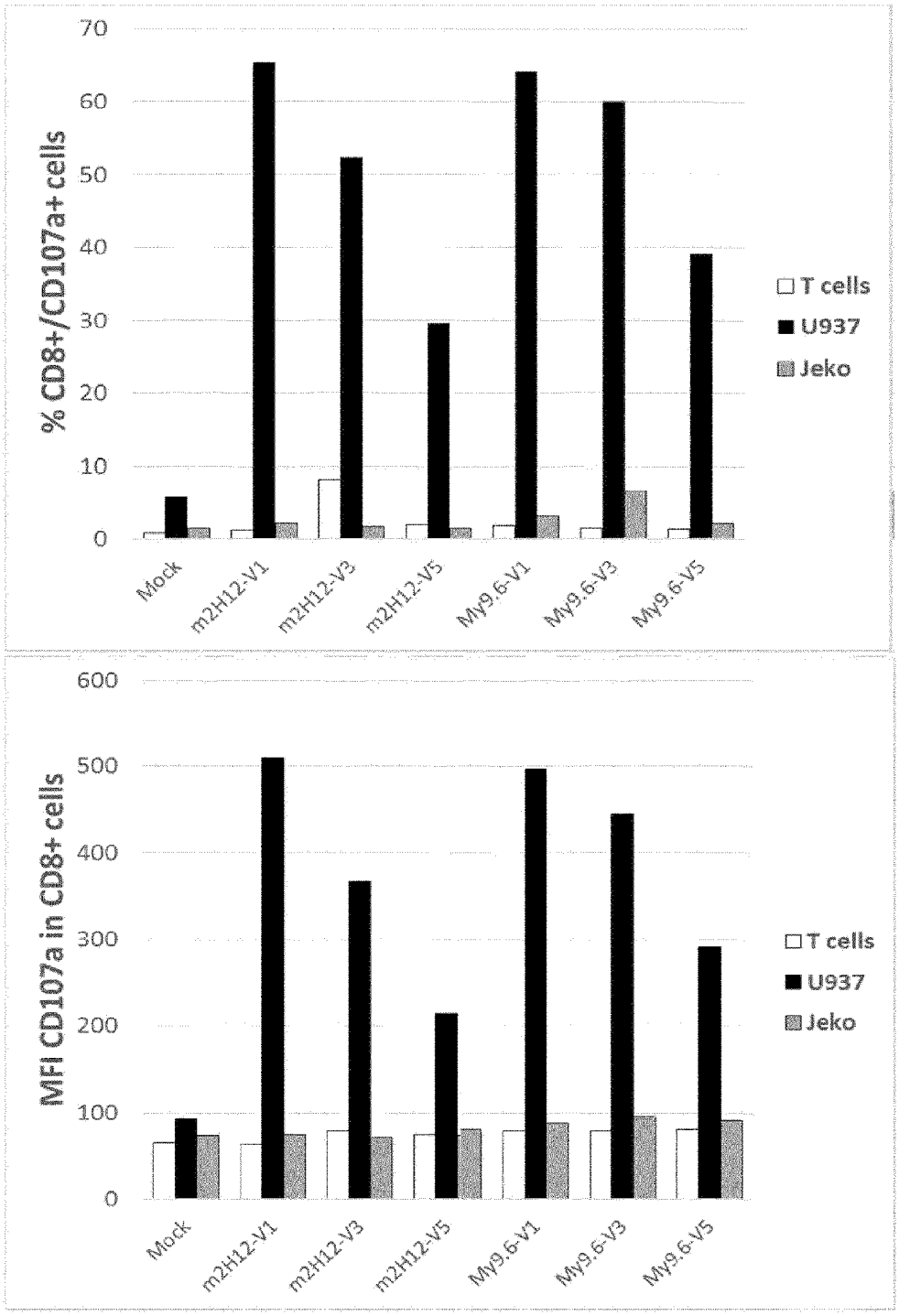
% CD8+/CD107a+ cells
70
60
50
40
30
20
10
0
Mock
m2H12-V1
m2H12-V3
m2H12-V5
My9.6-V1
My9.6-V3
My9.6-V5
T cells
U937
Jeko
MFI CD107a in CD8+ cells
600
500
400
300
200
100
0
Mock
m2H12-V1
m2H12-V3
m2H12-V5
My9.6-V1
My9.6-V3
My9.6-V5
T cells
U937
Jeko

Figure 5

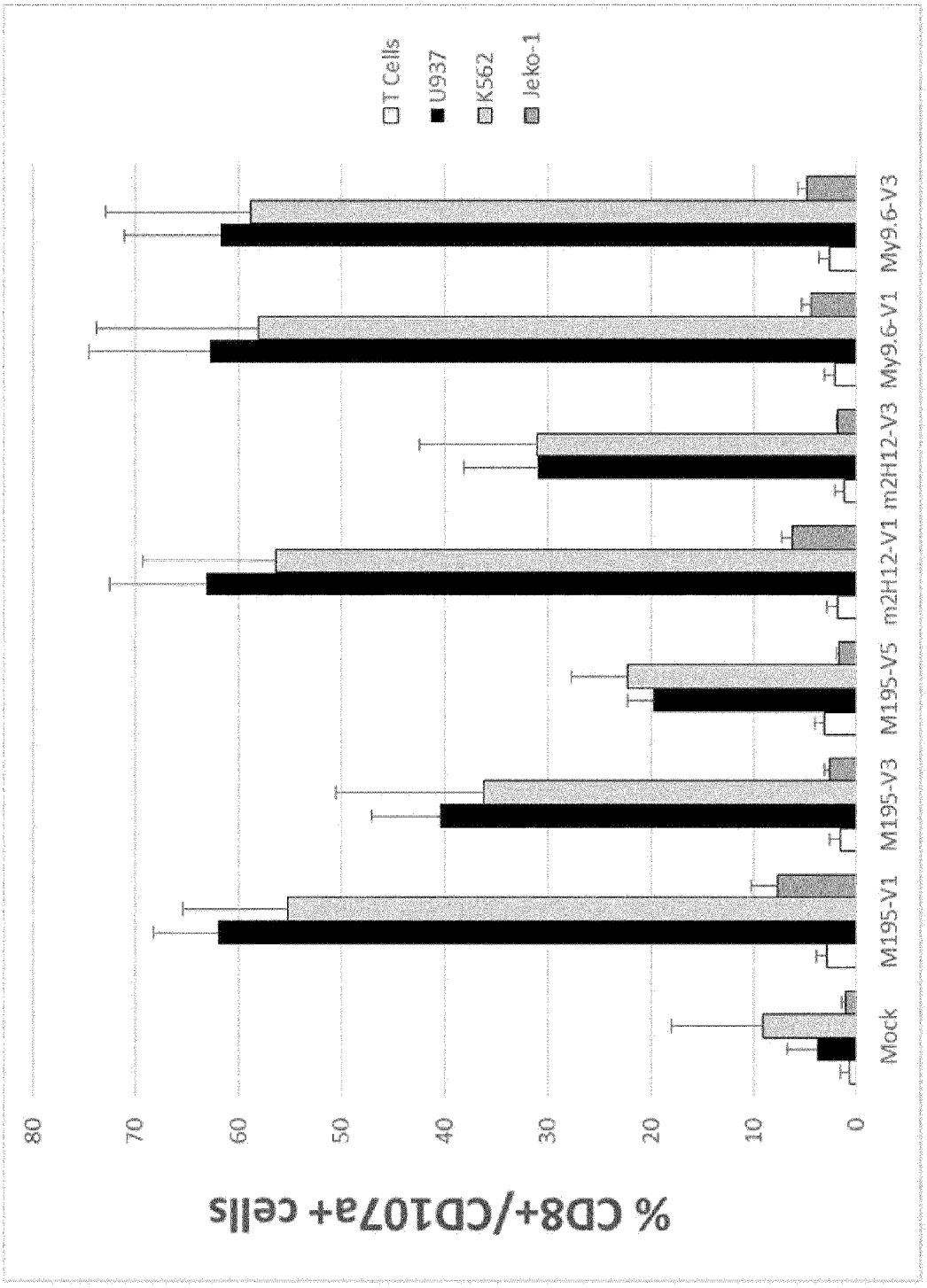
% CD8+/CD107a+ cells
80
70
60
50
40
30
20
10
0
Mock
M195-V1
M195-V3
M195-V5
m2H12-V1
m2H12-V3
My9.6-V1
My9.6-V3
T Cells
U937
K562
Jeko-1

Figure 6

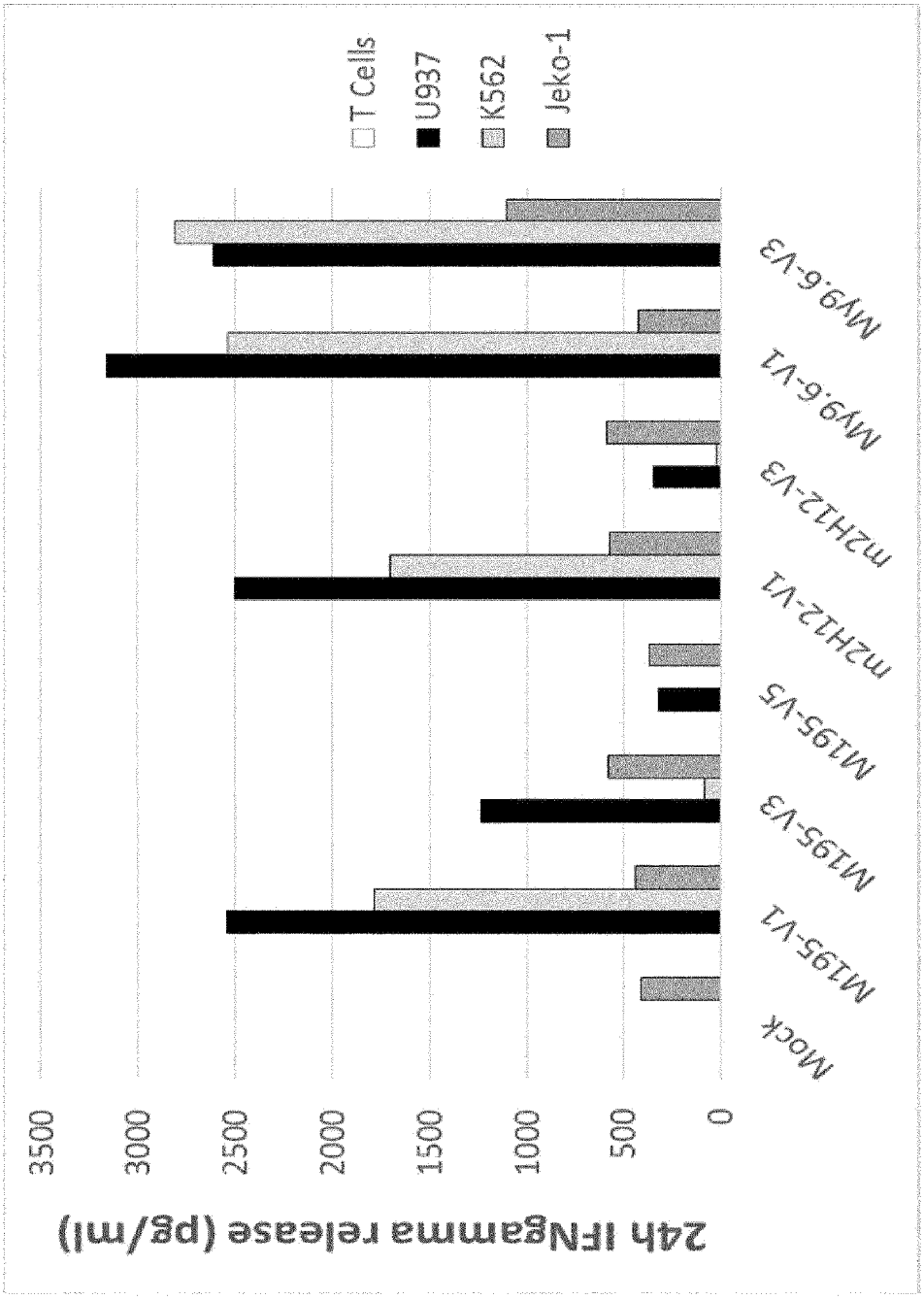
24h IFNgamma release (pg/ml)
3500
3000
2500
2000
1500
1000
500
0
Mock
M195-V1
M195-V3
M195-V5
m2H12-V1
m2H12-V3
MV9.6-V1
MV9.6-V3
T Cells
U937
K562
Jeko-1

Figure 7

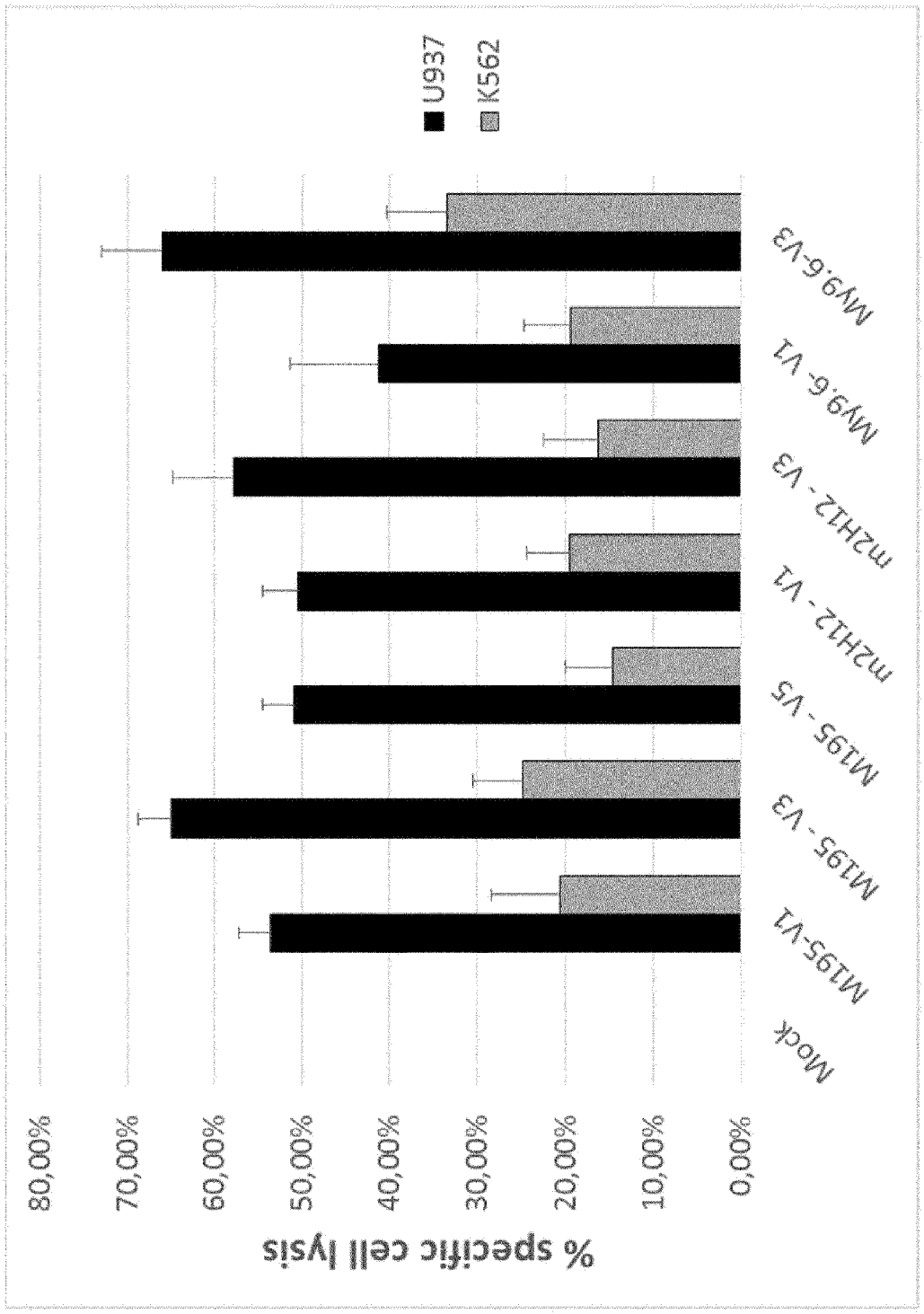
% specific cell lysis
80,00%
70,00%
60,00%
50,00%
40,00%
30,00%
20,00%
10,00%
0,00%
Mock
M195-V1
M195 - V3
M195 - V5
m2H12 - V1
m2H12 - V3
MY9.6- V1
MY9.6-V3
U937
K562

Figure 8