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(54) Title: CELLS FOR IMMUNOTHERAPY ENGINEERED FOR TARGETING CD38 ANTIGEN AND FOR CD38 GENE IN-
ACTIVATION

(57) Abstract: Methods of developing genetically engineered immune cells for immunotherapy, which can be endowed with Chi-
meric Antigen Receptors targeting an antigen marker that is common to both the pathological cells and said CD38 immune by the
fact that the genes encoding said markers are inactivated in said immune cells by a rare cutting endonuclease such as TALEN, Cas9
or argonaute.



**CELLS FOR IMMUNOTHERAPY ENGINEERED FOR TARGETING CD38 ANTIGEN AND FOR CD38 GENE
INACTIVATION**

Field of the invention

5 The present invention relates to methods of developing genetically engineered, preferably non-alloreactive, immune cells for immunotherapy, which are endowed with Chimeric Antigen Receptors targeting the CD38 antigen marker that is common to both the pathological cells and the immune cells).

10 The method comprises expressing a CAR directed against said antigen marker and inactivating the genes in the immune cells contributing to the presence of said antigen marker on the surface of said immune cells. This inactivation is typically performed by using transgenes encoding RNA-guided endonucleases (ex: Cas9/CRISPR), meganucleases, Zinc-finger nucleases or TAL nucleases. The engineered immune cells, preferably T-cells, direct their immune activity towards malignant, infected cells or defective immune cells, while avoiding their mutual destruction, auto-stimulation or
15 aggregation. The invention opens the way to standard and affordable adoptive immunotherapy strategies using immune cells for treating cancer, infections and auto-immune diseases.

Background of the invention

20 Adoptive immunotherapy, which involves the transfer of autologous antigen-specific immune cells generated *ex vivo*, is a promising strategy to treat viral infections and cancer. The T cells used for adoptive immunotherapy, for instance, can be generated either by expansion of antigen-specific T- cells or redirection of T-cells through genetic engineering (Park, Rosenberg et al. 2011).

25 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
30 cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. First generation CARs have been shown to successfully redirect T cell cytotoxicity, however, they failed to provide prolonged expansion and anti-tumor activity *in vivo*. Signaling domains from co-stimulatory molecules including CD28, OX-40

(CD134), and 4-1BB (CD137) have been added alone (second generation) or in combination (third generation) to enhance survival and increase proliferation of CAR modified T cells. CARs have successfully allowed T cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010).

5 The current protocol for treatment of patients using adoptive immunotherapy is based on autologous cell transfer. In this approach, T lymphocytes are recovered from patients, genetically modified or selected *ex vivo*, cultivated *in vitro* in order to amplify the number of cells if necessary and finally infused into the patient. In addition to lymphocyte infusion, the host may be manipulated in other ways that support the engraftment of the T cells or their participation in an immune response, for
10 example pre-conditioning (with radiation or chemotherapy) and administration of lymphocyte growth factors (such as IL-2). Each patient receives an individually fabricated treatment, using the patient's own lymphocytes (i.e. an autologous therapy). Autologous therapies face substantial technical and logistic hurdles to practical application, their generation requires expensive dedicated facilities and expert personnel, they must be generated in a short time following a patient's diagnosis, and in many cases,
15 pretreatment of the patient has resulted in degraded immune function, such that the patient's lymphocytes may be poorly functional and present in very low numbers. Because of these hurdles, each patient's autologous cell preparation is effectively a new product, resulting in substantial variations in efficacy and safety.

 Ideally, one would like to use a standardized therapy in which allogeneic therapeutic cells could
20 be pre-manufactured, characterized in detail, and available for immediate administration to patients. By allogeneic it is meant that the cells are obtained from individuals belonging to the same species but are genetically dissimilar. However, the use of allogeneic cells presently has many drawbacks. In immune-competent hosts allogeneic cells are rapidly rejected, a process termed host versus graft rejection (HvG), and this substantially limits the efficacy of the transferred cells. In immune-incompetent hosts,
25 allogeneic cells are able to engraft, but their endogenous T-cell receptors (TCR) specificities may recognize the host tissue as foreign, resulting in graft versus host disease (GvHD), which can lead to serious tissue damage and death.

 In order to provide allogeneic T-cells, the inventors previously disclosed a method to genetically engineer T-Cells, in which different effector genes, in particular those encoding T-cell receptors, were
30 inactivated by using specific TAL-nucleases, better known under the trade mark TALENTM (Collectis, 8, rue de la Croix Jarry, 75013 PARIS). This method has proven to be highly efficiency in primary cells using RNA transfection as part of a platform allowing the mass production of allogeneic T-cells (WO 2013/176915).

CD38 (cluster of differentiation 38), also known as cyclic ADP ribose hydrolase is a glycoprotein found on the surface of many immune cells (white blood cells), in particular T-cells, including CD4+, CD8+, B lymphocytes and natural killer cells. CD38 also functions in cell adhesion, signal transduction and calcium signaling. Structural information about this protein can be found in the UniProtKB/Swiss-Prot database under reference P28907. In humans, the CD38 protein is encoded by the CD38 gene which located on chromosome 4. CD38 is a multifunctional ectoenzyme that catalyzes the synthesis and hydrolysis of cyclic ADP-ribose (cADPR) from NAD⁺ to ADP-ribose. These reaction products are deemed essential for the regulation of intracellular Ca²⁺. Also, loss of CD38 function was associated with impaired immune responses and metabolic disturbances (Malavasi F., et al. (2008). "Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology". *Physiol. Rev.* 88(3): 841–86).

On another hand, CD38 protein is a marker of HIV infection, leukemias, myelomas, solid tumors, type II diabetes mellitus and bone metabolism, as well as some other genetically determined conditions. In particular, it has been used as a prognostic marker in leukemia (Ibrahim, S. et al. (2001) CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. *Blood* 98:181-186).

Mihara et al (2009) describes an anti-CD38 chimeric antigen receptor based on the TBH-7 scFv. These engineered T-cells do not contain any other genetic modification. It is stressed in this publication that the recovery of viable cells was very low.

Although, cells expressing CD38 could be regarded as an attractive target for CARs, the fact that such antigen markers are also expressed at the surface of most T-cells, has hampered significantly the selection of these markers to perform immunotherapy.

The inventors here provide strategies for immunotherapy involving pathological cells expressing CD38 specific antigen marker also present at the surface of T-cells.

Summary of the invention

The present invention discloses methods to engineer T-cells intended to target pathological cells, whereas said pathological cells express CD38 marker that are also present on the surface of T-cells. By antigen marker is meant the whole protein of an immune-reactive fragment thereof.

More particularly, the engineered immune cells of the invention relate to anti-CD38 specific chimeric antigen receptors (anti-CD38 CARs) having specific architectures of versions V1, V2 and V3 such as illustrated in Figure 8 ; this anti-CD38 CAR having as cytoplasmic domain the CD3 zeta signaling

domain and a co-stimulatory domain from 4-1BB, and having VH and VL chains deriving from 25A10, 28F5, 13F11, 16B5, 10F7, 27B6 or 29B4 monoclonal antibodies.

According to the invention, the T-cells are preferably engineered in order to inactivate the expression of the gene encoding such CD38 antigen marker. This inactivation is preferably performed by a genome modification, more particularly through the expression in the T-cell of a specific rare-cutting endonuclease able to target a genetic locus directly or indirectly involved in the production or presentation of said CD38 antigen marker at the surface of the T-cell. Different types of rare-cutting endonucleases can be used, such as meganucleases, TAL-nucleases, zing-finger nucleases (ZFN), or RNA/DNA guided endonucleases like Cas9/CRISPR or argonaute (Ago). Here, the use of TALENs has been found particularly suitable to inactivate the CD38 gene. A schematic representation is presented in Figure 1.

According to a preferred embodiment, the immune cells of the invention are engineered in order to inactivate the CD38 gene encoding for the CD38 surface antigen, and also to endow a specific anti-CD38 CAR, this double genetic modification aiming to specifically target cancerous CD38-expressing cells while reducing the risk for these anti-CD38 CAR immune cells to kill each other.

According to a still preferred embodiment, said double-genetically engineered anti-CD38 CAR cells have a CAR structure of V1, V2 or V3 such as presented in Figure 8, this anti-CD38 CAR having as cytoplasmic domain the CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB, and having VH and VL chains deriving from 25A10, 28F5, 13F11, 16B5, 10F7, 27B6 or 29B4 monoclonal antibodies.

According to another embodiment, the T-cells can be further engineered to make them allogeneic, especially by deleting genes involved into self-recognition, such as those, for instance, encoding components of T-cell receptors (TCR) or HLA complex.

According to another embodiment, the T-cells can be further engineered to integrate at least one epitope or mimotope in the extracellular binding domain of the chimeric antigen receptor in order to deplete *in vivo* such engineered T-cells in case of need.

The present invention encompasses the isolated cells or cell lines comprising the genetic modifications set forth in the detailed description, examples and figures, as well as any of the proteins, polypeptides or vectors useful to engineer said T-cells.

As a result of the invention, the engineered T-cells can be used as therapeutic products, ideally as an "off the shelf" product, in methods for treating or preventing cancer, infections or auto-immune disease. In particular, they are most suitable for the treatment of multiple myeloma (MM) or acute lymphoblastic lymphoma (ALL).

Preferred immune cells according to the present invention are the one resulting into the phenotype $[CAR\ CD38]^+[CD38]^-$, preferably also $[TCR]^-$ negative for their use as therapeutic products, preferably allogeneic ones.

5 Brief description of the figures

Figure 1: Schematic representation of an engineered T-cell according to the present invention disrupted for CD38 and endowed with a chimeric antigen receptor (represented as a single-chain CAR) targeting a malignant cell bearing the antigen marker CD38.

Figure 2: A: Schematic representation of in a mammalian expression vector (pCSL10794) under the control of the T7 promoter, which is used for the subcloning of each TALE-nuclease construct using restriction enzyme digestion (insert: SfaNI-BbvI and vector: BsmBI). **B:** Schematic representation of in a mammalian expression vector (pCLS9632) under the control of the T7 promoter, which is used for the subcloning of each CAR constructs using restriction enzyme digestion (Ascl et HindIII).

Figure 3: Expression of CD38 in T cells: Freshly isolated T cells were cultured with anti-CD3-CD28coated microbeads (Dynabeads, life technologies) + 20ng/ml human IL2 (Miltenyi). **A:** CD38 expression by T cells at Day 6 after activation. **B:** CD38 expression by T cells over 3 weeks after activation.

Figure 4: Time scales for 3 experiments performed in the present invention: **A:** TALEN-inactivation of the endogenous CD38 in T cells ; **B:** transfection of mRNA encoding CAR in WT T cells ; **C:** transfection of mRNA encoding CAR after T cell activation in purified CD38 deficient T cells

Figure 5: Sequences of the Exon 1 of the CD38 antigen and of the 3 targets tested for CD38 KO by TALEN; the 3 targets CD38-1, CD38-2 and CD38ex1-T2 correspond to the successive framed parts.

Figure 6: Percentage of CD38 negative T cells 7 days after TALEN mRNA transfection; the 3 pairs of TALEN CD38ex1-T2, CD38-1

Figure 7: A: Percentage of CD38 negative T cells over the 17 days of culture with IL-2 after TALEN mRNA transfection. **B:** T cell growth (i.e. factor of proliferation) over the 11 days of culture in presence of IL-2 after CD38 negative T cells purification -Day 6 after T cells electroporation and Day 10 after T cells activation- [legend: empty circle: No TALEN; full circle: TALEN CD38-1 and triangle: purified negative CD38 (TALEN CD38-1); N represents the number of tests performed for each case]

Figure 8: Representation of the 3 versions (V1, V2 and V3) of designed and tested anti-CD38 CARs depending of the hinge used.

Figure 9: Construct plasmids for subcloning of the anti-CD38 CARs, with the Kd (nM) of their respective scFvs.

Figure 10: Testing of 5 cell lines (MOLP8, Daudi, U266 CD38+, U266 CD38- and K562) for quantitative expression of CD38 antigen using Kifikit method (Dako) **A:** FAC analysis ; **B:** percentage of CD38 expression and number de CD38 antigens per cell.

Figure 11: Screening of all the 24 different CARs (8 pairs of scFvs X 3 versions V1, V2 and V3) after transfection of the mRNAs encoding the CARs 5 days after freshly isolated T cells. CAR expression analysis by flow cytometry using protein L, anti-Fab or CD38-Fc (N=1)

Figure 12: Screening of all the 24 different CARs (8 pairs of scFvs X 3 versions V1, V2 and V3) after transfection of the mRNAs encoding the CARs 5 days after activation of freshly isolated T cells. CD107a expression at the plasma membrane of T cells after incubation (5 hours) with target cell lines (N=1)

Figure 13: Comparison of wt, CD38-deficient, purified CD38-deficient anti-CD38 CAR T cells. **A:** T cells viability assessed by LUNA cell counter. **B:** CAR expression at day 1 and day 2 after mRNA transfection using CD38-Fc fusion protein (N=1).

Figure 14: Comparison of wt, CD38-deficient, purified CD38-deficient anti-CD38 CAR T cells. **A:** Percentage of CD107a+ CD8 T cells after T cells incubation with target cells. **B:** Target cells lysis normalized on no CAR T cells and K562 lysis. (N=1).

Figure 15: CAR expression at day 1 and day2 in purified CD38-deficient anti-CD38 CAR T cells after mRNA transfection using CD38-Fc fusion protein (N=3).

Figure 16: Percentage of CD107a+ CD8 T cells after T cells incubation with target cells (N=3, except for degranulation against autologous T cells (N=1 or 2)

Figure 17: Target cells lysis normalized on no-CAR T cells and K562 lysis (N=3, except for cytotoxicity against LT autologous N=1 or 2).

Figure 18: Schematic representation of FcεRI from which derivate the multi-chain CAR architecture according to the invention.

Figure 19: General structure of the polycistronic construct encoding the CD38 multi-chain CAR according to the invention.

Figure 20: Different architectures of the CD38 specific multi-chain CAR according to the invention. From left to right: polypeptide gamma (fused to ITAM of CD3zeta), polypeptide alpha (fused to ScFv), polypeptide beta (fused to co-stimulatory domain from either CD28 or 41BB). A and B: polypeptide beta is fused to co-stimulatory domain from 41BB, VL and VH fragments being in opposite

orders. C and D: polypeptide beta is fused to co-stimulatory domain from CD28, VL and VH fragments being in opposite orders.

Figure 21A and Figure 21B: Schematic representation of exemplary anti-CD38 specific CARs according to the invention involving different mAb-epitope tagging for T cell depletion, especially CD20 mimotope(s), which are designed to mitigate possible side effects associated with CAR positive cells injection.

(A) anti-CD38 specific CAR prototype according to the present invention not involving an epitope tagging sequence for sorting or depleting cells: V1 and v2 represents either VH or VL chain respectively of an antibody binding CD38, TM: transmembrane domain, L: linker, TM: Transmembrane domain (preferably CD8 α transmembrane domain), 4-1BB: intracellular co-stimulatory domain, CD3 ITAM: activation domain.

(B) anti-CD38 specific CAR architectures according to the invention further including at least one epitope inserted in the extracellular ligand binding domain of the CAR, wherein said epitope is inserted between the VH and VL chains; said epitope being bordered by different linkers.;

(C): anti-CD38 specific CAR architectures according to the invention, where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VH and VL chains, said 2nd epitope being also bordered by 2at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 2nd epitope.

(D): anti-CD38 specific CAR architectures according to the invention, where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the VH and VL chains; the other epitope is inserted between the VL chain and the hinge, each said epitope being also bordered by at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the 1st epitope.

(E): anti-CD38 specific CAR architecture according to the invention, where two epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VL chain and the hinge, said 2nd epitope being also bordered by such linkers.

(F): anti-CD38 specific CAR architectures according to the invention, where three epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the CAR

and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VH and VL chains, said epitope being also bordered by such linkers, and the third epitope being inserted between the VL chain et the hinge. These two architectures differ by the linkers used bordering the 2nd epitope.

5 (G): anti-CD38 specific CAR architectures according to the invention, where at least two epitopes (preferably CD20 epitopes) are inserted in the extracellular ligand binding domain between the hinge and the anti-CD38 VH and VL chains. In the third exemplary architecture, one CD34 epitope is included between two CD20 epitopes. Further architectures may be considered where CD34 replaces any other previous CD20 epitopes.

10 (H): anti-CD38 specific CAR architectures according to the invention, where at least two epitopes are inserted at the extremity of the extracellular ligand binding domain.

Figure 22: Evaluation of *in vivo* anti-tumor activity of T cells with CD38 knock out and endowing an anti CD19 CAR (CAR CD38-/- CAR-CD19 T cells); A) Timescale of the experiment; B) Bioluminescence imaging was assessed at Day 7, Day 14 and Day 21 in 4 groups of mice: no T cell was administrated, administration of T cells with KO CD38 and expressing RQR8 (CD38-/- RQR8), administration of T cells with KO CD38 and co-expressing CD19 CAR and RQR8, and administration of T cells WT CD38 and co-expressing CD19 CAR and RQR8. The missing mice at Day 14 and Day 21 are due to sacrificed ones for characterization purposes ; C) Evaluation of the tumor progression by bioluminescence imaging at Day 7, Day 14 and Day 21 in the 4 above groups of mice: D) Evaluation of survival percentage in function of time in the 4 above groups of mice.

Figure 23: Evaluation of CD38-KO efficacy in two independent experiments. The graph represents the % of CD38 negative cells 5 days after electroporation (TpT: buffer T= negative control).

Figure 24: Evaluation of the effect of the amount of CD38 TALEN mRNA on the KO efficiency A) and T cell proliferation rate B). The quantity of mRNA in the graph corresponds to the mRNA total used (10µg, 5µg and 2.5µg for each TALEN are equivalent to 2µg, 1µg and 0.5µg of mRNA per 10⁶ cells).

Figure 25: Evaluation of the transduction efficiency assessed by CD38-fc or RQR8 staining at Day 3 after transduction. Three MOIs (5, 10 and 15) for 4 different rLVs encoding for anti-CD38 CARs (25A10-V1 and V2, and 28F5 V1 and V2) were tested.

Figure 26: Time scaling for the screening process of T cells having undergone CD38 / TRAC double KO and anti-CD38 CAR transduction.

Figure 27: Evaluation of the efficiency of CD38/TRAC KO by TALEN mRNA electroporation in T cells, and of the CAR and RQR8 co-expression at the end of the culture process. A) three series of FACs corresponding to the expression of CD38 antigen and of anti-CD38 CAR -by RQR8 and CD38 Fc; these assays were performed on each of the 4 anti-CD38 CARs (25A10-V1 and V2, and 28F5 V1 and V2) which have undergone a CD38/TRAC double KO (DKO). B) Evaluation of the percentage of viable T cells having successfully undergone a CD38/TRAC double KO (DKO). C) Evaluation for the 4 above CARs of the anti-CD38 CAR expression based on RQR8 and CD38 Fc. NTD: non transduced.

Figure 28: Characterization of the phenotype of T cells having successfully undergone a double CD38/TRAC KO and expressing anti-CD38 CAR. A) Determination of the ratio of CD4/CD8 in T cells expressing RQR8 for each one of the 4 above anti-CD38 CARs. B) Determination of the ratio of T cells subsets: effector T cell (Teff), memory effector T cell (Tem), central memory T cells (Tcm), stem cell memory T cells (Tscm), another category of memory T cells (Temra) and naïve T cells (Tn), for each one of the 4 above anti-CD38 CARs

Figure 29: Evaluation of the activity for the 4 anti-CD38 CARs (25A10-V1 and V2, and 28F5 V1 and V2) A) Evaluation of their degranulation against MM cell lines : MOLP8, U266 (expressing or not CD38 antigen) and K562, against autologous T cells, and as positive control the case when no cell line was used (LT alone) : B) Evaluation of their cytotoxicity against MM CD38 expressing cell lines, the values for U266 which is a MM expressing CD38 cell line was normalized to that of U266 not expressing CD38 cell line. For these 2 graphs: the legends next to them from top to the bottom correspond to the bars from left to right.

Figure 30: Evaluation of the CD38 expression at the cell surface of three T acute lymphoblastic leukemia (T-ALL) cell lines (MOLT4, Loucy and CCRF-CEM), and of two MM cell lines (U266 CD38- and MOLP8).

Figure 31: Evaluation of the establishment of KO CD38 by determination of percentage of CD38 negative T cells in the CCRF-CEM (T-ALL) cell line before and after purification.

Figure 32: Evaluation of the anti-CD38 CAR activity for the 25A10-V1 A) Evaluation of their degranulation against T ALL cell lines : CCRF-CEM original cell and the one KO CD38, Loucy and MOLT4, a MM1S MM cell line expressing CD38, and as positive control the case when no cell line was used (LT alone) : B) Evaluation of their cytotoxicity against the above T ALL CD38 expressing cell lines (value for CCRF-CEM was normalized to the corresponding CD38 not expressing cell line). For these 2 graphs: the legends next to them from top to the bottom correspond to the bars from left to right.

Detailed description of the invention

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

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

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

The invention relates more particularly to anti-CD38 specific chimeric antigen receptors (anti-CD38 CARs) having one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure
30 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4).

5

Engineered T-cells expressing chimeric antigen receptors against pathological cells

The chimeric antigen receptors introduced into the T-cells according to the invention can adopt different design such as single-chain or multi-chain CARs. These different designs allow various strategies for improving specificity and binding efficiency towards the targeted pathological cells. Some of these strategies are illustrated in the figures of the present application. Single-chain CARs are the most classical version in the art. Multi-chain CAR architectures were developed by the applicant as allowing modulation of the activity of T-cells in terms of specificity and intensity. The multiple subunits can shelter additional co-stimulation domains or keep such domains at a distance, as well as other types of receptors, whereas classical single chain architecture can sometimes be regarded as too much sensitive and less permissive to multispecific interactions.

Single-Chain CAR

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.

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 including CD28, OX-40 (CD134), and 4-1BB (CD137) have been added alone (second generation) or in combination (third generation) to enhance survival and increase proliferation of CAR modified T cells. CARs have successfully allowed T cells to be redirected against antigens expressed at the surface of tumor cells from various malignancies including lymphomas and solid tumors (Jena, Dotti et al. 2010).

According to one embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4).

According to a preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10) and SEQ ID NO 62 and 58 (28F5).

According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1 and V2 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10) and, SEQ ID NO. 62 and 58 (28F5).

According to a preferred embodiment, said transmembrane domain of above anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO.79 (CD8 α TM).

5 According to a preferred embodiment, said V_H and V_L of above anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10) or SEQ ID NO. 62 and 58 (28F5).

10 According to a preferred embodiment, the CDRs sequences comprised in said V_H and V_L of said anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) are respectively SEQ ID NO.15-17 and SEQ ID NO.11-13; respectively SEQ ID NO.63-65 and SEQ ID NO.59-62; respectively SEQ ID NO.55-57 and SEQ ID NO.51-53; respectively SEQ ID NO.31-33 and SEQ ID NO.27-29; respectively SEQ ID NO.39-42 and SEQ ID NO.35-37; respectively SEQ ID NO.47-49 and SEQ ID NO.43-45; respectively SEQ ID NO.23-25 and SEQ ID NO.19-22.

15 According to a preferred embodiment, said hinge of above anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 76 (Fc ϵ RIII α), SEQ ID NO. 77 (CD8 α) and SEQ ID NO. 78 (IgG1).

20 According to a more preferred embodiment, said hinge of above anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 77 (CD8 α).

According to a preferred embodiment, said hinge of above anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 76 (Fc ϵ RIII α).

25 According to one embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) , SEQ ID NO. 88-90 (16B5), SEQ ID NO. 91-93 (10F7), SEQ ID NO.94-96 (27B6) and SEQ ID NO. 85-87 (29B4).

All above sequences of anti-CD38 CARs which are encompassed within the scope of the invention are presented in the following Table 1 (excepted the GMB005 CARs used as tool CAR for comparison). Are also disclosed sequences of the components which are used for their architectures (V1, V2 and V3 versions according to Figure 8).

Table 1: Polypeptide sequences of anti-CD38 CARs based on the V1, V2 and V3 versions in Figure 8, and of their corresponding components used to make them

Name of CAR	SEQ ID #	Polypeptide sequence
CD8 α -Signal peptide (SP)	74	MALPVTALLPLALLLHAARP
Fc ϵ RI γ - signal peptide (SP)	106	MIPAVVLLLLLLVEQAAA
Fc ϵ RI γ - signal peptide (SP)	110	MAPAMESPTLLCVALLFFAPDGVLA
GS linker	75	GGGSGGGSGGGGS
FCRIII α hinge	76	GLAVSTISSFFPPGYQ
CD8 α hinge	77	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD
IgG1 hinge	78	EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
CD8 α TM domain	79	IYIWAPLAGTCGVLLSLVITLYC
4-1 BB co-stimulatory domain	80	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
CD28 co-stimulatory domain (CD28-IC)	113	RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
CD3 ζ activation domain	81	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM QALPPR
Fc Receptor for IgE, alpha chain, transmembrane and intracellular domain (Fc ϵ RI α -TM-IC)	111	FFIPLLVVILFAVDTGLFISTQQQVTFLLKIKRTRKGFRLNPHPKPNPKNN
Fc Receptor for IgE, gamma chain, without ITAM) (Fc ϵ RI γ -ITAM)	107	LGEPQLCYILDAILFLYGIVLTLLYCRLKIQRKAAITSYEKS

Receptor for IgE, beta chain, without ITAM (FcεR1γ-ΔITAM)	112	MDTESNRRANLALPQEPSSVPAFEVLEISPQEVSSGRLLKSASSPPLHTWLTVLKKE QEFLGVTQILTAMICLCFGTVVCSVLDISHIEGDISSFKAGYPFWGAIFFSISGMLSI ISERRNATYLVRGSLGANTASSIAGGTGITILIINLKSLAYIHHSQCQFFETKCFMAS FSTEIVVMMLFLTILGLGSAVSLTICGAGEELKGNKVPE
GSG-P2A ribosomal skip peptide (GSG-P2A)	108	GSGATNFSLLKQAGDVEENPGP
GSG-T2A ribosomal skip peptide (GSG-T2A)	109	GSGEGRGSLLTCGDVEENPGP
25A10-V1 CAR	82	MALPVTALLPLALLLHAARPEVQLQQSGAELVRPGASVKLSCTASGFNIKDSLIH WVKQRPEQGLEWIGWIDPEDDKTKYAPKFQDKATLTADTSSNTAYLQLSTLTSED TAIYCVSRINYYFAYWGQGTTLTVSSGGGGSGGGGSGGGGSDIVMTQSPSSLT VTAGEKVTMSCKSSQSLHSGNQNRNYLTWYQQKPGQPPKLLIYWASTRESGVPD RFTGSGSGTDFTLTISVQAEDLAVYYCQNDYDYPYTFGGGTKEIKGLAVSTISSFF PPGYQIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGR DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLS TATKDTYDALHMQALPPR
25A10-V2 CAR	83	MALPVTALLPLALLLHAARPEVQLQQSGAELVRPGASVKLSCTASGFNIKDSLIH WVKQRPEQGLEWIGWIDPEDDKTKYAPKFQDKATLTADTSSNTAYLQLSTLTSED TAIYCVSRINYYFAYWGQGTTLTVSSGGGGSGGGGSGGGGSDIVMTQSPSSLT VTAGEKVTMSCKSSQSLHSGNQNRNYLTWYQQKPGQPPKLLIYWASTRESGVPD RFTGSGSGTDFTLTISVQAEDLAVYYCQNDYDYPYTFGGGTKEIKTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLY CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCELRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
25A10-V3 CAR	84	MALPVTALLPLALLLHAARPEVQLQQSGAELVRPGASVKLSCTASGFNIKDSLIH WVKQRPEQGLEWIGWIDPEDDKTKYAPKFQDKATLTADTSSNTAYLQLSTLTSED TAIYCVSRINYYFAYWGQGTTLTVSSGGGGSGGGGSGGGGSDIVMTQSPSSLT VTAGEKVTMSCKSSQSLHSGNQNRNYLTWYQQKPGQPPKLLIYWASTRESGVPD RFTGSGSGTDFTLTISVQAEDLAVYYCQNDYDYPYTFGGGTKEIKEPKSPDKTHT CPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCVMHEALHNHYTQKSLSLSPGK IYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
29B4-V1 CAR	85	MALPVTALLPLALLLHAARPEVQLVESGGGLVKPGGSLKLSAASGFTFSDYFMY WVRQTPEKRLEWVAIISDGGIYTYYPDSVKGRFTISRDNKNNLYLQMSSLSKSED AMYYCARDGRDDYDGYWYFDVWAGATTVTVSSGGGGSGGGGSGGGGSDIVMT QSHKFMSTSVGDRVNITCKASQDVNTAVAWYQQKPGQSPKLLIYWASTRHAGV PDRFTGSGSGTDYALTISVQAEDLALYYCQQHYSTPRTFGGGTKEIKGLAVSTISS FFPPGYQIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED

		GCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
29B4-V2 CAR	86	MALPVTALLPLALLLHAARPEVQLVESGGGLVKPGGSLKLSAASGFTFSDYFMYWVRQTPEKRLEWVAIISDGGIYTYPPDSVKGRFTISRDNANKNNLYLQMSLKS EDT AMYYCARDGRDDYDGWYFDVWGAGTTTVTVSSGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVNITCKASQDVNTAVAWYQQKPGQSPKLLIYWASTRHAGVPDRFTGSGSGTDYALTISVQAEDLALYYCQQHYSTPRTFGGGTKLEIKTTTPAPRPPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
29B4-V3 CAR	87	MALPVTALLPLALLLHAARPEVQLVESGGGLVKPGGSLKLSAASGFTFSDYFMYWVRQTPEKRLEWVAIISDGGIYTYPPDSVKGRFTISRDNANKNNLYLQMSLKS EDT AMYYCARDGRDDYDGWYFDVWGAGTTTVTVSSGGGSGGGGSGGGGSDIVMTQSHKFMSTSVGDRVNITCKASQDVNTAVAWYQQKPGQSPKLLIYWASTRHAGVPDRFTGSGSGTDYALTISVQAEDLALYYCQQHYSTPRTFGGGTKLEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
16B5-V1 CAR	88	MALPVTALLPLALLLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNLH WVKQTPGQGLEWIGIYIPGNGGTNYNQKFKGKATLTADTSSSTAYMQISSLTSED SAVYFCARGGIYYGSSLDYWGGQTTLTVSSGGGSGGGGSGGGGSGNIVLTQSPASLAVSLGQRATISCRASESDNYGTTFMYYWYQQKPGQPPKLLIYLASNLES GPVPA RFSGSGSGTDFTLTIDPVEADDAATYYCQQNKEDPWTFGGGTKEIKGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
16B5-V2 CAR	89	MALPVTALLPLALLLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNLH WVKQTPGQGLEWIGIYIPGNGGTNYNQKFKGKATLTADTSSSTAYMQISSLTSED SAVYFCARGGIYYGSSLDYWGGQTTLTVSSGGGSGGGGSGGGGSGNIVLTQSPASLAVSLGQRATISCRASESDNYGTTFMYYWYQQKPGQPPKLLIYLASNLES GPVPA RFSGSGSGTDFTLTIDPVEADDAATYYCQQNKEDPWTFGGGTKEIKTTTPAPRPPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
16B5-V3 CAR	90	MALPVTALLPLALLLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNLH WVKQTPGQGLEWIGIYIPGNGGTNYNQKFKGKATLTADTSSSTAYMQISSLTSED SAVYFCARGGIYYGSSLDYWGGQTTLTVSSGGGSGGGGSGGGGSGNIVLTQSPASLAVSLGQRATISCRASESDNYGTTFMYYWYQQKPGQPPKLLIYLASNLES GPVPA RFSGSGSGTDFTLTIDPVEADDAATYYCQQNKEDPWTFGGGTKEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVI TLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

		YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC FPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR
10F7-V1 CAR	91	MALPVTALLPLALLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNM HWVKQTPGQGLEWIGIYIPGNGGTNYNQKFKDKATLTADTSSSTAYMQISSLTSE DSAVYFCARGGQLGRPWFAYWGQGLTVTVSAGGGGSGGGGSGGGGSGNIVLTQ SPASLAASPGQRATISCRASESVDSYGNTFMWYQQKPGQPPKLLIYLASNLESGV PVRFSGSGSRTDFTLTIDPVEADDAATYYCQNNEDPWTFGGGTKEIKGLAVSTI SSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR
10F7-V2 CAR	92	MALPVTALLPLALLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNM HWVKQTPGQGLEWIGIYIPGNGGTNYNQKFKDKATLTADTSSSTAYMQISSLTSE DSAVYFCARGGQLGRPWFAYWGQGLTVTVSAGGGGSGGGGSGGGGSGNIVLTQ SPASLAASPGQRATISCRASESVDSYGNTFMWYQQKPGQPPKLLIYLASNLESGV PVRFSGSGSRTDFTLTIDPVEADDAATYYCQNNEDPWTFGGGTKEIKTTTPAP RPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSL VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
10F7-V3 CAR	93	MALPVTALLPLALLHAARPQAYLQQSGAELVRSGASVKMSCKASGYTFTSYNM HWVKQTPGQGLEWIGIYIPGNGGTNYNQKFKDKATLTADTSSSTAYMQISSLTSE DSAVYFCARGGQLGRPWFAYWGQGLTVTVSAGGGGSGGGGSGGGGSGNIVLTQ SPASLAASPGQRATISCRASESVDSYGNTFMWYQQKPGQPPKLLIYLASNLESGV PVRFSGSGSRTDFTLTIDPVEADDAATYYCQNNEDPWTFGGGTKEIKEPKSPD KTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
27B6-V1 CAR	94	MALPVTALLPLALLHAARPQVQLKQSGPGLVQPSQSLITCTVSGFSLTSYNIDW VRQSPGKGLEWLGVIWSGGSTDYNAAFISRLKISKDDSKSQVFFKMNSLQSDDTA IYYCARHSPLVSTPDWYFDVWGAGTTVTVSSGGGGSGGGGSGGGGSGQIVLTQSP AIMSASPGEKVTMTCTSSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFS GSGSGTSYSLTINNMEAEDAATYSCQQWSSYPPTFGGGTKLEIKGLAVSTISSFFPP GYQIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
27B6-V2 CAR	95	MALPVTALLPLALLHAARPQVQLKQSGPGLVQPSQSLITCTVSGFSLTSYNIDW VRQSPGKGLEWLGVIWSGGSTDYNAAFISRLKISKDDSKSQVFFKMNSLQSDDTA IYYCARHSPLVSTPDWYFDVWGAGTTVTVSSGGGGSGGGGSGGGGSGQIVLTQSP AIMSASPGEKVTMTCTSSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFS GSGSGTSYSLTINNMEAEDAATYSCQQWSSYPPTFGGGTKLEIKTTTPAPRPPTPA PTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCK

		RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQ QGGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR
27B6-V3 CAR	96	MALPVTALLPLALLLHAARPQVQLKQSGPGLVQPSQSLSITCTVSGFSLTSYNIDW VRQSPGKLEWLGVIWSSGGSTDYNAAFISRLKISKDDSKSQVFFKMNSLQSDDTA IYYCARHSPVSTPDWYFDVWGAGTTVTVSSGGGGSGGGSGGGGSQIVLTQSP AIMSASPGEKVTMTCTSSSVSYMHWYQQKSGTSPKRWIYDTSKLASGVPARFS GSGSGTSYSLTINNMEAEDAATYSCQQWSSYPPTFGGGTKLEIKEPKSPDKHTCP PCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT PPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKIYI WAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEE EGGCELRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTY DALHMQALPPR
13F11-V1 CAR	97	MALPVTALLPLALLLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFKKYGMN WVKQAPGKGLKWMGWINTNTGEPTYAEFEKGRFAFSLETSASTAYLQINNPKNE DTATYFCARWYWGSTPSSYTMDYWGQGTSTVTVSSGGGGSGGGSGGGGSETTV TQSPASLSVATGEKVTIRCITSTDIDDDMNWYQQKPGEPKVLISEGNTLRPGVPS RFSSSGYGTDFVFTIENTLSEVDADYYCLQSNNMPYTFGGGTKEIKGLAVSTISSFF PPGYQIYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGC SCRFPEEEEGGCELRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLS TATKDTYDALHMQALPPR
13F11-V2 CAR	98	MALPVTALLPLALLLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFKKYGMN WVKQAPGKGLKWMGWINTNTGEPTYAEFEKGRFAFSLETSASTAYLQINNPKNE DTATYFCARWYWGSTPSSYTMDYWGQGTSTVTVSSGGGGSGGGSGGGGSETTV TQSPASLSVATGEKVTIRCITSTDIDDDMNWYQQKPGEPKVLISEGNTLRPGVPS RFSSSGYGTDFVFTIENTLSEVDADYYCLQSNNMPYTFGGGTKEIKTTTPAPRPPT PAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLY CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAY QGGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR
13F11-V3 CAR	99	MALPVTALLPLALLLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFKKYGMN WVKQAPGKGLKWMGWINTNTGEPTYAEFEKGRFAFSLETSASTAYLQINNPKNE DTATYFCARWYWGSTPSSYTMDYWGQGTSTVTVSSGGGGSGGGSGGGGSETTV TQSPASLSVATGEKVTIRCITSTDIDDDMNWYQQKPGEPKVLISEGNTLRPGVPS RFSSSGYGTDFVFTIENTLSEVDADYYCLQSNNMPYTFGGGTKEIKEPKSPDKHT CPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK IYWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCELRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDT YDALHMQALPPR
28F5-V1 CAR	100	MALPVTALLPLALLLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTKYGMN WVKQTPGKGLKWMGWINTNSGEPTYAEFEKGRFAFSLETSASTAYLQINNPKNE DTATYFCARGAYRYDGEVSYAMDYWGQGTSTVTVSSGGGGSGGGSGGGGS QIILTQSPAIMSASLGERVTMTCTATSSSLSSYLHWYQQKPGSSPKLWIYSTSHLAS

		GVPARFSGGGSGTSYSLTISSMEAEDAATYYCHQYHLSPTYFGGGTKLEIKGLAVST ISSFFPPGYQIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDR RGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLY QGLSTATKDTYDALHMQALPPR
28F5-V2 CAR	101	MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTKYGMN WVKQTPGKGLKWMGWINTNSGEPTYAEFFKGRFAFSLETSASTAYLQINNKLNE DTATYFCARGAYRYDGEVSYAMDYWGQTSVTVSSGGGGSGGGSGGGGS QIILTQSPAIMASASLGERVTMTCTATSSSSYLHWYQQKPGSSPKLWIYSTSHLAS GVPARFSGGGSGTSYSLTISSMEAEDAATYYCHQYHLSPTYFGGGTKLEIKTTTPAP RPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLSL VITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
28F5-V3 CAR	102	MALPVTALLPLALLHAARPQIQLVQSGPELKKPGETVKISCKASGYTFTKYGMN WVKQTPGKGLKWMGWINTNSGEPTYAEFFKGRFAFSLETSASTAYLQINNKLNE DTATYFCARGAYRYDGEVSYAMDYWGQTSVTVSSGGGGSGGGSGGGGS QIILTQSPAIMASASLGERVTMTCTATSSSSYLHWYQQKPGSSPKLWIYSTSHLAS GVPARFSGGGSGTSYSLTISSMEAEDAATYYCHQYHLSPTYFGGGTKLEIKEPKSPD KTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNYHTQKLSL SPGKIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC RFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTA TKDTYDALHMQALPPR
GMB005-V1 CAR	103	MALPVTALLPLALLHAARPEVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMS WVRQAPGKGLEWVSAISGSGGGTYADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYFCAKDILWFGEPVFDYWGQGLTVTVSSASGGGGSGGGSGGGGSEIVL TQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYDASN RATGIPA RFSGSGSGTDFTLTISSELPEDFAVYYCQQRSNWPPTFGQGTKVEIKGLAVSTISSF FPPGYQIYIWAPLAGTCGVLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDG CSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGR RDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR
GMB005-V2 CAR	104	MALPVTALLPLALLHAARPEVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMS WVRQAPGKGLEWVSAISGSGGGTYADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYFCAKDILWFGEPVFDYWGQGLTVTVSSASGGGGSGGGSGGGGSEIVL TQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYDASN RATGIPA RFSGSGSGTDFTLTISSELPEDFAVYYCQQRSNWPPTFGQGTKVEIKTTTPAPRPPT PAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLSLVITLY CKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
GMB005-V3 CAR	105	MALPVTALLPLALLHAARPEVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMS WVRQAPGKGLEWVSAISGSGGGTYADSVKGRFTISRDN SKNTLYLQMNSLRAE DTAVYFCAKDILWFGEPVFDYWGQGLTVTVSSASGGGGSGGGSGGGGSEIVL TQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLIYDASN RATGIPA RFSGSGSGTDFTLTISSELPEDFAVYYCQQRSNWPPTFGQGTKVEIKEPKSPDKTH TCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVD

		GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEALHNHYTQKSLSLSPG KIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF EEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK DTYDALHMQALPPR
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According to a preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) and SEQ ID NO. 88-90 (16B5).

According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10) and SEQ ID NO. 100-102 (28F5).

According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 82-84 (25A10).

According to a even more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 82 (25A10-v1).

According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to NO. 100-102 (28F5).

According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 97-99 (13F11).

The present invention is more particularly drawn to immune cells that are endowed with a CAR presenting some identity with those described in the present application and that would bear rare-cutting endonuclease induced mutations in a gene encoding the cell marker targeted by said CAR (i.e. the CAR displays affinity with the product of said inactivated gene). By identity is meant at least 70%, preferably 80%, more preferably 90% and even more preferably 95% polynucleotide or polypeptide

identity as determined by the software such as FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.). BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical. 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 same applies with respect to polynucleotide sequences using BLASTN.

The present invention relates also to an engineered immune cell (preferably T cell) which expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) such as described previously, said immune cell having its endogenous CD38 gene genetically inactivated or mutated, said CD38 antigen being present both on the surface of said immune cell and the pathological cell.

In a preferred embodiment, said engineered immune cell expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR), said immune cell having its endogenous CD38 and TCR genes genetically inactivated or mutated.

In an embodiment, said engineered immune cell expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR), said immune cell having its endogenous CD38, TCR and dCK genes genetically inactivated or mutated.

In a preferred embodiment, said engineered immune cell expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) which has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4),

said immune cell (preferably T cell) having its endogenous CD38 gene genetically inactivated or mutated, said CD38 antigen being present both on the surface of said immune cell and the pathological cell.

In a more preferred embodiment, said engineered immune cell expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) which has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain

comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4),

said immune cell (preferably T cell) having its endogenous CD38 and TCR genes genetically inactivated or mutated, said CD38 antigen being present both on the surface of said immune cell and the pathological cell.

In the above embodiments, said genetic inactivation or mutation is performed preferably by the use of specific rare-cutting endonuclease such as described in the present invention.

The present invention relates also to a population comprising at least two an engineered immune cells (preferably T cells) which expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) such as described previously, said immune cell having its endogenous CD38 gene genetically inactivated or mutated, said CD38 antigen being present both on the surface of said immune cell and the pathological cell.

In a preferred embodiment, said population comprises at two engineered immune cells expressing an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) which has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4),

said immune cells (preferably T cells) having its endogenous CD38 gene genetically inactivated or mutated, said CD38 antigen being present both on the surface of said immune cell and the pathological cell.

Extracellular domain

The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

Single-chain variable antibodies (scFvs)

The antigen binding domain of the anti-CD38 CARs of the invention can be any domain that binds to the off-tissue antigen including but not limited to a monoclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof.

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

A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., J. Immunol., 169: 1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J. Biol. Chem., 272(16): 10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer

Res., 55(8): 1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959- 73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.).

Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a 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 GD3 using the functional assays described herein.

According to one embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprise V_H and V_L variable chain which polypeptide sequence display at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4).

Said above sequences of anti-CD38VH and VL chains and their respective corresponding CDRs are presented in the following Table 2.

Table 2: Sequences of VH and VL chains of the 8 couple of scFv anti-CD38 antibodies and their respective CDR.

Name	VH or VL chain	SEQ ID #	Polypeptide or nucleic acid sequence
25A10	VL	10	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLHSGNQARNYLT WYQQKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFTL TISSVQAEDLAVYYCQNDYDYPYTFGGGKLEIK
	CDR1	11	QSLHSGNQARNY
	CDR2	12	WAS
	CDR3	13	QNDYDYPYT
	VH	14	EVQLQQSGAELVRPGASVKLSCTASGFNIKDSLHWVKQR PEQGLEWIGWIDPEDDKTKYAPKFQDKATLTADTSSNTA YLQLSTLTSEDTAIYYCVSRYINYYFAYWGQGTTLTVSS
	CDR1	15	GFNIKDSL
	CDR2	16	IDPEDDKT
	CDR3	17	VSRYINYYFAY
29B4	VL	18	DIVMTQSHKFMSTSVGDRVNITCKASQDVNTAVAWYQ QKPGQSPKLLIYWASTRHAGVPDRFTGSGSGTDYALTSS VQAEDLALYYCQQHYSTPRTFGGGKLEIK
	CDR1	19	QDVNTA
	CDR2	20	WAS
	CDR3	21	QQHYSTPRT
	VH	22	EVQLVESGGGLVKPGGSLKLSAASGFTFSDYFMYWVRQ TPEKRLEWVAIISDGGIYTYPPDSVKGRFTISRDNKNNLYL QMSSLKSEDTAMYYCARDGRDDYDGWYFDVWGAGTTV TVSS
	CDR1	23	GFTFSDYF
	CDR2	24	ISDGGIYT
	CDR3	25	ARDGRDDYDGWYFDV
16B5	VL	26	NIVLTQSPASLAVSLGQRATISCRASESDNYGTTFFMYWY QQKPGQPPKLLIYLASNLESGVPARFSGSGSGTDFTLTIDP VEADDAATYYCQQNKEDPWTFGGGKLEIK
	CDR1	27	ESVDNYGTTF
	CDR2	28	LAS
	CDR3	29	QQNKEDPWT
	VH	30	QAYLQQSGAELVRSGASVKMSCKASGYTFTSYNLHWVK QTPGQGLEWIGIYIPGNGGTNYNQKFKGKATLTADTSSS TAYMQISSLTSEDSAVYFCARGGIYYGSSLDYWQGTTT TVSS
	CDR1	31	GYTFTSYN

10F7	CDR2	32	IYPGNNGGT
	CDR3	33	ARGGIYYYGSSLDY
	VL	34	NIVLTQSPASLAASPGQRATISCRASESVDSYGNTFMYWY QQKPGQPPKLLIYLASNLESGVPVRFSGSGSRTDFTLTIDP VEADDAATYYCQQNNEDPWTFGGGTKVEIK
	CDR1	35	ESVDSYGNTF
	CDR2	36	LAS
	CDR3	37	QQNNEDPWT
	VH	38	QAYLQQSGAELVRSGASVKMSCKASGYTFTSYNMHWVK QTPGQGLEWIGYIYPGNNGGTNYNQKFKDKATLTADTSSS TAYMQISSLTSEDSAVYFCARGGQLGRPWFAYWGQGT LTVSA
	CDR1	39	GYTFTSYN
	CDR2	40	IYPGNNGGT
	CDR3	41	ARGGQLGRPWFAY
27B6	VL	42	QIVLTQSPAIMASASPGEKVTMTCTSSSVSYMHWYQQKS GTSPKRWIYDTSKLAGVPARFSGSGSGTSYSLTINNMEA EDAATYSCQQWSSYPPTFGGGTKLEIK
	CDR1	43	SSVSY
	CDR2	44	DTS
	CDR3	45	QQWSSYPPT
	VH	46	QVQLKQSGPGLVQPSQSLITCTVSGFSLTSYNIDWVRQS PGKGLEWLGVIWSSGGSTDYNAAFISRLKISKDDSKSQVFF KMNSLQSDDTAIYYCARHSPLVSTPDWYFDVWGAGTTV TVSS
	CDR1	47	GFSLSYN
	CDR2	48	IWSGGST
	CDR3	49	ARHSPLVSTPDWYFDV
13F11	VL	50	ETTVTQSPASLSVATGEKVTIRCITSTDIDDDMNWYQQKP GEPKVLISEGNTLRPGVPSRFSSSGYGTDFVFTIENTLSED VADYYCLQSNMPYTFGGGTKLEIK
	CDR1	51	TDIDDD
	CDR2	52	EGN
	CDR3	53	LQSNMPYT
	VH	54	QIQLVQSGPELKKPGETVKISCKASGYTFKKYGMNWVKQ APGKGLKWMGWINTNTGEPTYAEFKGRFAFSLETSAST AYLQINNLIKNEDTATYFCARWYYGSTPSSYTMDYWGQG TSVTSS
	CDR1	55	GYTFKKYG
	CDR2	56	INTNTGEP
	CDR3	57	ARWYYGSTPSSYTMDY
28F5	VL	58	QIILTQSPAIMASASLGERVTMTCTATSSLSSTLHWYQQKP GSSPKLWIYSTSHLAGVPARFSGGGSGTSYSLTISSMEAE DAATYYCHQYHLSPTYFGGGTKLEIK
	CDR1	59	SSLSSSY
	CDR2	60	STS
	CDR3	61	HQYHLSPTY
	VH	62	QIQLVQSGPELKKPGETVKISCKASGYTFTKYGMNWVKQ TPGKGLKWMGWINTNSGEPTYAEFKGRFAFSLETSAST AYLQINNLIKNEDTATYFCARGAYRYDGEVSYAMDYWG

			QGTSVTVSS
	CDR1	63	GYTFTKYG
	CDR2	64	INTNSGEP
	CDR3	65	ARGAYRYDGEVSYAMDY
GMB005	VL	66	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPG QAPRLLIYDASNRATGIPARFSGSGSGTDFTLTSSLEPEDF AVYYCQQRSNWPPTFGQGTKVEIK
	CDR1	67	QSVSSY
	CDR2	68	DAS
	CDR3	69	QQRSNWPPT
	VH	70	EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQ APGKGLEWVSAISGSGGGTYADSVKGRFTISRDN SKNTL YLQMNSLRAEDTAVYFCAKDKILWFGE PVFDYWGQGT L VTVSSAS
	CDR1	71	GFTFNSFA
	CDR2	72	ISGSGGGT
	CDR3	73	AKDKILWFGE PVFDY

According to a preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprise V_H and V_L variable chain which polypeptide sequence display at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11) or SEQ ID NO. 30 and 26 (16B5).

5 According to a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprise V_H and V_L variable chain which polypeptide sequence display at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10) or SEQ ID NO. 62 and 58 (28F5).

10 According to one embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises V_H and V_L variable regions containing the CDRs sequences of respectively SEQ ID NO.15-17 and SEQ ID NO.11-13; respectively SEQ ID NO.63-65 and SEQ ID NO.59-62; respectively SEQ ID NO.55-57 and SEQ ID NO.51-53; respectively SEQ ID NO.31-33 and SEQ ID NO.27-29; respectively SEQ ID NO.39-42 and SEQ ID NO.35-37; respectively SEQ ID NO.47-49 and SEQ ID NO.43-45 or respectively SEQ ID NO.23-25 and SEQ ID NO.19-22.

15 In a preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises V_H and V_L variable regions containing the CDRs sequences of respectively SEQ ID NO.15-17 and SEQ ID NO.11-13; respectively SEQ ID NO.63-65 and SEQ ID NO.59-62; respectively SEQ ID NO.55-57 and SEQ ID NO.51-53 or respectively SEQ ID NO.31-33.

20 In a more preferred embodiment, the anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) of the invention comprises V_H and V_L variable regions containing the CDRs sequences of respectively SEQ ID NO.15-17 and SEQ ID NO.11-13 or respectively SEQ ID NO.63-65 and SEQ ID NO.59-62.

25 In addition to the CAR targeting the antigen marker, which is common to the pathological cells and the T-cells, such as CD38, it is envisioned to express further CARs directed towards other antigen markers not necessarily expressed by the T-cells, so as to enhancing T-cells specificity.

30 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.

Examples of chimeric antigen receptor that can be further expressed by the T-cells to create multi-specific cells, are antigen receptors directed against multiple myeloma or lymphoblastic leukemia antigen markers, such as TNFRSF17 (UNIPROT Q02223), SLAMF7 (UNIPROT Q9NQ25), GPRC5D (UNIPROT Q9NZD1), FKBP11 (UNIPROT Q9NYL4), KAMP3, ITGA8 (UNIPROT P53708), and FCRL5 (UNIPROT Q68SN8).

As further examples for conferring another specificity alongside CD38, the antigen of the target can be from any cluster of differentiation molecules (e.g. CD16, CD64, CD78, CD96, CLL1, CD116, CD117, CD71, CD45, CD71, CD123 and CD138), a tumor-associated surface antigen, such as ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), EGFR variant III (EGFRvIII), CD19, CD20, CD30, CD40, disialoganglioside GD2, ductal-epithelial mucine, gp36, TAG-72, glycosphingolipids, glioma-associated antigen, β -human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostate specific antigen (PSA), PAP, NY-ESO-1, LAGA-1a, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, CD22, insulin growth factor (IGF1)-I, IGF-II, IGF1 receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, ROR1, Nkp30, NKG2D, tumor stromal antigens, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1) and fibroblast associated protein (fap); a lineage-specific or tissue specific antigen such as CD3, CD4, CD8, CD24, CD25, CD33, CD34, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), GM-CSF, cytokine receptors, endoglin, a major histocompatibility complex (MHC) molecule, BCMA (CD269, TNFRSF 17), or a virus-specific surface antigen such as an HIV-specific antigen (such as HIV gp120); an EBV-specific antigen, a CMV-specific antigen, a HPV-specific antigen, a Lasse Virus-specific antigen, an Influenza Virus-specific antigen as well as any derivate or variant of these surface markers. Antigens are not necessarily surface marker antigens but can be also endogenous small antigens presented by HLA class I at the surface of the cells.

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 CD38 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.

CARs with a lower affinity for CD38

According to another embodiment, anti-CD38 of the invention comprises scFvs with a lower affinity for CD38 antigen.

Such CARs are designed to prevent a possible risk of toxicity linked to the use *in vivo* of such engineered immune cells.

By "affinity" is meant a measure of the binding strength between antibody and a simple hapten or antigen determinant. Without being bound to theory, affinity depends on the closeness of stereochemical fit between antibody combining sites and antigen determinants, on the size of the area of contact between them, and on the distribution of charged and hydrophobic groups. Affinity also includes the term "avidity," which refers to the strength of the antigen-antibody bond after formation of reversible complexes. Methods for calculating the affinity of an antibody for an antigen are known in the art, including use of binding experiments to calculate affinity. In the case of an antibody (Ab) binding to an antigen (Ag), the affinity constant is used (expressed as inverted dissociation constant). $K_a = \frac{[AbAg]}{[Ab][Ag]}$ The chemical equilibrium of antibody binding is also the ratio of the on-rate ($k_{forward}$) and off-rate (k_{back}) constants. Two antibodies can have the same affinity, but one may have both a high on- and off-rate constant, while the other may have both a low on- and off-rate constant. $k_{forward} = \text{on-rate}$ $k_{back} = \text{off-rate}$ Antibody activity in functional assays (e.g., cell lysis assay) is also reflective of antibody affinity. In various embodiments of the invention, the antigen recognizing receptor has low affinity. Low affinity includes micromolar and nanomolar affinities (e.g. 10^{-5} , 5×10^{-6} , 10^{-6} , 5×10^{-7} , 10^{-7} , 5×10^{-8} , 10^{-8} , 5×10^{-9} , 10^{-9} M). Antibody and affinities can be phenotypically characterized and compared using functional assay (e.g., cell lysis assay).

The present invention encompasses also anti-CD38 CARs with a low affinity for CD38 antigen, such as those comprising VH and VL chains in their scFvs corresponding to the monoclonal 2C2, 5G9, 9E2, 28B9, 26D8, 15D1, and 23F2 antibodies.

According to one preferred embodiment, said anti-CD38 CARs with a low affinity for CD38 antigen comprise VH and VL chains in their scFvs corresponding to the monoclonal 2C2 or 5G9 antibodies (called also m2C2 and m5G9).

According to an embodiment, V_H and V_L of said scFvs displaying a lower affinity for CD38 antigen comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively V_H and V_L chains of m2C2 antibody of SEQ ID NO. 132 (EVKLVESGGGLVQPGGSLKLSCATSGFTFSYYMFVWRQTPEKRLEWVAYISNGDGNTYYPDTLKGRFTISRDNANNT
 5 LYLQMSRLKSEDTAMYYCARSISRYFDVWGAGTTVTSS) and SEQ ID NO. 133 (DIVMTQSPATLSVTPGDRVSLSCRASQSISDFLHWYQQKSHESPRLLIKYVSQSISGIPSRFSGSGSGSDFTLINSVEPED VGVYYCQNAHSFPSTFGGGTKLEIK); or to respectively V_H and V_L chains of m5G9 antibody of SEQ ID NO.134

(EIHLQQSGPELVKPGASVKISCKASGYSFTDYNIWVVKQSHGESLEWVGIDPYNGGAYYNQKFKAMATLTVDKSSST
 10 AFMHLNSLTSEDSAVYYCARKGVYGLAYWGQGLTVTVSS) and SEQ ID NO.135 (DIQMTQSPASLSVSVGETVTITCRASESIYSNLAWYQQKQKSPQLLVYASTHLADGVPSRFSGSGSGAQYSLKINSIQ SEDFGSYQCQHFVGTPYTFGGGKLEIK);

or to respectively V_H and V_L chains of m9E2 antibody of SEQ ID NO.142 (EVQLQQSGPELEKPGASVKISCKASGYSFTDYNMNWVKQSNKGSLIEWIGNIDPDYGGTTYNQKFKGKATLTVDKSSS
 15 TAYMQLRSLTSEDSAVYYCARSGYRYGFVYWGQGLTVTVSA) and SEQ ID NO.143 (DIVMTQAAAFSNPVTLGTSASISCRSSKSLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRIS RVEAEDVGVYYCAQNLELPWTFGGGKLEIK),

or to respectively V_H and V_L chains of m28B9 antibody of SEQ ID NO.144 (EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYLHWMKQRPEQGLEWIGRIDPANGNTKSDPKFQDKATIDADTSSN
 20 TAYLQLNSLTSEDTAVYYCAKGPWFPYWGQGLTVTVSA) and SEQ ID NO.145 (DIQMTQSPASQSASLGESVTITCLASQTIGTWLAWYQQKPGKSPQLLIYAATSLADGVPSRFSGSGSGTKFSFKISLQA EDFVSYHCQQLYRTPLTFGGGKLEIK);

or to respectively V_H and V_L chains of m26D8 antibody of SEQ ID NO.146 (QVQLQQPGAEMVRPGASVKLSCKASGYTFTDYWMHWWKQRPQGQGLEWIGKIDPSDSETHYHQKFKDKATLTVDKS
 25 SSTAYLQFKSLTSEDSAVHYCARREGIWLRyamdyWGQGSVTVSS) and SEQ ID NO.147 (DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRSGVPDRFTGSGSGTDFTLVSS VQSEDLAEYFCQQYNSYPYTFGGGKLEIK).

or to respectively V_H and V_L chains of m15D1 antibody of SEQ ID NO.148
 30 (EVQLQQSGPELVKPGASVKMSCRASGYSITDYNMHWWKQSHGKSLEWIGYIDPDNGATNNNQKFKGKATLTVDKSS STAYMQLSSLTSEDSAVYYCARSEGYQYYYAMDYWGQGSVTVSS) and SEQ ID NO.149 (DIVMTQAAAFSNPVALGTSASISCSSSKSLHSNGITYLHWYLRPGQSPQLLIYRMSNLASGVDPDRFSGSGSGTDFTLRIS RVEAEDVGVYYCAQMVERPWTFGGGKLEIK),

or to respectively V_H and V_L chains of m23F2 antibody of SEQ ID NO.150 (QVQVQQPGAELVKPGASVKLSCKASGYTFTSYWINWVKRPGQGLEWIGNIYPGSSSTNHNEKFKSKATLTVDTSSTAYMQLSSLTSDDSAVYYCARRGSSPSYTM DYWGQGTSTVTVSS) and SEQ ID NO.151 (DIVLTQSPASLAVALGQRATISCRASKSVSTFGYSYMHWYQQKPGQAPKLLIYLASNLESGV PARFSGSGSGDFTLKIHPVEEEDAATYYCLHSRELPWTFGGGKLEIK).

According to a preferred embodiment, V_H and V_L of said scFvs displaying a lower affinity for CD38 antigen comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively V_H and V_L chains of m2C2 antibody of SEQ ID NO. 132 (EVKLVESGGGLVQPGGSLKLS CATSGFTFSDYYMFVWRQTPEKRLEWVAYISNGDGNTYYPDTLKGRFTISRDNANNTLYLQMSRLKSEDTAMYYCARSISRYFDVWGAGTTVTVSS) and SEQ ID NO. 133 (DIVMTQSPATLSVTPGDRVSLSCRASQSISDFLHWYQQKSHESPRLLIKYVSQSIGIPSRFSGSGSGSDFTLSINSVEPED VGVYYCQNAHSFPSTFGGGKLEIK); and to respectively V_H and V_L chains of m5G9 antibody of SEQ ID NO.134

(EIHLQQSGPELVKPGASVKISCKASGYSTFDYNIYWVKQSHGESLEWVG YIDPYNGGAYYNQKFKAMATLTVDKSSSTAFMHLNSLTSEDSAVYYCARKGVYGLAYWGQGT LTVTVSS) and SEQ ID NO.135 (DIQMTQSPASLSVSVGETVTITCRASESIYSNLAWYQQKQKGSPQLLVYASTHLADGVPSRFSGSGSGAQYSLKINS LQSEDFGSYYCQHFWGTPYTFGGGKLEIK).

According to one embodiment, the anti-CD38 CARs with a low affinity for CD38 antigen of the present invention comprise an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a signaling domain –preferably CD3 zeta signaling domain, and a co-stimulatory domain -preferably from 4-1BB-,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively V_H and V_L chains of m2C2 antibody of SEQ ID NO. 132

(EVKLVESGGGLVQPGGSLKLS CATSGFTFSDYYMFVWRQTPEKRLEWVAYISNGDGNTYYPDTLKGRFTISRDNANNTLYLQMSRLKSEDTAMYYCARSISRYFDVWGAGTTVTVSS) and SEQ ID NO. 133 (DIVMTQSPATLSVTPGDRVSLSCRASQSISDFLHWYQQKSHESPRLLIKYVSQSIGIPSRFSGSGSGSDFTLSINSVEPED VGVYYCQNAHSFPSTFGGGKLEIK) ; and to respectively V_H and V_L chains of m5G9 antibody of SEQ ID NO.134

(EIHLQQSGPELVKPGASVKISCKASGYSTFDYNIYWVKQSHGESLEWVG YIDPYNGGAYYNQKFKAMATLTVDKSSSTAFMHLNSLTSEDSAVYYCARKGVYGLAYWGQGT LTVTVSS) and SEQ ID NO.135

(DIQMTQSPASLSVSVGETVTITCRASESIYSNLAWYQQKQKGKSPQLLVYASTHLADGVPSRFSGSGSGAQYSLKINSIQ
SEDFGSYYCQHFWGTPYTFGGGKLEIK).

According to a particular embodiment, an anti-CD38 specific chimeric antigen receptor (anti-
CD38 CAR) having one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure
8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a
monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a
CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%,
at least 98% or at least 99% identity to respectively VH and VL chains of m2C2 antibody of SEQ ID NO.

132
(EVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMFVWRQTPEKRLEWVAYISNGDGNTYYPDTLKGRFTISRDNANNT
LYLQMSRLKSEDTAMYICARSISRYFDVWGAGTTVTVSS) and SEQ ID NO. 133
(DIVMTQSPATLSVTPGDRVSLSCRASQSISDFLHWYQQKSHESPRLLIKYVSQSISGIPSRFSGSGSGSDFTLINSVEPED
VGVYYCQNAHSFPSTFGGGKLEIK) ; and to respectively VH and VL chains of m5G9 antibody of SEQ ID
NO.134

(EIHLLQQSGPELVKPGASVKISCKASGYSFTDYNIIWVKQSHGESLEWVGIDPYNGGAYYNQKFKAMATLTVDKSSST
AFMHLNSLTSEDSAVYYCARKGVYGLAYWGQGLTVTVSS) and SEQ ID NO.135
(DIQMTQSPASLSVSVGETVTITCRASESIYSNLAWYQQKQKGKSPQLLVYASTHLADGVPSRFSGSGSGAQYSLKINSIQ
SEDFGSYYCQHFWGTPYTFGGGKLEIK).

According to a more particular embodiment, the anti-CD38 specific chimeric antigen receptor
(anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at least
95%, at least 98% or at least 99% identity to SEQ ID NO.136-138 (corresponding respectively to m5G9
versions V1, V2 and V3).

According to another more particular embodiment, the anti-CD38 specific chimeric antigen
receptor (anti-CD38 CAR) of the invention comprises a polypeptide sequence displaying at least 90 %, at
least 95%, at least 98% or at least 99% identity to SEQ ID NO.139-141 (corresponding respectively to
m2C2 versions V1, V2 and V3).

According to another embodiment, the immune cell of the present invention is engineered to
express anti-CD38 CAR having a low affinity for CD38 antigen.

According to another embodiment, the immune cell which is engineered to express anti-CD38
CAR having a low affinity for CD38 antigen, is further engineered to have its endogenous CD38 gene
inactivated by knock-out, preferably by using a rare cutting endonuclease.

Transmembrane domain

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

A CAR according to the invention generally further comprises a transmembrane domain (TM) such as CD8 α and 4-1BB and more particularly CD8 α , showing at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO.79.

The transmembrane domain can further comprise a hinge region between said extracellular ligand-binding domain and said transmembrane domain.

Hinge

The term "hinge region" used herein generally means any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, hinge region are used to provide more flexibility and accessibility for the extracellular ligand-binding domain. A hinge region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Hinge region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively the hinge region may be a synthetic sequence that corresponds to a naturally occurring hinge sequence, or may be an entirely synthetic hinge sequence. In a preferred embodiment said hinge domain comprises a part of human CD8 alpha chain, Fc γ RIII α receptor or IgG1 respectively referred to in this specification as SEQ ID NO. 77, SEQ ID NO. 76 and SEQ ID NO.78, or hinge

polypeptides which display preferably at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with these polypeptides.

Intracellular domain

5 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
10 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.

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
15 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
20 known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non-limiting examples those derived from TCRzeta, FcRgamma, FcRbeta, FcRepsilon, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d. In a preferred embodiment, the signaling transducing
25 domain of the CAR can comprise the CD3zeta signaling domain which has amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90 %, 95 % 97 % or 99 % sequence identity with amino acid sequence of SEQ ID NO: 81.

In particular embodiment the signal transduction domain of the CAR of the present invention comprises a co-stimulatory signal molecule. A co-stimulatory molecule is a cell surface molecule other
30 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.

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

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

Multi-subunit CAR

Chimeric antigen receptors from the prior art introduced in T-cells have been formed of single chain polypeptides that necessitate serial appending of signaling domains. However, by moving signaling domains from their natural juxtamembrane position may interfere with their function. To overcome this drawback, the applicant recently designed a multi-chain CAR derived from FcεRI (Figure 18) to allow normal juxtamembrane position of all relevant signaling domains. In this new architecture, the high affinity IgE binding domain of FcεRI alpha chain is replaced by an extracellular ligand-binding domain such as scFv to redirect T-cell specificity against cell targets and the N and/or C-termini tails of FcεRI beta chain are used to place costimulatory signals in normal juxtamembrane positions. The multi-chain CAR (mcCAR) construct may have a polycistronic structure such as depicted in Figure 19.

Accordingly, the CAR expressed by the engineered T-cell according to the invention can be a multi-chain chimeric antigen receptor (CAR) particularly adapted to the production and expansion of engineered T-cells of the present invention. Such multi-chain CARs comprise at least two of the following components:

- 5 a) one polypeptide comprising the transmembrane domain of FcεRI alpha chain and an extracellular ligand-binding domain,
- b) one polypeptide comprising a part of N- and C- terminal cytoplasmic tail and the transmembrane domain of FcεRI beta chain and/or
- c) at least two polypeptides comprising each a part of intracytoplasmic tail and the
- 10 transmembrane domain of FcεRI gamma chain, whereby different polypeptides multimerize together spontaneously to form dimeric, trimeric or tetrameric CAR.

According to such architectures, ligands binding domains and signaling domains are born on separate polypeptides. The different polypeptides are anchored into the membrane in a close proximity allowing interactions with each other. In such architectures, the signaling and co-stimulatory domains

15 can be in juxtamembrane positions (i.e. adjacent to the cell membrane on the internal side of it), which is deemed to allow improved function of co-stimulatory domains. The multi-subunit architecture also offers more flexibility and possibilities of designing CARs with more control on T-cell activation. For instance, it is possible to include several extracellular antigen recognition domains having different specificity to obtain a multi-specific CAR architecture. It is also possible to control the relative ratio

20 between the different subunits into the multi-chain CAR. This type of architecture has been recently described by the applicant in PCT/US2013/058005 (WO2014/039523).

The assembly of the different chains as part of a single multi-chain CAR is made possible, for instance, by using the different alpha, beta and gamma chains of the high affinity receptor for IgE (FcεRI) (Metzger, Alcaraz et al. 1986) to which are fused the signaling and co-stimulatory domains. The gamma

25 chain comprises a transmembrane region and cytoplasmic tail containing one immunoreceptor tyrosine-based activation motif (ITAM) (Cambier 1995).

The multi-chain CAR can comprise several extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In one embodiment, the extracellular ligand-binding domains can be placed in tandem on the

30 same transmembrane polypeptide, and optionally can be separated by a linker. In another embodiment, said different extracellular ligand-binding domains can be placed on different transmembrane polypeptides composing the multi-chain CAR. In another embodiment, the present invention relates to a population of multi-chain CARs comprising each one different extracellular ligand

binding domains. In a particular, the present invention relates to a method of engineering immune cells comprising providing an immune cell and expressing at the surface of said cell a population of multi-chain CAR each one comprising different extracellular ligand binding domains. In another particular embodiment, the present invention relates to a method of engineering an immune cell comprising

5 providing an immune cell and introducing into said cell polynucleotides encoding polypeptides composing a population of multi-chain CAR each one comprising different extracellular ligand binding domains. In a particular embodiment the method of engineering an immune cell comprises expressing at the surface of the cell at least a part of FcεRI beta and/or gamma chain fused to a signal-transducing domain and several part of FcεRI alpha chains fused to different extracellular ligand binding domains. In

10 a more particular embodiment, said method comprises introducing into said cell at least one polynucleotide which encodes a part of FcεRI beta and/or gamma chain fused to a signal-transducing domain and several FcεRI alpha chains fused to different extracellular ligand binding domains. By population of multi-chain CARs, it is meant at least two, three, four, five, six or more multi-chain CARs each one comprising different extracellular ligand binding domains. The different extracellular ligand

15 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 multi-chain CARs each one comprising different extracellular ligand binding domains.

The signal transducing domain or intracellular signaling domain of the multi-chain CAR of the

20 invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the multi-chain CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of

25 cytokines.

In the present application, 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.

Preferred examples of signal transducing domain for use in single or multi-chain CAR can be the

30 cytoplasmic sequences of the Fc receptor or T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivate or variant of these sequences and any synthetic sequence that as the same functional capability. Signal transduction domain comprises two distinct classes of cytoplasmic signaling sequence, those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a

secondary or co-stimulatory signal. Primary cytoplasmic signaling sequence can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non-limiting examples those derived from TCRzeta, FcRgamma, FcRbeta, FcRepsilon, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d. In a preferred embodiment, the signaling transducing domain of the multi-chain CAR can comprise the CD3zeta signaling domain, or the intracytoplasmic domain of the FcεRI beta or gamma chains.

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

Ligand binding-domains can be any antigen receptor previously used, and referred to, with respect to single-chain CAR referred to in the literature, in particular scFv from monoclonal antibodies.

Bispecific or multi-specific CARs as described in WO 2014/4011988 are incorporated by reference.

Similarly as described before with respect to single-chain CARs, the present invention encompasses immune cells endowed with multi-chain CARs which target specifically the CD38 cell surface marker. According to a preferred embodiment of the invention the CARs described above are expressed in immune cells, whereas inactivation of the endogenous genes encoding said surface marker is induced by expression of a rare-cutting endonuclease.

According to a preferred embodiment, the multi-chain CAR (mcCAR) of the invention comprises at a transmembrane polypeptide from the alpha chain of high-affinity IgE receptor (FcεRI) fused to an extracellular CD38 ligand binding domain.

According to a more preferred embodiment, said anti-CD38 multi-chain CAR comprises further a second transmembrane polypeptide from the gamma or beta chain of FcεRI fused to a signal transducing domain.

According to an even more preferred embodiment, said anti-CD38 multi-chain CAR comprises a third transmembrane polypeptide from the gamma or beta chain of FcεRI comprising a co-stimulatory domain.

According to a preferred embodiment, wherein said CD38 ligand binding domain of above anti-CD38 mcCAR, which is fused to said alpha chain of FcεRI, is a single-chain variable fragment (scFv) comprising heavy (V_H) and light (V_L) chains conferring specificity to CD38.

In a more preferred embodiment, said V_H of above anti-CD38 mcCAR comprises a polypeptide sequence displaying at least 90 % identity to one selected from SEQ ID NO. 14, 62, 54 and 30.

In another more preferred embodiment, said V_L of above anti-CD38 mcCAR comprises a polypeptide displaying at least 90 % identity to one selected from SEQ ID NO. 10, 58, 50 and 26.

5 In an embodiment, said alpha chain of Fc ϵ RI of above anti-CD38 mcCAR is fused to said extracellular ligand-binding domain by a hinge from CD8 α , IgG1 or FcRIII α proteins.

In an embodiment, said signal transducing domain of above anti-CD38 mcCAR is fused to the gamma or beta chain of Fc ϵ RI is from the TCR zeta chain, the FC ϵ R β chain, the Fc ϵ RI γ chain, or includes an immunoreceptor tyrosine-based activation motif (ITAM).

10 In a preferred embodiment, said signal transducing domain of above anti-CD38 mcCAR is from CD3zeta, and preferably comprising a polypeptide sequence displaying at least 90 % identity to SEQ ID NO.81.

In an embodiment, said second or third polypeptide of above anti-CD38 mcCAR comprises a co-stimulatory domain from the cytoplasmic domain of a costimulatory molecule selected from CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, CD8, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

In a preferred embodiment, said above co-stimulatory domain is from 4-1BB and comprises a polypeptide sequence displaying at least 90 % identity to SEQ ID NO.80.

20 Examples of different architectures of the CD38 specific multi-chain CAR according to the invention are presented in Figure 20.

Constructions of exemplary anti-CD38 mcCAR comprising the three polypeptide gamma, alpha and beta are presented in the following Table 3.

Table 3: Exemplary polypeptides forming anti-CD38 multi-chain CARs

Multi chain CAR Designation	Precursor BCMA multi-chain CAR polypeptide structure											
	Gamma polypeptide			Alpha polypeptide								Beta polypeptide
	FcεRI γ-SP	FcεRI γ-ΔITAM	CD3ζ-IC	P2A	FcεRI α-SP	CD8α hinge	VH	G4SX3 Linker	VL	FcεRI α-TM-IC	T2A	
25A10 anti-CD38mcCAR (4-1BB costimul. domain)	SEQ ID NO.106	SEQ ID NO.107	SEQ ID NO.10	SEQ ID NO.108	SEQ ID NO.110	SEQ ID NO.77	SEQ ID NO.14	SEQ ID NO.75	SEQ ID NO.10	SEQ ID NO.111	SEQ ID NO.109	SEQ ID NO.80
28F5 anti-CD38 mcCAR (4-1BB costimul. domain)	SEQ ID NO.106	SEQ ID NO.107	SEQ ID NO.10	SEQ ID NO.108	SEQ ID NO.110	SEQ ID NO.77	SEQ ID NO.62	SEQ ID NO.75	SEQ ID NO.58	SEQ ID NO.111	SEQ ID NO.109	SEQ ID NO.80
13F11 anti-CD38 mcCAR (4-1BB costimul. domain)	SEQ ID NO.106	SEQ ID NO.107	SEQ ID NO.10	SEQ ID NO.108	SEQ ID NO.110	SEQ ID NO.77	SEQ ID NO.54	SEQ ID NO.75	SEQ ID NO.50	SEQ ID NO.111	SEQ ID NO.109	SEQ ID NO.80
16B6 anti-CD38 mcCAR (4-1BB costimul. domain)	SEQ ID NO.106	SEQ ID NO.107	SEQ ID NO.10	SEQ ID NO.108	SEQ ID NO.110	SEQ ID NO.77	SEQ ID NO.30	SEQ ID NO.75	SEQ ID NO.26	SEQ ID NO.111	SEQ ID NO.109	SEQ ID NO.80
25A10 anti-CD38mcCAR	SEQ ID NO.106	SEQ ID NO.107	SEQ ID NO.10	SEQ ID NO.108	SEQ ID NO.110	SEQ ID NO.77	SEQ ID NO.14	SEQ ID NO.75	SEQ ID NO.10	SEQ ID NO.111	SEQ ID NO.109	SEQ ID NO.113

Activation and expansion of T cells

The method according to the invention generally includes a further step of activating and/or expanding the T-cells. This can be done prior to or after genetic modification of the T cells, using the 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. According to these methods, the T cells of the invention can be expanded by contact with a surface having attached thereto an agent that stimulates a CD3 TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells.

In particular, T cell populations may be stimulated *in vitro* such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either CD4+ T cells or CD8+ T cells, an anti-CD3 antibody and an anti-CD28 antibody. For example, the agents providing each signal may be in solution or coupled to a surface. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. In further embodiments of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. Cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one embodiment the cells (for example, 4 to 10 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, preferably PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. The mixture may be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for 21 days. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-g , 1L-4, 1L-7, GM-CSF, -10, - 2, 1L-15, TGFp, and TNF- or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of

cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, A1M-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C) and atmosphere (e.g., air plus 5% CO₂). T-cells that have been exposed to varied stimulation times may exhibit different characteristics.

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

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

According to one embodiment, said T cells of the invention can undergo robust *in vivo* T cell expansion upon administration to a patient, and can persist in the body fluids for an extended amount of time, preferably for a week, more preferably for 2 weeks, even more preferably for at least one month. Although the T-cells according to the invention are expected to persist during these periods, their life span into the patient's body are intended not to exceed a year, preferably 6 months, more preferably 2 months, and even more preferably one month.

Gene inactivation of CD38 antigen

The antigen marker CD38 has been used as specific markers in diagnostic methods for a while, especially with respect to Leukemia pathological cells, but not in therapy. Indeed, although this marker was identified in the art as quite specific marker, it could not be used as target for immunotherapy because antibodies directed against this marker would have destroyed or interfered with patients' T-cells.

In a first embodiment, the method of the invention concerns a method of preparing appropriate immune cells, preferably T-cells for immunotherapy comprising the step of:

(a) Genetically inactivating or mutating a gene encoding the CD38 gene in an immune cell, which is involved in the expression or presentation of the CD38 antigen marker, said CD38 antigen marker being present both on the surface of said immune cell and the pathological cell;

(b) Expressing into said immune cell a transgene encoding a chimeric antigen receptor directed against said CD38 antigen marker such as presented in the previous sections, said antigen marker being present at the surface of said pathological cell.

In another embodiment, said method for engineered CD38 antigen-inactivated and CD38 CAR
5 expressing appropriate immune cells, preferably T-cells for immunotherapy comprises the step of:

(a) Genetically inactivating or mutating a gene encoding the CD38 gene in an immune cell as described in the present invention, combined with the inactivation or mutation of another gene or other genes; said gene(s) being selected in the group consisting of gene(s) involved in engraftment of allogeneic immune cells, as immune checkpoints, in conferring drug resistance or in conferring
10 resistance to immunosuppressive agent;

(b) Expressing into said immune cell a transgene encoding a chimeric antigen receptor directed against said CD38 antigen marker such as described in other sections and in claim 1.

In a particular embodiment, said method to engineer cells comprises at least one of the following steps:

15 (a) providing an immune cell, preferably T-cell, from a blood sample;

(b) introducing into said immune cell a rare-cutting endonuclease able to selectively inactivate by DNA cleavage, preferably by double-strand break respectively:

- said gene encoding CD38 antigen, and
- at least one gene encoding a component involved in engraftment of allogeneic immune
20 cells, as immune checkpoints, in conferring drug resistance or in conferring resistance to immunosuppressive agent).

(c) expanding said cells.

In a preferred embodiment, said additional gene(s) to be inactivated or mutated during step a) is or are selected in the group consisting of TCR, beta2M, PD-1, CTLA-4, dCK, DHFR, MGMT, IMPDH2,
25 MDR1, CD52, GR.

In another embodiment, said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease, a TALE-nuclease, a Casp9 nuclease or a Cpf1 nuclease. In a preferred embodiment, said rare-cutting endonuclease is a TALE-nuclease.

In a particular embodiment, inactivation of CD38 antigen and of gene(s) involved in engraftment
30 of allogeneic immune cells, as immune checkpoints, in conferring drug resistance or in conferring

resistance to immunosuppressive agent can be done at a precise genomic location targeted by a specific rare-cutting endonuclease such as TALE-nuclease, wherein said specific endonuclease catalyzes a cleavage and wherein an exogenous nucleic acid successively comprising at least a region of homology and a sequence to inactivate CD38 targeted gene and said above other gene(s) which is integrated by homologous recombination. In another embodiment, several genes can be, successively or at the same time, inactivated by using several specific rare-cutting endonucleases respectively and specifically targeting one defined gene and several specific polynucleotides for specific gene inactivation.

In a preferred embodiment, said method for engineered KO CD38 and anti-CD38 CAR expressing immune cells comprises the step of:

(a) Genetically inactivating the CD38 gene in an immune cell, said target CD38 being selected in the group consisting of SEQ ID 1, 4 and 7, by using respectively the left and right TALE nuclease of SEQ 2-3, SEQ 5-6, and SEQ 8-9;

(b) Expressing into said immune cell a transgene encoding a chimeric antigen receptor directed against CD38 antigen, said specific anti-CD38 CAR having one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4).

In another preferred embodiment, said method for engineered KO CD38 and CD38+ expressing immune cells comprises the step of:

(a) Genetically inactivating the CD38 gene in an immune cell, said target CD38 being selected in the group consisting of SEQ ID 1, 4 and 7, by using respectively the left and right TALE nuclease of SEQ 2-3, SEQ 5-6, and SEQ 8-9;

(b) Expressing into said immune cell a transgene encoding an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR), wherein said V_H and V_L comprise the CDRs sequences of respectively SEQ ID NO.15-17 and SEQ ID NO.11-13; respectively SEQ ID NO.63-65 and SEQ ID NO.59-62; respectively

SEQ ID NO.55-57 and SEQ ID NO.51-53; respectively SEQ ID NO.31-33 and SEQ ID NO.27-29, respectively SEQ ID NO.39-41 and SEQ ID NO.35-37, respectively SEQ ID NO.47-49 and SEQ ID NO.45-43, respectively SEQ ID NO.55-57 and SEQ ID NO.51-53, respectively SEQ ID NO.63-65 and SEQ ID NO.59-61, respectively SEQ ID NO.71-73 and SEQ ID NO.67-69.

5

In more preferred embodiment, said method for engineered KO CD38 and anti-CD38 CAR expressing immune cells comprises the step of:

(a) Genetically inactivating the CD38 gene in an immune cell, said target CD38 of SEQ ID 4, by using respectively the left and right TALE nuclease of SEQ 5-6;

10 (b) Expressing into said immune cell a transgene encoding an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) and SEQ ID NO. 88-90 (16B5).

15 In even more preferred embodiment, said method for engineered KO CD38 and anti-CD38 CAR expressing immune cells comprises the step of:

(a) Genetically inactivating the CD38 gene in an immune cell, said target CD38 of SEQ ID 4, by using respectively the left and right TALE nuclease of SEQ 5-6;

20 (b) Expressing into said immune cell a transgene encoding an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10) and SEQ ID NO. 100-102 (28F5).

In still even more preferred embodiment, said method for engineered KO CD38 and anti-CD38 CAR expressing immune cells comprises the step of:

25 (a) Genetically inactivating the CD38 gene in an immune cell, said target CD38 of SEQ ID 4, by using respectively the left and right TALE nuclease of SEQ 5-6;

(b) Expressing into said immune cell a transgene encoding an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 82 (25A10-v1 CAR).

The immune cells according to the invention are endowed with a anti-CD38 chimeric antigen receptor directed to the CD38 antigen marker that is commonly expressed by the pathological cells and immune cells, or known to be present on the surface of said T Cells. The expression "known to be present" means that the antigen marker is reported to be found on the surface of the immune cells
5 grown in natural conditions *in-vivo*, especially in the blood, but not necessarily when they are cultured *in-vitro*. In any event, the method of the invention results into the absence of the CD38 antigen marker on the surface of the immune cell, thereby preventing the chimeric antigen receptor from reacting with the engineered T-cell surface. In this respect, the method may include a further step of purifying the resulting T-cells by excluding the cells presenting said marker antigen on their surface.

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

25 According to a preferred embodiment of the invention, the gene mutation or inactivation of step a) of the above method is performed using a rare-cutting endonuclease.

The term "rare-cutting endonuclease" refers to a 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. Particularly, said nuclease can be an endonuclease, more preferably a rare-cutting endonuclease which is highly specific, recognizing nucleic acid target sites ranging from 10 to 45
30 base pairs (bp) in length, usually ranging from 10 to 35 base pairs in length, more usually from 12 to 20 base pairs. The endonuclease according to the present invention recognizes at specific polynucleotide sequences, further referred to as "target sequence" and cleaves nucleic acid inside these target sequences or into sequences adjacent thereto, depending on the molecular structure of said

endonuclease. The rare-cutting endonuclease can recognize and generate a single- or double-strand break at specific polynucleotides sequences.

In a particular embodiment, said rare-cutting endonuclease according to the present invention is a RNA-guided endonuclease such as the Cas9/CRISPR complex. RNA guided endonucleases constitute a new generation of genome engineering tool where an endonuclease associates with a RNA molecule. In this system, the RNA molecule nucleotide sequence determines the target specificity and activates the endonuclease (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013).

TAL-nucleases

In a preferred embodiment, the inactivation of the CD38 gene in step a) of the previously presented method is performed by the use of TAL-nuclease.

In a more preferred embodiment, said TAL-nuclease targets a CD38 gene sequence of SEQ ID NO.1, 4 or 7.

According a still more preferred embodiment, said inactivation of CD38 antigen is performed by using the TALE-nucleases of SEQ ID NO.2-3, 5-6 or 8-9.

Said above sequences for CD38 targets and their corresponding left and right TALE nuclease are presented in the following Table 4.

Table 4: Sequences of two other CD38 targets and the corresponding TALENs for their inactivation

Name	SEQ ID #	Nucleic acid sequence and repeats sequence
CD38ex1-T2 target	1	TGAGGTGGGTTGGCGACTaaggcgcaccggTGGGCACTGCGGGGACA
CD38ex1-T2 Left TALEN	2	MGDPKKKRKVIDYPYDVPDYAIDIADLRTLGYSSQQQEKIKPKVRSTVAQHHEALV GHGFTHAHIVALSQHHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARAL EALLTVAGELRGPPQLDGTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTQQQ VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLP VLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNN GGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQ VVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLL PVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN NGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQAL ETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPALESIVAQLSRPDPALAALTNH LVALACLGGRPALDAVKKGLGDPISRSQLVKSELEKKSELRHKLKYVPHEYIELIEIA RNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKA YSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWVKVYPSSVTEFKFLFVSGHF KGNKYAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFAA D Repeat sequence TALEN: NN-NI-NN-NN-NG-NN-NN-NN-NG-NG-NN-NN- HD-NN-NI-NG
CD38ex1-T2 Right TALEN	3	MGDPKKKRKVIDKETAAAKFERQHMDSIDIADLRTLGYSSQQQEKIKPKVRSTVA QHHEALVGHGFTHAHIVALSQHHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQ WSGARALEALLTVAGELRGPPQLDGTGQLLKIAGRGGVTAVEAVHAWRNALTGA PLNLTQQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQ ALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRL LPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN NGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQAL ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQ VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLL PVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHD GGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPALESIVAQLSRPDPALA ALTNHDLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEKKSELRHKLKYVPHE YIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGV IVDTKAYS GGYNLPIGQADEMQRYVEENQTRNKHINPNEWVKVYPSSVTEFKFLF VSGHFKGNKYAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNNG EINFAAD Repeat sequence TALEN : NN-NG-HD-HD-HD-NN-HD-NI-NN-NG-NN- HD-HD-HD-NG

CD38-1 target	4	TGCGAGTTCAGCCCGGtggtccggggacaaacccTGCTGCCGGCTCTCTA
CD38-1 Left TALEN	5	MGDPKKKKRKVIDYPYDVPDYAIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALV GHGFTHAHIVALSQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARAL EALLTVAGELRGPPQLDGTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTQQ VVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQALL PVLCQAHGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASN NGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNIGGKQALETVQRLLPVLCQAH GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQ VVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNIGGKQALETVQRLL PVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASH DGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA HGLTPQQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQA LETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQ VVAIASNNGGGRPALESIVAQLSRPDPALAALNDHLVALACLGGRPALDAVKKGLG DPISRSQLVKSELEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKV YGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVE ENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNKYAQLTRLNHITNCNGA VLSVEELLIGGEMIKAGTLTLEEVRRKFNNGEINFAAD Repeat sequence TALEN: NG-NN-HD-NN-NI-NN-NG-NG-HD-NI-NN-HD- HD-HD-NN-NN-NG
CD38- 1_Right TALEN	6	MGDPKKKKRKVIDKETAAAKFERQHMSIDIADLRTLGYSSQQQKEIKPKVRSTVA QHHEALVGHGFTHAHIVALSQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQ WSGARALEALLTVAGELRGPPQLDGTGQLLKIAGRGGVTAVEAVHAWRNALTGA PLNLTQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQ ALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPE QVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIAS HDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQ AHGLTPEQVVAIASNNGGKQALETVQALLPVLCQAHGLTPQQVVAIASHDGGKQ ALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQ QVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQR LLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIAS NNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNIGGGRPALESIVAQLSRPDPAL AALNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEKKSELRHKLKYVP HEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDY GIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKF LFVSGHFKGNKYAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRRKFNN GEINFAAD Repeat sequence TALEN: NG-NN-HD-NG-NN-HD-HD-NN-NN-HD-NG-HD- NG-HD-NG-NI

Such catalytic domain usually comes from enzymes, such as for instance I-TevI, CoIE7, NucA and Fok-I. TALE-nuclease can be formed under monomeric or dimeric forms depending of the selected catalytic domain (WO2012138927). Such engineered TALE-nucleases are commercially available under the trade name TALENTM (Collectis, 8 rue de la Croix Jarry, 75013 Paris, France).

5 According to a preferred embodiment of the invention, the DNA binding domain is derived from a Transcription Activator like Effector (TALE), wherein sequence specificity is driven by a series of 33-35 amino acids repeats originating from *Xanthomonas* or *Ralstonia* bacterial proteins AvrBs3, PthXo1, AvrHah1, PthA, Tal1c as non-limiting examples.

10 These repeats differ essentially by two amino acids positions that specify an interaction with a base pair (Boch, Scholze et al. 2009; Moscou and Bogdanove 2009). Each base pair in the DNA target is contacted by a single repeat, with the specificity resulting from the two variant amino acids of the repeat (the so-called repeat variable dipeptide, RVD). TALE binding domains may further comprise an N-terminal translocation domain responsible for the requirement of a first thymine base (T0) of the targeted sequence and a C-terminal domain that containing a nuclear localization signals (NLS). A TALE
15 nucleic acid binding domain generally corresponds to an engineered core TALE scaffold comprising a plurality of TALE repeat sequences, each repeat comprising a RVD specific to each nucleotides base of a TALE recognition site. In the present invention, each TALE repeat sequence of said core scaffold is made of 30 to 42 amino acids, more preferably 33 or 34 wherein two critical amino acids (the so-called repeat variable dipeptide, RVD) located at positions 12 and 13 mediates the recognition of one nucleotide of
20 said TALE binding site sequence; equivalent two critical amino acids can be located at positions other than 12 and 13 specially in TALE repeat sequence taller than 33 or 34 amino acids long. 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. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards
25 nucleotides A, T, C and G and in particular to enhance this specificity. A TALE nucleic acid binding domain usually comprises between 8 and 30 TALE repeat sequences. More preferably, said core scaffold of the present invention comprises between 8 and 20 TALE repeat sequences; again more preferably 15 TALE repeat sequences. It can also comprise an additional single truncated TALE repeat sequence made of 20 amino acids located at the C-terminus of said set of TALE repeat sequences, i.e. an additional C-
30 terminal half- TALE repeat sequence.

Other engineered DNA binding domains can be used as alternative sequences to form so-called modular base-per-base specific nucleic acid binding domains (MBBBD) as described in WO 2014/018601. Said MBBBD can be engineered, for instance, from newly identified proteins, namely EAV36_BURRH, E5AW43_BURRH, E5AW45_BURRH and E5AW46_BURRH proteins from the recently sequenced genome

of the endosymbiont fungi *Burkholderia Rhizoxinica* (Lackner, Moebius et al. 2011). These nucleic acid binding polypeptides comprise modules of about 31 to 33 amino acids that are base specific. These modules display less than 40 % sequence identity with *Xanthomonas* TALE common repeats and present more polypeptides sequence variability. The different domains from the above proteins (modules, N and C-terminals) from *Burkholderia* and *Xanthomonas* are useful to engineer new proteins or scaffolds having binding properties to specific nucleic acid sequences and may be combined to form chimeric TALE-MBBBD proteins.

The present invention encompasses a method for engineered T-cells in order to inactivate the expression of the genes encoding CD38 antigen marker by using specific TALE-nucleases.

Particularly suitable for the realization of the invention, TALE-nucleases such as the ones in SEQ ID NO: 2-3; 5-6; 8-9 for the CD38 gene. These specific TALE-nucleases, their sequence target and the protocol used are presented more thoroughly in the following Example 1.

Cas 9

In another embodiment, the gene inactivation of CD38 in step a) of the previously presented method is performed using the Cas 9 RNA-guided endonuclease.

In another embodiment, said RNA-guided endonuclease is split into at least 2 polypeptides, one comprising RuvC and another comprising HNH.

Cas9, also named Csn1 (COG3513) is a large protein that participates in both crRNA biogenesis and in the destruction of invading DNA. Cas9 has been described in different bacterial species such as *S. thermophiles*, *Listeria innocua* (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012) and *S. Pyogenes* (Deltcheva, Chylinski et al. 2011). The large Cas9 protein (>1200 amino acids) contains two predicted nuclease domains, namely HNH (McrA-like) nuclease domain that is located in the middle of the protein and a splitted RuvC-like nuclease domain (RNase H fold) (Makarova, Grishin et al. (2006).

By "Cas9" is meant an engineered endonuclease or a homologue of Cas9 which is capable of processing target nucleic acid sequence. In particular embodiment, Cas9 can induce a cleavage in the nucleic acid target sequence which can correspond to either a double-stranded break or a single-stranded break. Cas9 variant can be a Cas9 endonuclease that does not naturally exist in nature and that is obtained by protein engineering or by random mutagenesis. Cas9 variants according to the invention can for example be obtained by mutations i.e. deletions from, or insertions or substitutions of at least one residue in the amino acid sequence of a *S. pyogenes* Cas9 endonuclease (COG3513). In the frame aspects of the present invention, such Cas9 variants remain functional, i.e. they retain the capacity of

processing a target nucleic acid sequence. Cas9 variant can also be homologues of *S. pyogenes* Cas9 which can comprise deletions from, or insertions or substitutions of, at least one residue within the amino acid sequence of *S. pyogenes* Cas9. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity, in particular the capacity of binding a guide RNA or nucleic acid target sequence.

RuvC/RNaseH motif includes proteins that show wide spectra of nucleolytic functions, acting both on RNA and DNA (RNaseH, RuvC, DNA transposases and retroviral integrases and PIWI domain of Argonaut proteins). In the present invention the RuvC catalytic domain of the Cas9 protein can be characterized by the sequence motif: D-[I/L]-G-X-X-S-X-G-W-A, wherein X represents any one of the natural 20 amino acids and [I/L] represents isoleucine or leucine. In other terms, the present invention relates to Cas9 variant which comprises at least D-[I/L]-G-X-X-S-X-G-W-A sequence, wherein X represents any one of the natural 20 amino acids and [I/L] represents isoleucine or leucine.

HNH motif is characteristic of many nucleases that act on double-stranded DNA including colicins, restriction enzymes and homing endonucleases. The domain HNH (SMART ID: SM00507, SCOP nomenclature:HNH family) is associated with a range of DNA binding proteins, performing a variety of binding and cutting functions. The ones with known function are involved in a range of cellular processes including bacterial toxicity, homing functions in groups I and II introns and inteins, recombination, developmentally controlled DNA rearrangement, phage packaging, and restriction endonuclease activity (Dalgaard, Klar et al. 1997). These proteins are found in viruses, archaeobacteria, eubacteria, and eukaryotes. Interestingly, as with the LAGLI-DADG and the GIY-YIG motifs, the HNH motif is often associated with endonuclease domains of self-propagating elements like inteins, Group I, and Group II introns (Dalgaard, Klar et al. 1997). The HNH domain can be characterized by the presence of a conserved Asp/His residue flanked by conserved His (amino-terminal) and His/Asp/Glu (carboxy-terminal) residues at some distance. A substantial number of these proteins can also have a CX2C motif on either side of the central Asp/His residue. Structurally, the HNH motif appears as a central hairpin of twisted β -strands, which are flanked on each side by an α helix (Kleanthous, Kuhlmann et al. 1999). The large HNH domain of Cas9 is represented by SEQ ID NO.5. In the present invention, the HNH motif can be characterized by the sequence motif: Y-X-X-D-H-X-X-P-X-S-X-X-X-D-X-S, wherein X represents any one of the natural 20 amino acids. The present invention relates to a Cas9 variant which comprises at least Y-X-X-D-H-X-X-P-X-S-X-X-X-D-X-S sequence wherein X represents any one of the natural 20 amino acids.

This invention can be of particular interest to easily do targeted multiplex gene modifications and to create an inducible nuclease system by introduction of the guide RNA to the Cas9 cells. For the purpose of the present invention, the inventors have established that Cas9 protein can be divided into

two separate split Cas9 RuvC and HNH domains which can process target nucleic acid sequence together or separately with the guide RNA.

Also the RuvC and HNH domains from different RNA guided endonucleases or Cas homologues may be assembled to improve nuclease efficiency or specificity. The domains from different species can be either split into two proteins or fused to each other to form a variant Cas protein. The Cas9 split system is deemed particularly suitable for an inducible method of genome targeting and to avoid the potential toxic effect of the Cas9 overexpression within the cell. Indeed, a first split Cas9 domain can be introduced into the cell, preferably by stably transforming said cell with a transgene encoding said split domain. Then, the complementary split part of Cas9 can be introduced into the cell, such that the two split parts reassemble into the cell to reconstitute a functional Cas9 protein at the desired time.

The reduction of the size of the split Cas9 compared to wild type Cas9 ease the vectorization and the delivery into the cell, for example, by using cell penetrating peptides. Re-arranging domains from different Cas proteins, allows to modulate the specificity and nuclease activity, for instance, by targeting PAM motifs that are slightly different from *S. pyogenes* Cas9

Split Cas9 system

The previous characterization of the RuvC and HNH domains has prompted the inventors to engineer Cas9 protein to create split Cas9 protein. Surprisingly, the inventors showed that these two split Cas9 could process together or separately the nucleic acid target. This observation allows developing a new Cas9 system using split Cas9 protein. Each split Cas9 domains can be prepared and used separately. Thus, this split system displays several advantages for vectorization and delivery of the RNA guided endonuclease in T-cells, allowing delivering a shorter and/or inactive protein, and is particularly suitable to induce genome engineering in T-cells at the desired time and thus limiting the potential toxicity of an integrated Cas9 nuclease.

By "Split Cas9" is meant here a reduced or truncated form of a Cas9 protein or Cas9 variant, which comprises either a RuvC or HNH domain, but not both of these domains. Such "Split Cas9" can be used independently with guide RNA or in a complementary fashion, like for instance, one Split Cas9 providing a RuvC domain and another providing the HNH domain. Different split RNA guided endonucleases may be used together having either RuvC and/or HNH domains.

Each Cas9 split domain can be derived from the same or from different Cas9 homologues. Many homologues of Cas9 have been identified in genome databases.

Said Cas9 split domains (RuvC and HNH domains) can be simultaneously or sequentially introduced into the cell such that said split Cas9 domain(s) process the target nucleic acid sequence in

the cell. Said Cas9 split domains and guide RNA can be introduced into the cell by using cell penetrating peptides or other transfection methods as described elsewhere.

In another aspect of the invention, only one split Cas9 domain, referred to as compact Cas9 is introduced into said cell. Indeed, surprisingly the inventors showed that the split Cas9 domain comprising the RuvC motif as described above is capable of cleaving a target nucleic acid sequence independently of split domain comprising the HNH motif. Thus, they could establish that the guideRNA does not need the presence of the HNH domain to bind to the target nucleic acid sequence and is sufficiently stable to be bound by the RuvC split domain. In a preferred embodiment, said split Cas9 domain alone is capable of nicking said target nucleic acid sequence.

Each split domain can be fused to at least one active domain in the N-terminal and/or C-terminal end, said active domain can be selected from the group consisting of: nuclease (e.g. endonuclease or exonuclease), polymerase, kinase, phosphatase, methylase, demethylase, acetylase, desacetylase, topoisomerase, integrase, transposase, ligase, helicase, recombinase, transcriptional activator(e.g. VP64, VP16), transcriptional inhibitor (e. g; KRAB), DNA end processing enzyme (e.g. Trex2, Tdt), reporter molecule (e.g. fluorescent proteins, lacZ, luciferase).

HNH domain is responsible for nicking of one strand of the target double-stranded DNA and the RuvC-like RNaseH fold domain is involved in nicking of the other strand (comprising the PAM motif) of the double-stranded nucleic acid target (Jinek, Chylinski et al. 2012). However, in wild-type Cas9, these two domains result in blunt cleavage of the invasive DNA within the same target sequence (proto-spacer) in the immediate vicinity of the PAM (Jinek, Chylinski et al. 2012). Cas 9 can be a nickase and induces a nick event within different target sequences.

As non-limiting example, Cas9 or split Cas9 can comprise mutation(s) in the catalytic residues of either the HNH or RuvC-like domains, to induce a nick event within different target sequences. As non-limiting example, the catalytic residues of the Cas9 protein are those corresponding to amino acids D10, D31, H840, H868, N882 and N891 or aligned positions using CLUSTALW method on homologues of Cas Family members. Any of these residues can be replaced by any other amino acids, preferably by alanine residue. Mutation in the catalytic residues means either substitution by another amino acids, or deletion or addition of amino acids that induce the inactivation of at least one of the catalytic domain of cas9. (cf.. In a particular embodiment, Cas9 or split Cas9 may comprise one or several of the above mutations. In another particular embodiment, split Cas9 comprises only one of the two RuvC and HNH catalytic domains. In the present invention, Cas9 from different species, Cas9 homologues, Cas9 engineered and functional variant thereof can be used. The invention envisions the use of any RNA guided endonuclease or split RNA guided endonucleases variants to perform nucleic acid cleavage in a genetic sequence of interest.

Meganucleases

Rare-cutting endonuclease can also be a homing endonuclease, also known under the name of meganuclease. Such homing endonucleases are well-known to the art (Stoddard 2005). Homing endonucleases are highly specific, recognizing DNA target sites ranging from 12 to 45 base pairs (bp) in length, usually ranging from 14 to 40 bp in length. The homing endonuclease according to the invention may for example correspond to a LAGLIDADG endonuclease, to a HNH endonuclease, or to a GIY-YIG endonuclease. Preferred homing endonuclease according to the present invention can be an I-Cre1 variant. A "variant" endonuclease, i.e. an endonuclease that does not naturally exist in nature and that is obtained by genetic engineering or by random mutagenesis can bind DNA sequences different from that recognized by wild-type endonucleases (see international application WO2006/097854).

Said rare-cutting endonuclease can be a modular DNA binding nuclease. By modular DNA binding nuclease is meant any fusion proteins comprising at least one catalytic domain of an endonuclease and at least one DNA binding domain or protein specifying a nucleic acid target sequence. The DNA binding domain is generally a RNA or DNA-binding domain formed by an independently folded polypeptide or protein domain that contains at least one motif that recognizes double- or single-stranded polynucleotides. Many such polypeptides have been described in the art having the ability to bind specific nucleic acid sequences. Such binding domains often comprise, as non-limiting examples, helix-turn helix domains, leucine zipper domains, winged helix domains, helix-loop-helix domains, HMG-box domains, Immunoglobulin domains, B3 domain or engineered zinc finger domain.

Zinc-Finger Nucleases

Initially developed to cleave DNA in vitro, "Zinc Finger Nucleases" (ZFNs) are a fusion between the cleavage domain of the type IIS restriction enzyme, FokI, and a DNA recognition domain containing 3 or more C2H2 zinc finger motifs. The heterodimerization at a particular position in the DNA of two individual ZFNs in precise orientation and spacing leads to a double-strand break (DSB) in the DNA. The use of such chimeric endonucleases have been extensively reported in the art as reviewed by Urnov et al. (Genome editing with engineered zinc finger nucleases (2010) *Nature reviews Genetics* 11:636-646).

Standard ZFNs fuse the cleavage domain to the C-terminus of each zinc finger domain. In order to allow the two cleavage domains to dimerize and cleave DNA, the two individual ZFNs bind opposite strands of DNA with their C-termini a certain distance apart. The most commonly used linker sequences between the zinc finger domain and the cleavage domain requires the 5' edge of each binding site to be separated by 5 to 7 bp.

The most straightforward method to generate new zinc-finger arrays is to combine smaller zinc-finger "modules" of known specificity. The most common modular assembly process involves combining three separate zinc fingers that can each recognize a 3 base pair DNA sequence to generate a 3-finger array that can recognize a 9 base pair target site. Numerous selection methods have been used to generate zinc-finger arrays capable of targeting desired sequences. Initial selection efforts utilized phage display to select proteins that bound a given DNA target from a large pool of partially randomized zinc-finger arrays. More recent efforts have utilized yeast one-hybrid systems, bacterial one-hybrid and two-hybrid systems, and mammalian cells.

Delivery methods

The inventors have considered any means known in the art to allow delivery inside cells or subcellular compartments of said cells the polynucleotides expressing the endonucleases, their possible co-effectors (e.g. guide RNA or DNA associated with Cas9 or Argonaute nucleases) as well as the chimeric antigen receptors. These means include viral transduction, electroporation and also liposomal delivery means, polymeric carriers, chemical carriers, lipoplexes, polyplexes, dendrimers, nanoparticles, emulsion, natural endocytosis or phagocytose pathway as non-limiting examples.

As a preferred embodiment of the invention, polynucleotides encoding the endonucleases of the present invention are transfected under mRNA form in order to obtain transient expression and avoid chromosomal integration of foreign DNA, for example by electroporation. The inventors have determined different optimal conditions for mRNA electroporation in T-cell displayed in Table 5. The inventor used the cytoPulse technology which allows, by the use of pulsed electric fields, to transiently permeabilize living cells for delivery of material into the cells (U.S. patent 6,010,613 and WO 2004/083379). Pulse duration, intensity as well as the interval between pulses can be modified in order to reach the best conditions for high transfection efficiency with minimal mortality. Basically, the first high electric field pulses allow pore formation, while subsequent lower electric field pulses allow to moving the polynucleotide into the cell. In one aspect of the present invention, the inventor describe the steps that led to achievement of >95% transfection efficiency of mRNA in T cells, and the use of the electroporation protocol to transiently express different kind of proteins in T cells. In particular the invention relates to a method of transforming T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence consisting of:

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

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

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

In particular embodiment, the method of transforming T cell comprising contacting said T cell with RNA and applying to T cell an agile pulse sequence consisting of:

(a) one electrical pulse with a voltage of 2250, 2300, 2350, 2400, 2450, 2500, 2550, 2400, 2450, 2500, 2600, 2700, 2800, 2900 or 3000V per centimeter, a pulse width of 0.1 ms and a pulse interval of 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ms between the electrical pulses of step (a) and (b);

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

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

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

Table 5 : Different cytopulse programs used to determine the minimal voltage required for electroporation in PBMC derived T-cells.

<u>Cyto-pulse program</u>	Group 1				Group 2				Group 3			
	Pul- ses	V	duration (ms)	Interval (ms)	Pul- ses	V	duration (ms)	Interval (ms)	Pul- ses	V	duration (ms)	Interval (ms)
1	1	600	0.1	0.2	1	600	0.1	100	4	130	0.2	2
2	1	900	0.1	0.2	1	900	0.1	100	4	130	0.2	2
3	1	1200	0.1	0.2	1	1200	0.1	100	4	130	0.2	2
4	1	1200	0.1	10	1	900	0.1	100	4	130	0.2	2
5	1	900	0.1	20	1	600	0.1	100	4	130	0.2	2

Viral transduction

According to the present invention, the use of retroviral vectors and more preferably of lentiviral vectors is particularly suited for expressing the chimeric antigen receptors into the T-cells.

Methods for viral transduction are well known in the art (Walther et *al.* (2000) Viral Vectors for Gene Transfer. *Drugs*. 60(2):249-271). Integrative viral vectors allow the stable integration of the polynucleotides in the T-cells genome and to expressing the chimeric antigen receptors over a longer period of time.

5 According to another embodiment, said CD38 CARs selected are cloned in a lentiviral vector encoding for the suicide gene RQR8 which is be under the control of promotor such as EF1a.

According to a preferred embodiment, the CD38 CARs are transduced on T cells on CD38 deficient T cells. The expression of the CAR is usually assessed by flow cytometry and the anti-CD38 CAR T cells phenotype and function are tested. This is described in more details thereafter.

10 Non alloreactive T cells

Although the method of the invention could be carried out *in-vivo* as part of a gene therapy, for instance, by using viral vectors targeting T-cells in blood circulation, which would include genetic sequences expressing a specific rare-cutting endonuclease along with other genetic sequences
15 expressing a CAR, the method of the invention is more generally intended to be practiced *ex-vivo* on cultured T-cells obtainable from patients or donors.

According to one embodiment, the immune cell to be engineered such as presented in the present invention is derived from a primary stem cell, iPS or hES cell.

In another embodiment, said immune cell is derived from a patient affected by the development
20 of pathological cells.

The engineered T-cells engineered *ex-vivo* can be either re-implanted into a patient from where they originate, as part of an autologous treatment, or to be used as part of an allogeneic treatment. In this later case, it is preferable to further engineer the cells to make them non-alloreactive to ensure their proper engraftment. Accordingly, the method of the invention may include additional steps of
25 procuring the T-cells from a donor and to inactivate genes thereof involved in MHC recognition and or being targets of immunosuppressive drugs such as described for instance in WO 2013/176915.

T cell receptors (TCR) are cell surface receptors that participate in the activation of T cells in response to the presentation of antigen. The TCR is generally made from two chains, alpha and beta, which assemble to form a heterodimer and associates with the CD3-transducing subunits to form the T-
30 cell receptor complex present on the cell surface. Each alpha and beta chain of the TCR consists of an immunoglobulin-like N-terminal variable (V) and constant (C) region, a hydrophobic transmembrane domain, and a short cytoplasmic region. As for immunoglobulin molecules, the variable region of the alpha and beta chains are generated by V(D)J recombination, creating a large diversity of antigen

specificities within the population of T cells. However, in contrast to immunoglobulins that recognize intact antigen, T cells are activated by processed peptide fragments in association with an MHC molecule, introducing an extra dimension to antigen recognition by T cells, known as MHC restriction. Recognition of MHC disparities between the donor and recipient through the T cell receptor leads to T cell proliferation and the potential development of GVHD. It has been shown that normal surface expression of the TCR depends on the coordinated synthesis and assembly of all seven components of the complex (Ashwell and Klusner 1990). The inactivation of TCRalpha or TCRbeta can result in the elimination of the TCR from the surface of T cells preventing recognition of alloantigen and thus GVHD.

Thus, still according to the invention, engraftment of the T-cells may be improved by inactivating at least one gene encoding a TCR component. TCR is rendered not functional in the cells by inactivating TCR alpha gene and/or TCR beta gene(s).

With respect to the use of Cas9/CRISPR system, the inventors have determined appropriate target sequences within the 3 exons encoding TCR, allowing a significant reduction of toxicity in living cells, while retaining cleavage efficiency. The preferred target sequences are presented in Table 2 in the application WO2014/191128.

MHC antigens are also proteins that played a major role in transplantation reactions. Rejection is mediated by T cells reacting to the histocompatibility antigens on the surface of implanted tissues, and the largest group of these antigens is the major histocompatibility antigens (MHC). These proteins are expressed on the surface of all higher vertebrates and are called HLA antigens (for human leukocyte antigens) in human cells. Like TCR, the MHC proteins serve a vital role in T cell stimulation. Antigen presenting cells (often dendritic cells) display peptides that are the degradation products of foreign proteins on the cell surface on the MHC. In the presence of a co-stimulatory signal, the T cell becomes activated, and will act on a target cell that also displays that same peptide/MHC complex. For example, a stimulated T helper cell will target a macrophage displaying an antigen in conjunction with its MHC, or a cytotoxic T cell (CTL) will act on a virally infected cell displaying foreign viral peptides.

Thus, in order to provide less alloreactive T-cells, the method of the invention can further comprise the step of inactivating or mutating one HLA gene.

The class I HLA gene cluster in humans comprises three major loci, B, C and A, as well as several minor loci. The class II HLA cluster also comprises three major loci, DP, DQ and DR, and both the class I and class II gene clusters are polymorphic, in that there are several different alleles of both the class I and II genes within the population. There are also several accessory proteins that play a role in HLA functioning as well. The Tap1 and Tap2 subunits are parts of the TAP transporter complex that is essential in loading peptide antigens on to the class I HLA complexes, and the LMP2 and LMP7

proteosome subunits play roles in the proteolytic degradation of antigens into peptides for display on the HLA. Reduction in LMP7 has been shown to reduce the amount of MHC class I at the cell surface, perhaps through a lack of stabilization (Fehling et al. (1999) *Science* 265:1234-1237). In addition to TAP and LMP, there is the tapasin gene, whose product forms a bridge between the TAP complex and the HLA class I chains and enhances peptide loading. Reduction in tapasin results in cells with impaired MHC class I assembly, reduced cell surface expression of the MHC class I and impaired immune responses (Grande et al. (2000) *Immunity* 13:213-222 and Garbi et al. (2000) *Nat. Immunol.* 1:234-238). Any of the above genes may be inactivated as part of the present invention as disclosed, for instance in WO 2012/012667.

In another embodiment, the method of preparing immune cells includes a further step of inactivating a gene encoding $\beta 2m$. Beta-2 microglobulin, also known as B2M, is the light chain of MHC class I molecules, and as such an integral part of the major histocompatibility complex. In human, B2M is encoded by the b2m gene which is located on chromosome 15, opposed to the other MHC genes which are located as gene cluster on chromosome 6. The human protein is composed of 119 amino acids (SEQ ID NO: 1) and has a molecular weight of 11.800 Daltons. Mice models deficient for beta-2 microglobulin have shown that B2M is necessary for cell surface expression of MHC class I and stability of the peptide binding groove. It was further shown that haemopoietic transplants from mice that are deficient for normal cell-surface MHC I expression are rejected by NK1.1+ cells in normal mice because of a targeted mutation in the beta-2 microglobulin gene, suggesting that deficient expression of MHC I molecules renders marrow cells susceptible to rejection by the host immune system (Bix et al. 1991).

Insertion of at least one epitope in the extracellular domain of the anti-CD38-single chain CAR

An anti-CD38 CAR of the invention may include at least the insertion of at least one epitope in the extracellular domain of said CAR. This is intended to tentatively deplete the immune cells endowed with the CAR in the event of in vivo adverse effects such as a cytokine storm. Moreover, such insertion of epitope or "epitope-tagging" may be useful to sort or purify the engineered immune cells in-vitro during their manufacturing process. Said at least one epitope may be any antigenic peptide which is enough immunogenic to be bound by a specific antibody recognizing such peptide. For instance, this can be obtained, for instance, by inserting at least one, and preferably two copies of a CD20 mimotope, preferably of sequence CPYSNPSLCS (SEQ ID NO.114), into the CAR polypeptide sequence. For purpose of simplification hereafter, the order of the scFvs from the N terminal end to the C terminal end is presented as follows: the VH chain and then the VL chain. However, it can be envisioned in the scope of the present invention that this order is inversed: VL chain and then the VL chain.

Different positions of the at least one CD20 mimotope within the anti-CD38 CAR of the invention are schematized in Figure 21A and Figure 21B. Said two copies of a CD20 mimotope can be linked to each other and also to the V_L by a linker. They can also be inserted between the anti-CD38 scFv and the hinge (such as CD8alpha), by using an optional linker. The CD20 mimotopes can be bound by anti-CD20 antibodies, such as Rituximab (McLaughlin P, et al. 1998).

Accordingly, the anti-CD38 CAR of the present invention may comprise V_H and a V_L chains which are able to bind to CD38 cell surface antigen, optionally humanized, a linker L, a suicide domain, a hinge or part of it, a transmembrane domain, a co-stimulatory domain and a stimulatory domain.

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

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

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

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

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

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

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

$$V_1-L_1-V_2-(L)_x-\text{Epitope1}-(L)_x;$$

$$V_1-L_1-V_2-(L)_x-\text{Epitope1}-(L)_x-\text{Epitope2}-(L)_x;$$

$$V_1-L_1-V_2-(L)_x-\text{Epitope1}-(L)_x-\text{Epitope2}-(L)_x-\text{Epitope3}-(L)_x;$$

$$(L)_x-\text{Epitope1}-(L)_x-V_1-L_1-V_2;$$

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

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

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

5 $(L)_x$ -Epitope1- $(L)_x$ -V₁-L₁-V₂- $(L)_x$ -Epitope2- $(L)_x$ -Epitope3- $(L)_x$;

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

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

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

10 V₁- $(L)_x$ -Epitope1- $(L)_x$ -V₂;

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

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

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

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

15 $(L)_x$ -Epitope1- $(L)_x$ -V₁- $(L)_x$ -Epitope2- $(L)_x$ -V₂- $(L)_x$ -Epitope3- $(L)_x$;

V₁-L₁-V₂-L-Epitope1;

V₁-L₁-V₂-L-Epitope1-L;

V₁-L₁-V₂-L-Epitope1-L-Epitope2;

20 V₁-L₁-V₂-L-Epitope1-L-Epitope2-L;

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

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

V₁-L₁-V₂-Epitope1;

V₁-L₁-V₂-Epitope1-L;

V₁-L₁-V₂-Epitope1-L-Epitope2;

V₁-L₁-V₂-Epitope1-L-Epitope2-L;

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

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

Epitope1-V₁-L₁-V₂;

Epitope1-L-V₁-L₁-V₂;

L-Epitope1-V₁-L₁-V₂;

10 L-Epitope1-L-V₁-L₁-V₂;

Epitope1-L-Epitope2-V₁-L₁-V₂;

Epitope1-L-Epitope2-L-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-V₁-L₁-V₂;

L-Epitope1-L-Epitope2-L-V₁-L₁-V₂;

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

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

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

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

20 V₁-L-Epitope1-L-V₂;

L-Epitope1-L-V₁-L-Epitope2-L-V₂;

V₁-L-Epitope1-L-V₂-L-Epitope2-L;

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

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

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

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

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

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

L-Epitope1-L-V₁-L₁-V₂-L-Epitope2-L;

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

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

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

wherein,

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

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

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

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

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

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

V_H-L₁-V_L-L-Epitope1-L-Epitope2-L;

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

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

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

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

5

In some embodiments, L₁ is a linker comprising Glycine and/or Serine. In some embodiment, L₁ is a linker comprising the amino acid sequence (Gly-Gly-Gly-Ser)_n or (Gly-Gly-Gly-Gly-Ser)_n, where n is 1, 2, 3, 4 or 5. In some embodiments L₁ is (Gly₄Ser)₄ or (Gly₄Ser)₃.

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

In some embodiments, Epitope 1, Epitope 2 and Epitope 3 are identical or different and are selected from mAb-specific epitopes having an amino acid sequence of anyone of SEQ ID NO 114 to SEQ
20 ID NO 121 such as presented in the following Table 6.

Table 6: Mimotopes and epitopes with their corresponding sequences

Rituximab		
Mimotope	SEQ ID NO 114	CPYSNPSTLC
Palivizumab		
Epitope	SEQ ID NO 115	NSELLSLINDMPITNDQKKL MSNN
Cetuximab		
Mimotope 1	SEQ ID NO 116	CQFDLSTRRLKC
Mimotope 2	SEQ ID NO 117	CQYNLSSRALKC
Mimotope 3	SEQ ID NO 118	CVWQRWQKSYVC
Mimotope 4	SEQ ID NO 119	CMWDRFSRWYKC
Nivolumab		
Epitope A	SEQ ID NO 120	SFVLNWYRMSPSNQTDKL AAFPEDR
Epitope B	SEQ ID NO 121	SGTYLCGAISLAPKAQIKE

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

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

In a preferred embodiment, the epitope introduced within the chimeric scFv is the CD20 mimotope (SEQ ID NO.114) and the infused mAb presenting an affinity to this mimotope -for sorting and/or depletion purpose(s)- is rituximab.

In one embodiment, said at least one epitope is inserted between the VH and VL chains of the anti-CD38 CAR, optionally linked to said VH and VL chains by one linker.

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

In an embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 or V3, as illustrated in Figure 8, wherein one CD20 mimotope is inserted between the VH and VL chains of the anti-CD38 CAR, optionally linked to said VH and VL chains by one linker.

In a preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4)

and wherein one CD20 mimotope is inserted between the VH and VL chains of the anti-CD38 CAR, optionally linked to said VH and VL chains by one linker.

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

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

In a preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the V_L chain and to the hinge by one linker.

In a more preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4) and wherein one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the V_L chain and to the hinge by one linker.

In a preferred embodiment, at least two epitopes are inserted in the extracellular domain of the anti-CD38 CAR of the present invention.

In an embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4),

said extra-binding domain comprising VH and VL chains directed against CD38 and a Fc γ RIII α or CD8 α or IgG1 hinge;

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

5 a linker being interspaced between the 2 epitopes and/or between the VH and the 2 epitopes.

In an embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-
10 stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and two CD20 mimotopes,

15 said extra-binding domain comprising VH and VL chains directed against CD38 and a Fc γ RIII α or CD8 α or IgG1 hinge;

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

and optionally, a linker being interspaced between the 2 epitopes and/or at the N terminal end
20 of the CAR.

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

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

In a preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38

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

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

In a more preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

According to another embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises an extracellular binding domain wherein at least two epitopes are inserted in the extracellular domain in such a way that the V_H and V_L chains are located between them, all these components being optionally interspaced by at least one linker.

In a preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID

NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a more preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In another embodiment, three epitopes are inserted in the extracellular domain of the anti-CD38 CAR of the present invention.

According to a particular embodiment, said CD38 specific CAR of the invention contains an extracellular binding domain wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains are located between them, all these components being optionally interspaced by at least one linker.

In a more preferred embodiment, said CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), all these components being optionally interspaced by at least one linker.

In another embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), and three CD20 epitopes,

said extra-binding domain comprising V_H and V_L chains directed against CD38 and a FcγRIIIα or CD8α or IgG1 hinge;

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

a linker being interspaced between the 3 epitopes and/or between the V_H and the 3 epitopes.

In another embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), two CD20 epitopes, and one CD34 epitope;

5 said extra-binding domain comprising V_H and V_L chains directed against CD38 and a FcγRIIIα or CD8α or IgG1 hinge;

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

 and said CD34 epitope being inserted between the said 2 CD20 epitopes, all components being optionally interspaced between them by a linker .

10

 In some embodiment, Epitope 1 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 114 or 116-119.

 In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 114 or 116-119.

15 In some embodiment, Epitope 3 and Epitope 4 are mAbs-specific epitope having an amino acid sequence of SEQ ID NO 114 or 116-119.

 In some embodiment, one of Epitope 1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID NO 122 or 123. In some embodiment, one of Epitope1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID NO 122 or 123 and the other
20 mAb specific epitopes are CD20 mimotopes, preferably mimotope of SEQ ID NO 114.

 In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling
25 domain and a co-stimulatory domain from 4-1BB,

 wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO. 46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), two CD20 epitopes having an amino acid

sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V2 as illustrated in Figure 8, said structure comprising an extra
5 cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a CD8 α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR
10 comprising two CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.124.

15 In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a FcRIII α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

20 wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 62 and 58 (28F5), said anti-CD38 CAR comprising two CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR)
25 comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.126.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V2 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a CD8 α
30 hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR comprising two CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different.

5 In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.126.

10 In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V2 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a FcRIIIα hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

15 wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR comprising two CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.128.

20 In one embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

25 wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4), three CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119,said CD20 epitopes being
30 identical or different, and one CD34 epitope having an amino acid sequence of SEQ ID NO.122 or 123.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V2 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a CD8 α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 62 and 58 (28F5), said anti-CD38 CAR comprising three CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different, and one CD34 epitope having an amino acid sequence of SEQ ID NO.122 or 123.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.125.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a FcRIII α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 62 and 58 (28F5), said anti-CD38 CAR comprising three CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119; said CD20 epitopes being identical or different, and one CD34 epitope having an amino acid sequence of SEQ ID NO.122 or 123.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.127.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V2 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a CD8 α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR comprising three CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119; said CD20 epitopes being identical or different, and one CD34 epitope having an amino acid sequence of SEQ ID NO.122 or 123.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.129.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a CD8 α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR comprising two CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119; said CD20 epitopes being identical or different.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.130.

In a preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) has one of the polypeptide structure selected from V1 as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a FcR111 α hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), said anti-CD38 CAR comprising three CD20 epitopes having an amino acid sequence selected in the group consisting of SEQ ID NO 114 or 116-119, said CD20 epitopes being identical or different, and one CD34 epitope having an amino acid sequence of SEQ ID NO.122 or 123.

In more preferred embodiment, CD38 specific chimeric antigen receptor (anti-CD38 CAR) comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO.131.

5 Method of engineering drug-resistant T-cells

To improve cancer therapy and selective engraftment of allogeneic T-cells, drug resistance can be conferred to the engineered T-cells to protect them from the toxic side effects of chemotherapy or immunosuppressive agents. Indeed, the inventors have observed that most patients were treated with chemotherapy and immune depleting agents as a standard of care, prior to receiving T-cell
10 immunotherapy. Also they found that they could take advantage of these treatments to help the selection of the engineered T-cells, either by adding chemotherapy drugs in culture media for expansion of the cells *ex-vivo* prior to treatment, or by obtaining a selective expansion of the engineered T-cells *in-vivo* in patients under chemotherapy or immunosuppressive treatments.

Also the drug resistance of T-cells also permits their enrichment *in* or *ex vivo*, as T-cells which
15 express the drug resistance gene, will survive and multiply relative to drug sensitive cells. In particular, the present invention relates to a method of engineering allogeneic and drug resistance T-cells resistant for immunotherapy comprising:

- (a) Providing a T-cell;
 - (b) Selecting at least one drug;
 - 20 (c) Modifying T-cell to confer drug resistance to said T-cell;
 - (d) Expanding said engineered T-cell in the presence of said drug, and optionally
- the preceding steps may be combined with the steps of the methods as previously described.

Drug resistance can be conferred to a T-cell by inactivating one or more gene(s) responsible for the cell's sensitivity to the drug (drug sensitizing gene(s)), such as the hypoxanthine-guanine
25 phosphoribosyl transferase (HPRT) gene (Genbank: M26434.1). In particular HPRT can be inactivated in engineered T-cells to confer resistance to a cytostatic metabolite, the 6-thioguanine (6TG) which is converted by HPRT to cytotoxic thioguanine nucleotide and which is currently used to treat patients with cancer, in particular leukemias (Hacke, Treger et al. 2013). Another example if the inactivation of the CD3 normally expressed at the surface of the T-cell can confer resistance to anti-CD3 antibodies
30 such as teplizumab.

In an embodiment, the resistance gene to be inactivated is the one which encodes the deoxycytidine kinase (dCk). Deoxycytidine kinase (DCK) is required for the phosphorylation of several deoxyribonucleosides and their nucleoside analogs. Deficiency of DCK is associated with resistance to

antiviral and anticancer chemotherapeutic agents. Conversely, increased deoxycytidine kinase activity is associated with increased activation of these compounds to cytotoxic nucleoside triphosphate derivatives. DCK is clinically important because of its relationship to drug resistance and sensitivity (Hazra S, Szewczak A, Ort S, Konrad M, Lavie A (2011) "Post-translational phosphorylation of serine 74
5 of human deoxycytidine kinase favors the enzyme adopting the open conformation making it competent for nucleoside binding and release". *Biochemistry* **50** (14): 2870–8).

Drug resistance can also be conferred to a T-cell by expressing a drug resistance gene. Said drug resistance gene refers to a nucleic acid sequence that encodes "resistance" to an agent, such as a chemotherapeutic agent (e.g. methotrexate). In other words, the expression of the drug resistance gene
10 in a cell permits proliferation of the cells in the presence of the agent to a greater extent than the proliferation of a corresponding cell without the drug resistance gene. A drug resistance gene of the invention can encode resistance to anti-metabolite, methotrexate, vinblastine, cisplatin, alkylating agents, anthracyclines, cytotoxic antibiotics, anti-immunophilins, their analogs or derivatives, and the like.

Variant alleles of several genes such as dihydrofolate reductase (DHFR), inosine monophosphate dehydrogenase 2 (IMPDH2), calcineurin or methylguanine transferase (MGMT) have been identified to confer drug resistance to a cell. Said drug resistance gene can be expressed in the cell either by introducing a transgene encoding said gene into the cell or by integrating said drug resistance gene into the genome of the cell by homologous recombination. Several other drug resistance genes have been
15 identified that can potentially be used to confer drug resistance to targeted cells (Takebe, Zhao et al. 2001; Sugimoto, Tsukahara et al. 2003; Zielske, Reese et al. 2003; Nivens, Felder et al. 2004; Bardenheuer, Lehmborg et al. 2005; Kushman, Kabler et al. 2007).

DHFR is an enzyme involved in regulating the amount of tetrahydrofolate in the cell and is essential to DNA synthesis. Folate analogs such as methotrexate (MTX) inhibit DHFR and are thus used
25 as anti-neoplastic agents in clinic. Different mutant forms of DHFR which have increased resistance to inhibition by anti-folates used in therapy have been described. In a particular embodiment, the drug resistance gene according to the present invention can be a nucleic acid sequence encoding a mutant form of human wild type DHFR (GenBank: AAH71996.1) which comprises at least one mutation conferring resistance to an anti-folate treatment, such as methotrexate. In particular embodiment,
30 mutant form of DHFR comprises at least one mutated amino acid at position G15, L22, F31 or F34, preferably at positions L22 or F31 ((Schweitzer, Dicker et al. 1990); International application WO 94/24277; US patent US 6,642,043).

As used herein, "antifolate agent" or "folate analogs" refers to a molecule directed to interfere with the folate metabolic pathway at some level. Examples of antifolate agents include, e.g.,

methotrexate (MTX); aminopterin; trimetrexate (Neutrexin™); edatrexate; N10-propargyl-5,8-dideazafolic acid (CB3717); ZD1694 (Tumodex), 5,8-dideazaisofolic acid (IAHQ); 5,10-dideazatetrahydrofolic acid (DDATHF); 5-deazafolic acid; PT523 (N alpha-(4-amino-4- deoxypteroyl)-N delta-hemiphthaloyl-L-ornithine); 10-ethyl-10-deazaaminopterin (DDATHF, lomatrexol); piritrexim; 10-EDAM; ZD1694; GW1843; Pemetrexate and PDX (10-propargyl-10- deazaaminopterin).

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

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

In another particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer b at positions: V120, N123, L124 or K125, preferably double mutations at positions L124 and K125. Correspondence of amino acid positions described herein

is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer b polypeptide (GenBank: ACX34095.1).

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

Another drug resistance gene can be multidrug resistance protein 1 (MDR1) gene. This gene encodes a membrane glycoprotein, known as P-glycoprotein (P-GP) involved in the transport of metabolic byproducts across the cell membrane. The P-Gp protein displays broad specificity towards several structurally unrelated chemotherapy agents. Thus, drug resistance can be conferred to cells by the expression of nucleic acid sequence that encodes MDR-1 (NP_000918).

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

The T-cells can also be made resistant to immunosuppressive agents. An immunosuppressive agent is an agent that suppresses immune function by one of several mechanisms of action. In other words, an immunosuppressive agent is a role played by a compound which is exhibited by a capability to diminish the extent and/or voracity of an immune response. As non-limiting example, an immunosuppressive agent can be a calcineurin inhibitor, a target of rapamycin, an interleukin-2 α -chain blocker, an inhibitor of inosine monophosphate dehydrogenase, an inhibitor of dihydrofolic acid reductase, a corticosteroid or an immunosuppressive antimetabolite. Classical cytotoxic immunosuppressants act by inhibiting DNA synthesis. Others may act through activation of T-cells or by inhibiting the activation of helper cells. The method according to the invention allows conferring immunosuppressive resistance to T cells for immunotherapy by inactivating the target of the immunosuppressive agent in T cells. As non-limiting examples, targets for immunosuppressive agent can be a receptor for an immunosuppressive agent such as: CD52, glucocorticoid receptor (GR), a FKBP family gene member and a cyclophilin family gene member.

In immunocompetent hosts, allogeneic cells are normally rapidly rejected by the host immune system. It has been demonstrated that, allogeneic leukocytes present in non-irradiated blood products will persist for no more than 5 to 6 days. Thus, to prevent rejection of allogeneic cells, the host's

immune system must be effectively suppressed. Glucocorticoidsteroids are widely used therapeutically for immunosuppression. This class of steroid hormones binds to the glucocorticoid receptor (GR) present in the cytosol of T cells resulting in the translocation into the nucleus and the binding of specific DNA motifs that regulate the expression of a number of genes involved in the immunologic process.

5 Treatment of T cells with glucocorticoid steroids results in reduced levels of cytokine production leading to T cell anergy and interfering in T cell activation. Alemtuzumab, also known as CAMPATH1-H, is a humanized monoclonal antibody targeting CD52, a 12 amino acid glycosylphosphatidyl-inositol- (GPI) linked glycoprotein (Waldmann and Hale, 2005). CD52 is expressed at high levels on T and B lymphocytes and lower levels on monocytes while being absent on granulocytes and bone marrow
10 precursors. Treatment with Alemtuzumab, a humanized monoclonal antibody directed against CD52, has been shown to induce a rapid depletion of circulating lymphocytes and monocytes. It is frequently used in the treatment of T cell lymphomas and in certain cases as part of a conditioning regimen for transplantation. However, in the case of adoptive immunotherapy the use of immunosuppressive drugs will also have a detrimental effect on the introduced therapeutic T cells. Therefore, to effectively use an
15 adoptive immunotherapy approach in these conditions, the introduced cells would need to be resistant to the immunosuppressive treatment.

As a preferred embodiment of the above steps, said gene of step (b), specific for an immunosuppressive treatment, is CD52, and the immunosuppressive treatment of step (d) comprises a humanized antibody targeting CD52 antigen. As another embodiment, said gene of step (b), specific for
20 an immunosuppressive treatment, is a glucocorticoid receptor (GR) and the immunosuppressive treatment of step d) comprises a corticosteroid such as dexamethasone. As another embodiment, said target gene of step (b), specific for an immunosuppressive treatment, is a FKBP family gene member or a variant thereof and the immunosuppressive treatment of step (d) comprises FK506 also known as Tacrolimus or fujimycin. As another embodiment, said FKBP family gene member is FKBP12 or a variant
25 thereof. As another embodiment, said gene of step (b), specific for an immunosuppressive treatment, is a cyclophilin family gene member or a variant thereof and the immunosuppressive treatment of step (d) comprises cyclosporine.

In a particular embodiment of the invention, the genetic modification step of the method relies on the inactivation of two genes selected from the group consisting of CD52 and GR, CD52 and TCR
30 alpha, CDR52 and TCR beta, GR and TCR alpha, GR and TCR beta, TCR alpha and TCR beta. In another embodiment, the genetic modification step of the method relies on the inactivation of more than two genes. The genetic modification is preferably operated ex-vivo using at least two RNA guides targeting the different genes.

By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form.

Engineering highly active T cells for immunotherapy

5 According to the present invention, the T-cells can be 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. They can be extracted from blood or derived from stem cells. The stem cells can be adult stem cells, embryonic stem cells, more particularly non-human stem cells,
10 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. 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. T-cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord
15 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
20 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.

As a further aspect of the invention, the T-cells according to the invention may be further engineered, preferably genetically engineered, to enhance their activity and/or activation, especially by modulating the expression of proteins involved in overall T-cell regulation, referred to as "immune-
25 checkpoints".

Immune check points

It will be understood by those of ordinary skill in the art, that the term "immune checkpoints" means a group of molecules expressed by T cells. These molecules effectively serve as "brakes" to down-
30 modulate or inhibit an immune response. Immune checkpoint molecules include, but are not limited to Programmed Death 1 (PD-1, also known as PDCD1 or CD279, accession number: NM_005018), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4, also known as CD152, GenBank accession number AF414120.1), LAG3 (also known as CD223, accession number: NM_002286.5), Tim3 (also known as HAVCR2, GenBank

accession number: JX049979.1), BTLA (also known as CD272, accession number: NM_181780.3), BY55 (also known as CD160, GenBank accession number: CR541888.1), TIGIT (also known as IVSTM3, accession number: NM_173799), LAIR1 (also known as CD305, GenBank accession number: CR542051.1, {Meygaard, 1997 #122}), SIGLEC10 (GeneBank accession number: AY358337.1), 2B4 (also known as
5 CD244, accession number: NM_001166664.1), PPP2CA, PPP2CB, PTPN6, PTPN22, CD96, CRTAM, SIGLEC7 {Nicolli, 1999 #123}, SIGLEC9 {Zhang, 2000 #124; Ikehara, 2004 #125}, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBR2, TGFBR1, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF {Quigley, 2010 #121}, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3 which directly inhibit immune cells. For
10 example, CTLA-4 is a cell-surface protein expressed on certain CD4 and CD8 T cells; when engaged by its ligands (B7-1 and B7-2) on antigen presenting cells, T-cell activation and effector function are inhibited. Thus the present invention relates to a method of engineering T-cells, especially for immunotherapy, comprising genetically modifying T-cells by inactivating at least one protein involved in the immune check-point, in particular PD1 and/or CTLA-4 or any immune-checkpoint proteins referred to in Table 7.

Table 7: List of genes encoding immune checkpoint proteins.

Pathway		Genes that can be inactivated In the pathway
Co-inhibitory receptors	CTLA4 (CD152)	CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22
	PDCD1 (PD-1, CD279)	PDCD1
	CD223 (lag3)	LAG3
	HAVCR2 (tim3)	HAVCR2
	BTLA(cd272)	BTLA
	CD160(by55)	CD160
	IgSF family	TIGIT
		CD96
		CRTAM
	LAIR1(cd305)	LAIR1
	SIGLECs	SIGLEC7
		SIGLEC9
	CD244(2b4)	CD244
Death receptors	TRAIL	TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7
	FAS	FADD, FAS
Cytokine signalling	TGF-beta signaling	TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1
	IL10 signalling	IL10RA, IL10RB, HMOX2
	IL6 signalling	IL6R, IL6ST
Arginine/tryptophan starvation		EIF2AK4
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

Therapeutic applications

In a general aspect, the present invention relates to methods for new adoptive immunotherapy strategies in treating diseases linked with the development of pathological cells, such as cancer, infections and auto-immune diseases.

As a main objective of the invention is the possibility to target pathological cells that bear specific antigen markers in common with T-cells. By pathological cell is meant any types of cells present in a patient, which are deemed causing health deterioration.

In general, pathological cells are malignant or infected cells that need to be reduced or eliminated to obtain remission of a patient.

These anti-CD38 CARs immune cells, particularly when they have undergone a CD38 gene inactivation, are useful as medicament can for treating a CD38-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a CD38-expressing cell, such as MM, MM, RRMM, ALL, NHL lymphoma (as referred above), their related complication, and their related conditions.

In an embodiment, said anti-CD38 CAR in immune cells CD8 gene-inactivated are used as medicament and they comprise a polypeptide sequence displaying at least 90%, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11), SEQ ID NO. 88-90 (16B5), SEQ ID NO. 91-93 (10F7), SEQ ID NO.94-96 (27B6) and SEQ ID NO. 85-87(29B4), more preferably from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) and SEQ ID NO. 88-90 (16B5), and more preferably from SEQ ID NO. 82-84 (25A10) and SEQ ID NO. 100-102 (28F5).

In a preferred embodiment, said anti-CD38 CAR in immune cells CD8 gene-inactivated are used as medicament and they comprise a polypeptide sequence displaying at least 90%, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10) and SEQ ID NO. 100-102 (28F5).

In a more preferred embodiment, said anti-CD38 CAR in immune cells CD8 gene-inactivated are used as medicament and they comprise a polypeptide sequence displaying at least 90%, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82 (25A10-V1 CAR).

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.

5 The immune cells, such as T-cells, engineered according to one of the previous methods may be pooled, frozen, and administrated to one or several patients. Accordingly the present invention encompass a method for treating an immune disease by directing engineered T-cells as previously described against patient's own T-cells. When they are made non-alloreactive, they are available as an "off the shelf" therapeutic product, which means that they can be universally infused to patients in need
10 thereof.

In one 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 a preferred 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
15 in need thereof.

In a preferred embodiment, the cancer that may be treated using the anti-CD38 CAR -expressing cells of the present invention is leukemia or lymphoma, a disease associated to leukemia or lymphoma or a complication thereof.

In a particular embodiment, an anti-CD38 CAR expressing T cell is provided as a medicament for
20 the treatment of CD38+ hematological malignancies and in particular to those which have progressed on or after standard therapy or for whom there is no effective standard therapy (refractory/relapsed patients).

By "Relapsed": it is referred to a subject in whom the hematological malignancy has been treated and improved but in whom the hematological malignancy recurred.

25 By "Refracted": it is referred to a subject in whom the hematological malignancy has been treated without any improvement and the hematological malignancy thus progressed.

In the context of the disclosure the CD38+ hematological malignancy is in particular selected from the group consisting of non-Hodgkin's lymphoma (NHL) (including, e.g. Burkitt's lymphoma (BL) and T cell lymphoma (TCL)), multiple myeloma (MM), chronic lymphocytic leukemia (CLL) (such as e.g. B
30 chronic lymphocytic leukemia (B-CLL) or hairy cell leukemia (HCL)), B and T acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), Hodgkin's Lymphoma (HL), and chronic myeloid leukemia (CML), wherein the cancerous cells are or comprise CD38+ cells.

In particular, CD38+ hematological malignancies are B-cell non-Hodgkins Lymphoma (NHL), multiple myeloma (MM), acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (B-cell ALL) and/or chronic lymphocytic leukaemia (CLL), more particularly multiple myeloma (MM), most particularly relapsed and/or refractory multiple Myeloma.

5 Methods to identify a hematological malignancy are known to the skilled in the art and include as a first step a complete blood count (CBC) and a test of the peripheral blood smear. Definitive diagnosis usually requires an adequate bone marrow aspiration and/or biopsy for morphology studies eventually complemented by flow cytometry analysis, cytogenetics and further molecular techniques. Techniques to confirm that the cells derived from this hematological malignancy are CD38⁺ are known to
10 the skilled in the art and include standard molecular biology techniques such as, for example, polymerase chain reaction (PCR) and/or immunochemical methods such as Western Blot analysis.

In a preferred embodiment, the invention provides a treatment for CD38+ hematological malignancies such as presented above in patients over 60 years or in patients of less than 20 years.

In a preferred embodiment, said medicament can be used for the treatment of multiple
15 myeloma (MM) of an MM subtype such as refractory/relapsed multiple myeloma (RRMM).

In another embodiment, said medicament can be used for the treatment of the acute lymphoblastic leukemia (ALL).

Multiple myeloma

20 Multiple myeloma may be detected by the presence of monoclonal proteins (M proteins). "M-Protein" refers to a paraprotein (a monoclonal protein, or M protein). This paraprotein is an immunoglobulin or immunoglobulin light-chain that is produced in excess by the clonal proliferation of plasma cells. Amounts higher than a certain threshold indicate multiple Myeloma. The M-protein is usually quantified in the serum as well as in the urine. The M-protein level in the serum is measured by
25 typically serum electrophoresis or by for example specific immunoglobulin assays; however, specific immunoglobulin quantification always overestimates the M-protein because normal immunoglobulins are included in the result. For this reason, baseline and follow-up measurements of the M-protein should be done by the same method (Riches P G et al., 1991).

Dosages/ group of patients with MM to be treated

30 In one embodiment, M-protein in serum of higher than 0.5 g/dL indicates multiple myeloma.

In one embodiment, M-protein in urine of higher than 200 mg in a 24-hr urine indicate multiple Myeloma (MM).

In another embodiment, elevated serum free light chains (FLC) with FLC greater than about 10 mg/dL and with abnormal FLC ratio indicates multiple Myeloma. MM might be further identified by immunoglobulin light chain found in the urine, this paraprotein is called Bence Jones protein and is a urinary paraprotein composed of free light chains, wherein the light chains are lambda (λ) and/or kappa (κ) free light chains. These free light chains (FLC) may be measured by commercial tests. The free light chain measurement refers to the measurement of the FLC kappa and FLC lambda free light chains giving a free light chain ratio (FLC) of FLC kappa to FLC lambda (FLC κ/λ ratio), wherein a normal FLC κ/λ ratio ranges from 0.26 to 1.65.

In patients with multiple myeloma, either of the light chains, kappa or lambda, may be dominantly produced which results in changes of the FLC κ/λ ratio. Abnormal FLC κ/λ ratios indicating multiple myeloma are thus FLC κ/λ ratios lower than 0.26 or higher than 1.65

Therefore, in one embodiment, the subject having multiple myeloma has a) measurable serum M-protein of greater than about 0.5 g/dL, and/or b) urine M-protein of greater than about 200 mg (24-hr urine), and/or c) elevated serum free light chains (FLC) with FLC greater than about 10 mg/dL with abnormal FLC ratio.

In one embodiment, subjects to be treated by the engineered immune cells of the invention have multiple myeloma and certain genetic features, such as a translocation between chromosomes 9 and 22, known as the Philadelphia chromosome; or a translocation between chromosomes 4 and 11 [t(4;11)(q21;q23)]; a hyperdiploidy such as trisomy 4, 10, 17), or chromosome 9p deletion.

Therefore in one embodiment, the subject to be treated by the isolated cells of the invention has a 17p deletion, t (4, 14), t (14, 16), t (14, 20) and/or more than 3 copies of 1q21. It is known in the art, that subjects having multiple myeloma and certain genetic features, such as the chromosomal deletion 17p, the translocations t (4, 14), t (14, 16), t (14, 20) or amplifications such as more than 3 copies of 1q21 are associated with a worse outcome (Avet-Loiseau H et al., 2011). Researchers such as Van Laar et al. (2014) have developed a genomic profiling test for subjects with multiple Myeloma. This type of test allows doctors to classify subjects with multiple myeloma based on its genomic expression profile and not just a few chromosomal abnormalities.

In one embodiment, the subject may have a high-risk gene expression profiling (GEP) signature. For instance, this topic is described in more details in Shaughnessy et al (2007).

The subject may have any combination of the above mentioned features.

Acute lymphoblastic leukemia (ALL)

In one embodiment, the leukemia which can be treated by the medicament of the present invention is acute lymphoblastic leukemia (ALL).

In another embodiment of any of the above, the leukemia is pediatric (childhood) ALL.

In another embodiment of any of the above, the leukemia is relapsed ALL.

5 In another embodiment of any of the above, the leukemia is refractory ALL.

In another embodiment of any of the above, the leukemia is drug-resistant ALL.

In a further embodiment, the leukemia is glucocorticoid- resistant ALL.

In still another embodiment, said medicament can be used for the treatment of a B-cell non-Hodgkin's lymphoma (NHL) patients such as mantle cell leukemia (MCL).

10

Other CD38-mediated pathological conditions

According to another embodiment, the engineered immune cells of the invention are used to treat CD38+cell-mediated solid tumors such as prostate, seminal vesicle, appendix or to a pathology such as diabetes (Antonelli et al; 2004).

15

Associated treatments

In some embodiments, the subject has been previously treated with an anti-cancer therapy. In particular said, said previous anti-cancer therapy may be selected from the group constituted of chemotherapy, targeted cancer therapies, radiotherapy, bone marrow and/or stem cell transplantation and immunotherapy. In a more preferred embodiment, the present invention provides a pediatric treatment, in particular a pediatric treatment against MM, RRMM or-related diseases or complications.

20

In one embodiment, the subject has been previously treated with bortezomib and/or lenalidomide.

"Radiation therapy" or "radiation" uses high-energy radiation to remove cancer cells. Radiation therapy might be used before a bone marrow or peripheral blood stem cell transplant.

25

"Bone marrow and/or stem cell transplantation" refers to a cell transplantation aimed to restore stem cells that were destroyed by high doses of chemotherapy and/or radiation therapy. Sources of stem cells include bone marrow, peripheral blood or umbilical cord blood. Depending on the source of stem cells that are transplanted, the procedure might be distinguished into bone marrow transplant (BMT) or peripheral blood stem cell transplant (PBSCT) or umbilical cord blood transplantation (UCBT).

30

Furthermore bone marrow and/or stem cell transplantation might refer to an autologous stem cell transplantation and/or an allogeneic transplantation.

Resistance to drug or immunosuppressive agent

The engineered immune cells, as previously described, when they are made resistant to chemotherapy drugs and immunosuppressive drugs that are used as standards of care, especially methotrexate and the combination of fludarabine and Cyclophosphamide, are particularly suited for treating various forms of cancer. Indeed, the present invention preferably relies on cells or population of cells. In this aspect, it is expected that the chemotherapy and/or immunosuppressive treatment should help the selection and expansion of the engineered T-cells in-vivo.

In certain embodiments of the present invention, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizimab treatment for MS patients or efalizimab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Citr. Opin. mm n. 5:763-773, 93). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or 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. Said modified cells obtained by any one of the methods described here can be used in a particular aspect of the invention for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating patients in

need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified cells comprising inactivated TCR alpha and/or TCR beta genes.

5 Administration

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

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

10

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
15 intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

15

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
20 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
25 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.

25

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

30

An embodiment of the present invention is related to a method for treating a patient comprising:

(a) Diagnosing said patient for the presence of pathological cells presenting CD38-specific antigen markers in common with immune cells;

5 (b) Preparing a population of engineered immune cells according to the method presented in details previously,

(c) Administering said engineered immune cells to said patient diagnosed for said pathological cells.

The step of diagnostic of well-known for the skill man in the art, for instance reference can be
10 made to Dürig J, Naschar M, Schmücker U, Renzing-Köhler K, Hölter T, Hüttmann A, Dührsen U, (2002) "CD38 expression is an important prognostic marker in chronic lymphocytic leukaemia" Leukemia. 16(1):30-5.

Example of steps to engineer T-cells according to the invention for immunotherapy

15

For a better understanding of the invention, it is provided below an example of the steps to follow to produce T-cells directed against leukemia CD38 positive cells:

1. Providing T-cells from a cell culture or from a blood sample from one individual patient or from blood bank and activating said T cells using anti-CD3/C28 activator beads (Dynabeads®). The
20 beads provide both the primary and co-stimulatory signals that are required for activation and expansion of T cells.

2. Transducing said cells with a retroviral vector comprising a transgene encoding a Chimeric antigen receptor consisting of the fusion of CD3zeta activation domain, 4-1BB co-stimulation domain, a transmembrane domain and a hinge from CD28 α , Fc ϵ 11y or IgG1 fused to a sequence
25 encoding the variable chain of an anti-CD38 antibody. For security improvement of the transformed T-cell, a suicide gene sensitive to rituximab may further be introduced as described in WO 2013/153391 into the lentiviral vector separated by T2A splitting sequences.

3. (optionally) Engineering non alloreactive and/or resistant T cells:

a) It is possible to Inactivate TCR alpha in said cells to eliminate the TCR from the surface of the cell and prevent recognition of host tissue as foreign by TCR of allogenic and thus to avoid GvHD by following the protocols set forth in WO 2013/176915.

b) It is also possible to inactive one gene encoding target for an immunosuppressive agent or a chemotherapy drug to render said cells resistant to immunosuppressive or chemotherapy treatment to prevent graft rejection without affecting transplanted T cells. In this example, target of immunosuppressive agents is CD52 and immunosuppressive agent is a humanized monoclonal anti-CD52 antibody (ex: Alemtuzumab) as described in WO 2013/176915.

4. Gene Inactivation is performed by electoporating T-cells with mRNA encoding specific TAL-endonuclease (TALENTM – Collectis, 8 rue de la Croix Jarry, France). Inactivated T cells are sorted using magnetic beads. For example, T cells still expressing the CD38 targeted gene (can be removed by fixation on a solid surface, and inactivated cells are not exposed of the stress of being passed through a column. This gentle method increases the concentration of properly engineered T-cells.

5. Expansion *in vitro* of engineered T-cells prior to administration to a patient or *in vivo* following administration to a patient through stimulation of CD3 complex. Before administration step, patients can be subjected to an immunosuppressive treatment such as CAMPATH1-H, a humanized monoclonal anti-CD52 antibody.

6. Optionally exposed said cells with bispecific antibodies *ex vivo* prior to administration to a patient or *in vivo* following administration to a patient to bring the engineered cells into proximity to a target antigen.

GENERAL METHODS

- Primary T-cell cultures

T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using EasySepTM Direct Human T Cell Isolation Kit (Stem cells). Purified T cells were activated in X-VivoTM-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).

- CD38 TALEN transfection

A schematic representation is shown in Figure 8.

Heterodimeric TALE-nuclease targeting two 17-bp long sequences (called half targets) separated by an 15-bp spacer within CD38 gene were designed and produced. Each half target is recognized by

repeats of the half TALE-nucleases listed in Table 3. The sequences of the CD38 targets are provided in Figure 5. Each TALE-nuclease construct was subcloned using restriction enzyme digestion (insert : SfaNI-BbvI and vector : BsmBI) in a mammalian expression vector (pCSL10794) under the control of the T7 promoter. mRNA encoding TALE-nuclease cleaving CD38 genomic sequence 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 30°C with 5% CO₂. IL-2 (from Miltenyi Biotec was added 2h after electroporation at 20ng/mL. 18 hours later, cells were transferred at 37°C with 5% CO₂.

- *CD38 negative T cells purification*

Six days after CD38 TALEN transfection, CD38 negative cells were purified by magnetic separation using CD38 microbeads kit according to the manufacturer's specifications (Miltenyi).

- *CAR mRNA transfection*

Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15µg of mRNA encoding the different 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% CO₂. IL-2 (from Miltenyi Biotec was added 2h after electroporation at 20ng/mL.

- *Degranulation assay (CD107a mobilization)*

T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing various levels of the CD38 protein. Co-cultures were maintained in a final volume of 100µl of X-Vivo™-15 medium (Lonza) for 6 hours at 37°C with 5% CO₂. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody at the beginning of the co-culture, together with 1µg/ml of anti-CD49d, 1µg/ml of anti-CD28, and 1x Monensin solution. After the 5h incubation period, cells were stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and anti-CD3 and analyzed by flow cytometry. The degranulation activity was determined as the % of viable/CD3+/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.

- *Cytotoxicity assay*

5 T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing CD38) and 10,000 control (CD38neg) 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% CO₂. After this incubation period, cells were labelled with a fixable viability dye and analyzed by flow cytometry. Viability of each
10 cellular population (target cells or CD38neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48h after mRNA transfection.

Example 1: Inactivation of CD38 antigen in T cells by knock-out (KO) using TALE nuclease

15 CD38 is also highly expressed by activated T cells. CD38 expression by T cells after activation with CD3/CD28 beads and IL-2 was analyzed by FACS every 3-4 days for 17days (Figure 3A). It was observed that more than 90% of T cells express CD38 between day 6 and day 17 after activation (Figure 3B).

Thus in order to avoid killing of activated T cells by anti-CD38 CAR⁺ T cells, CD38 surface
20 expression in T cells needs to be prevented. This may be accomplished by the inactivation of the CD38 gene using TALE-nucleases. The experiment was performed as presented in Figure 4A.

Heterodimeric TALE-nucleases targeting two 17-pb long sequences separated by a 13-pb spacer within the CD38 gene were designed and produced. Each half target is recognized by repeats of the half TALE-nucleases listed in the Table 4 and Figure 5.

25 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 CD38 were synthesized from plasmids carrying the coding sequence downstream from the T7 promoter.

Purified T cells activated during 4 days with anti CD3/CD28 coated beads and recombinant IL-2 were transfected by electroporation (Cytopulse) with each of the 2 mRNAs (10ug each) encoding both
30 half TALE-nucleases. To investigate, the CD38 KO, the percentage of CD38 negative T cells was assessed by flow cytometry at day 7 (Figure 6). The three TALENs were able to induce CD38 KO but the CD38-1 was the most efficient (60.95% +/- 7.6 CD38^{neg} T cells).

The CD38 inactivation induced by the CD38-1 TALEN was stable in culture for 3 weeks (Figure 7A). CD38 deficient T cells could be easily sorted using the anti-CD38 microbeads (Miltenyi) (Figure 7A).

The proliferation rate of purified CD38 deficient T cells was comparable with non-transfected or non-purified T cells (Figure 7B).

In two independent experiments, CD38 TALEN transfection were performed by using 2 µg of RNA per 10⁶ of T cells and using electroporation, it is shown in Figure 23 that such transfection induce CD38 KO with high frequency. In another experiment, a comparison of 2µg and 1µg or 0.5µg per million cells was tested. The amount of mRNA TALEN didn't modify the proliferation rate of T cells (Figure 24B) but decrease in the % of CD38 negative cells (Figure 24A).

Example 2: Study of anti-CD38 CARs activity after mRNA transfection in WT T cells

CAR Structure

Eight pairs of scFvs have been tested, their sequences SEQ ID NO: 10 to 73 including their corresponding CDRs are presented in Table 2.

For each pair of scFvs, 3 different CARs constructs have been designed with the 41BB costimulatory domain, the CD3ζ activation domain, the CD8α transmembrane domain and 3 different hinges of sequences SEQ ID NO: 76, 77 and 78) respectively:

V1: FcεRIIIα hinge

V2: CD8α hinge

V3: IgG1 hinge.

The Figure 9 and Figure 2A shows respectively the name of plasmids of the different versions of the CAR created for the subcloning and the one used as backbone. Also the value of Kd for their respective scFvs are provided.

The Table 1 shows the sequences SEQ ID NO: 82 to 105 for all the 24 different anti-CD38 CARs (8 pairs of scFv X 3 versions V1, V2&V3) and of their constituents (except scFvs presented in Table 2).

Selection of target cells

The CAR molecules generated were screened for degranulation and cytotoxic activity toward target cell lines expressing CD38 following transient transfection of T cells with CAR mRNA. Target cell lines expressing different expression levels of CD38 (figure 3B) were used for activity testing (Figure 10A and Figure 10B).

The number of CD38 molecules per cell was evaluated by Qifikit assay (DAKO Company) for the following cell lines:

- U266 CD38+ B-cell myeloma (48216 molecules/cell) and U266 CD38- (230 molecules/cell) obtained from U266B1 cell line (#ATCC® TIB-196™) by magnetic separation using anti-CD38 microbeads;

- MOLP8, a multiple myeloma cell line (#DSMZ ACC 569) expressing high levels of CD38 (259889 molecules/cell);

- Daudi (#ATCC® CCL-213™), a cell line derived from Burkitt lymphoma expressing high levels of CD38;

- K562 (#ATCC® CCL-243), a cell line CD38 negative cell line derived from chronic myelogenous leukemia.

CAR mRNA transfection in WT T cells at Day 5 after T cell activation

T cells were purified from buffy coat samples and activated using anti-CD3/CD28 coated beads. Cells were transfected 5 days after activation with 15 µg of mRNA encoding anti-CD38 CAR (time scale of the experiment is presented in Figure 4C). The CAR expression and the degranulation capacity of CART cells were assessed 24 hours after the transfection.

Despite CD38 expression on T cells, significant T cell mortality after CAR mRNA transfection at Day 5 was not observed.

Expression

Three detection methods were assessed on cells transfected 5 days after activation: an anti-Fab antibody, the L-protein staining and a CD38-Fc protein (produced by LakePharma) staining (Figure 11). The anti-CD38 CARs 13F11-V2, 16B5-V2&V3, 25A10-V1&V2, 28F5-V1&V2, GMB005-V1&V2&V3 were detectable by at least one method. Some CARs were undetectable by any of these methods but were able to degranulate in a CD38 dependent manner (see next section).

Degranulation capacity

The CAR T cell degranulation was evaluated by flow cytometry. The read-out is the CD107a expression at the T cell plasma membrane after 5 hours incubation with target cells (Figure 12). Most of the scFv's induce T cell degranulation in a CD38 dependent manner except 10F7, 27B6 and 29B4

The 3 versions (V1, V2 and V3) of the scFv's 13F11, 16B5, 25A10, 28F5 and the tool CAR GMB005-V1 were selected for screening at day 12.

Example 3: Evaluation of the effect of CD38 KO and purification of CD38 negative T cells on the activity of a serial anti-CD38 CARs obtained by mRNA transfection

i. Comparison CAR mRNA screening in WT / CD38-deficient / purified CD38 deficient T cells

5 T cells were purified from buffy coat samples and activated using anti-CD3/CD28 coated beads (time scale of the experiment is presented in Figure 4B). Cells were transfected 12 days after activation with 15 µg of mRNA encoding anti-CD38 CAR and 24h after CAR mRNA transfection, a significant mortality of T cells was observed.

10 The CD38 KO and CD38 negative purification has been tested and evaluated. CD38-1 TALEN mRNA was transfected (or not) in T cells at day 4 after activation with anti-CD3/CD28 coated beads. 6 days after CD38 negative cells were purified (or not) by using magnetic separation (Miltenyi's protocol). The day after, CAR mRNAs were transfected. The CAR expression and degranulation capacity of CART cells were assessed 24 hours after the transfection. CAR expression and cytotoxic capacity of CART cells were assessed 48 hours after the transfection. The anti-CD38 CARs, 13F11-V2 and -V3 were used for the
15 analysis. After 24hours of CAR mRNAs transfection, T cell viability was increased in CD38 KO T cells with purified CD38 KO T cells displaying the best viability (Figure 13A).

Expression

20 CAR expression was analyzed by flow cytometry using CD38-Fc protein. CAR expression was detectable at higher levels at day 1 and day 2 in CD38-deficient T cells. No significant difference was observed between the purified or non-purified CD38 KO T cells (Figure 13B).

Degranulation capacity

25 The percentage of T cells that have degranulated after incubation with target cell lines is CD38-independent in wt CAR T cells but is CD38-dependent when CAR T cells are CD38-deficient (Figure 14A). The purification step has no effect on CAR T cell degranulation in the tested conditions.

Cytotoxic activity

30 Forty-eight hours after CAR mRNAs transfection, there was no cytotoxic activity of CAR T cells against target cell lines. This cytotoxic activity was restored in CD38 deficient CAR T cells. This cytotoxic activity was CD38-dependent but no difference was observed between purified and non-purified CD38-deficient T cells (Figure 14B).

Altogether, these results indicate the improved effect of CD38 inactivation (induced by CD38-1 TALEN) on the cytotoxic activity before anti-CD38 CAR mRNA transfection in T cells. Next experiments were performed by using purified CD38-deficient T cells.

5 **ii. CAR mRNA transfection at day 12 after T cell activation in purified CD38 deficient T cells**

T cells were purified from buffy coat samples and activated using anti-CD3/CD28 coated beads and IL-2. CD38-1 TALEN mRNA was transfected in T cells at day 4 after activation, 6 days later CD38 negative cells were purified by using magnetic separation (Miltenyi). The day after, CAR mRNAs were transfected. The CAR expression and degranulation capacity of CART cells were assessed 24 hours after the transfection. CAR expression and cytotoxic capacity of CART cells were assessed 48 hours after the transfection.

Expression

All CARs were detected 24h after transfection except the 25A10-V3. However, the latter expressed anti-CD38 CAR above the background. The 13F11-V2, 25A10-V1, 25A10-V2 and 28F5-V2 were highly expressed (more than 80%). The CAR-V2 and -V3 expression were more stable at 48h than -V1 (Figure 15).

Degranulation capacity

Most of the CART cells were able to degranulate against CD38 expressing cell lines. Only the 16B5-V3, 28F5-V3, 25A10-V3 show low degranulation capacity. The CART cells that present the best ratios (>2) of degranulation against U266 CD38⁺ and U266 CD38⁻ were 13F11-V1, -V3, 16B5-V1, -2 25A10-V1, 28F5-V1,-V2 and GMB005-V1 (Figure 16). The CAR-V1 and -V3 were able to degranulate at lower level against autologous T cells CD38⁺ than CAR-V2.

Cytotoxic activity

Most of the CAR T cells were able to kill efficiently (more than 20% in 4 hours) CD38⁺ target cells lines (Figure 17). The 16B5-V2, 25A10-V2 and 28F5-V2 that are highly cytotoxic against CD38⁺ target cells lines are also highly cytotoxic against autologous activated T cells. Interestingly, the -V1 version of the 25A10 and 28F5 were cytotoxic against CD38^{hi} cells lines but presented a lower cytotoxic activity for the autologous T cells.

From all these results et from all the CARs tested, it appears that the CARs 25A10-V1, 25A10-V2, 28F5-V1 and 28F5-V2 –particularly when they have their CD38 gene inactivated- show a higher potential

in terms of cytotoxicity towards cancerous CD38-expressing cells, while showing a reduced effect in the interaction with the T cells (i.e. other activated CD38 T cells from the same donor).

5 Example 4: Evaluation of the impact of CD38-KO for anti-CD19 CAR activity in vivo

To evaluate the impact of CD38-KO for CAR activity, NOG mice were sublethally irradiated 8 days before injection of T-cells. At Day (-7) 5×10^5 Daudi Luciferase cells/mice were iv injected. Mice were then infused with wt or CD38-/- CAR-CD19 RQR8 T-cells. The tool CD19 CAR comprising FMC63 scfv
10 correspond to the polynucleotide having the sequence SEQ ID NO.16 disclosed in WO2014/184143. CAR-CD19 RQR8 T-cells were obtained by co-expression of both anti-CD19 and RQR8 accordingly to methods disclosed in prior art such as Donnelly ML et al, (2001) *J Gen Virol.*;82(Pt 5):1027-1041. Bioluminescent signal was assessed at D(-1), D7, D14, D21 and D28 post injection of T-cells (Figure 22A and Figure 22B). When the tumor progression and the survival rate are assessed, it is shown that the
15 CD38-/- CAR-CD19 RQR8 cells presented the same anti-tumor activity in vivo than CAR-CD19 RQR8 T cells (Figure 22C) and T cells endowing CD19 CAR and KO CD38 deficient survived longer (Figure 22D). Thus, CD38-KO doesn't affect the anti-tumor activity of CAR T-cells *in vivo*.

20 Example 5: Evaluation of activity of anti-CD38 CAR obtained by lentiviral transduction in double KO TRAC/CD38 T cells

CAR Cloning in expression plasmid

The 4 anti-CD38 CARs 25A10-v1, 25A10-v2, 28F5-v1 and 28F5-v2 were cloned using the restriction enzyme XmaI into the final rLV backbone containing RQR8 (pCCL-RQR8-T2A-CAR) under the control of the EF1a promoter. The sequences of these plasmids have been analyzed and the plasmids
25 have been sent to Vectalys (France) for rLV production.

rLV CD38 CAR transduction in function of rLV MOI

The CAR CD38 rLV dose needed to transduce primary T cells was assessed at day 7 after T cell activation.

30 T cells were transfected with CD38 TALEN mRNA at day 3 after activation and transduced 4 days later with the 4 CAR candidate rLVs (25A10-v1, -v2, 28F5-v1 and -v2) at MOI 5, 10 and 15.

The transduction efficiency was assessed 3 days after transduction by detection of the percentages of positive CAR or positive RQR8 cells. No significant difference of transduction efficiency between the 3 MOIs was observed (Figure 25A and Figure 25B respectively). The following rLV screening will be performed with a transduction at MOI 5.

5

Screening with CD38-KO and TCR-KO attributes

The outline of the process is shown in Figure 26. The parameters are the following : T cells were activated by using Dynabeads CD3-CD28, the culture were made by using medium Xvivo+5% human serum heat inactivated + IL-2 20ng/ml, a double KO (DKO) CD38-TRAC was performed by using a ratio of 2µg TALEN mRNA per million of cells (5 million cells /cuvette); the transduction was performed by using rLV from Vectalys(France) at a MOI of 5.

10

To assess the cytotoxic capacities of the CARs, the MOLP8, Daudi, U266 CD38+ and U266 CD38- were used as target cells previously used during the mRNA screen. These cell lines are mostly multiple myeloma cell lines (except Daudi) expressing different levels of CD38.

15

The rLV CAR screen has been done in 3 different experiments, using 3 different PBMC donors. The CAR expression, enrichment of CD38 negative cells after CAR transduction and the cytotoxic capacity of the 4 CARs 25A10-v1, -v2, 28F5-v1 and -v2 were analysed.

Evaluation of anti-CD38 CAR expression and CART phenotyping

20

The screening of the 4 CARs 25A10-v1, -v2, 28F5-v1 and -v2 after rLV transduction in DKO T cells has shown that all the CARs were expressed at the T cell surface with a high efficiency (>40%) and 3 of them above 80% (Figure 27C). RQR8 expression was correlated with CAR expression (Figure 27A). Most of the T cells were CD38 negative when T cells have been transduced by CD38 CARs (Figure 27A). It is shown also that 60-85% of T cells endowed with the above 4 CARs were DKO TRAC/CD38 (Figure 27B).

25

The DKO CD38 CART cells were mainly CD8+ (Figure 28A) and presented an effector/effector memory phenotype. There was no difference between CAR constructs (Figure 28B).

Evaluation of CAR activity in vitro

The CART degranulation was evaluated by flow cytometry. The read-out is the CD107a expression at the T cell plasma membrane after 5 hours of incubation with target cells. These CARs were

30

able to degranulate with a high efficiency against CD38+ multiple myeloma cell lines as well as against CD38+ T cells (Figure 29A). The 4 CD38 CAR candidates were able to induce lysis of CD38+ multiple myeloma cell lines in a CD38 dose dependent manner (Figure 29B).

5 Example 6: Evaluation of anti-CD38 CAR T cells against T cells acute lymphoblastic leukemia (T-ALL)

As shown in Figure 30, T-ALL cells expressed a high expression level of CD38. This pathology could be another application for the treatment based on the administration of anti-CD38 T cells in patient.

10 *Development of a new T-ALL cell line with CD38-KO*

All T-ALL cells lines tested were CD38+. To evaluate the CAR activity against T-ALL a negative cell line was needed as control. The CD38-KO CCRF-CEM cell lines has been generated by transfection of the CCRF-CEM cells with CD38 TALEN mRNA. CD38 negative cells were purified by magnetic separation (anti-CD38 microbeads, Miltenyi- Figure 31). The new cell line has been used as negative control for the
15 cytotoxicity assay.

Evaluation of anti-CD38 CAR T cell activity against T-ALL cell lines

Anti-CD38 CAR T cell activity against T-ALL cell lines was assessed by analysis of their degranulation after coculture with several T-ALL cell lines expressing CD38. The high majority (>70%) of
20 UCART38 were able to degranulate in presence of T-ALL CD38+ cells (Figure 32A). The cytotoxicity of UCART38 against T-ALL cells in vitro has been evaluated by coculture of UCART38 with T-ALL cells during 4h. UCART38 cells were able to induce lysis of CD38+ T-ALL cells (Figure 32B).

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CLAIMS

1) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) having one of the polypeptide structure selected from V1, V2 and V3, as illustrated in Figure 8, said structure comprising an extra cellular ligand binding-domain comprising V_H and V_L from a monoclonal anti-CD38 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB,

wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11), SEQ ID NO. 30 and 26 (16B5), SEQ ID NO. 38 and 34 (10F7), SEQ ID NO.46 and 42 (27B6) or SEQ ID NO. 22 and 18 (29B4).

2) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 1, wherein the transmembrane domain comprises a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO.79 (CD8 α TM).

3) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 1 or claim 2, wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10), SEQ ID NO. 62 and 58 (28F5), SEQ ID NO. 54 and 50 (13F11) or SEQ ID NO. 30 and 26 (16B5).

4) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to anyone of claim 1 to 3, wherein said V_H and V_L comprise a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to respectively SEQ ID NO. 14 and 10 (25A10) or SEQ ID NO. 62 and 58 (28F5).

5) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to anyone of claim 1 to 4, wherein said V_H and V_L comprise the CDRs sequences of respectively SEQ ID NO.15-17 and SEQ ID NO.11-13; respectively SEQ ID NO.63-65 and SEQ ID NO.59-62; respectively SEQ ID NO.55-57 and SEQ ID NO.51-53; respectively SEQ ID NO.31-33 and SEQ ID NO.27-29; respectively SEQ ID NO.39-42 and SEQ ID NO.35-37; respectively SEQ ID NO.47-49 and SEQ ID NO.43-45; respectively SEQ ID NO.23-25 and SEQ ID NO.19-22.

6) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to anyone of claim 1 to 5, comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) , SEQ ID NO. 88-90 (16B5), SEQ ID NO. 91-93 (10F7), SEQ ID NO.94-96 (27B6) and SEQ ID NO. 85-87(29B4).

- 7) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 6, comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10), SEQ ID NO. 100-102 (28F5), SEQ ID NO. 97-99 (13F11) and SEQ ID NO. 88-90 (16B5).
- 8) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 6 or claim 7, comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 82-84 (25A10) and SEQ ID NO. 100-102 (28F5).
- 9) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 6 to 8, comprising a polypeptide sequence displaying at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 82 (25A10-v1).
- 10) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 1 to 9, said extracellular binding domain further comprising at least one mAb specific-epitope.
- 11) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 10, wherein said at least one mAb specific-epitope has an amino acid sequence selected in the group consisting of SEQ ID 114 to 121.
- 12) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 10 or claim 11, wherein two CD20 mimotopes of SEQ ID NO.114 are inserted in said extra cellular ligand binding-domain.
- 13) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 10, wherein two CD20 mimotopes of SEQ ID NO.114 are inserted in the hinge of said extra cellular ligand binding-domain.
- 14) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to claim 10, wherein three CD20 mimotopes of SEQ ID NO.114 and one CD34 epitope of SEQ ID NO.122 or 123 are inserted in said extra cellular ligand binding-domain.
- 15) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 1 to 14, comprising a hinge which polypeptide displays at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 76 (FcεRIIIα), SEQ ID NO. 77 (CD8α) and SEQ ID NO. 78 (IgG1).

16) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 1 to 15, comprising a hinge which polypeptide displays at least 90 %, at least 95%, at least 98% or at least 99% identity to SEQ ID NO. 77 (CD8 α).

17) An anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 1 to 15, comprising a transmembrane domain which polypeptide displays at least 90 %, at least 95%, at least 98% or at least 99% identity to one selected from SEQ ID NO. 76 (Fc ϵ RIII α).

18) A method of preparing immune cells, preferably T-cells, for immunotherapy against pathological cells comprising the step of:

(a) Genetically inactivating or mutating a gene encoding the CD38 gene in an immune cell, which is involved in the expression or presentation of the CD38 antigen marker, said CD38 antigen marker being present both on the surface of said immune cell and the pathological cell;

(b) Expressing into said immune cell a transgene encoding a chimeric antigen receptor directed against said CD38 antigen marker according to anyone of claim 1 to 17, said antigen marker being present at the surface of said pathological cell.

19) A method according to any one of claims 18, wherein step a) is performed using a rare-cutting endonuclease.

20) A method according to claim 19, wherein step a) is performed using a TAL-nuclease.

21) A method according to claim 20, wherein said TAL-nuclease targets a sequence of SEQ ID NO.1, 4 or 7.

22) A method according to claim 21, wherein said TAL-nuclease targets a sequence of SEQ ID NO. 4 (CD38-1 target).

23) A method according to any one of claim 19 to 21, wherein said inactivation of CD38 antigen is performed by using the TALE-nucleases of SEQ ID NO.2-3, 5-6 or 8-9.

24) A method according to claim 23, wherein said inactivation of CD38 antigen is performed by using the TALE-nucleases of SEQ ID NO. 5-6.

25) A method according to any one of claims 18 to 24, wherein said method includes a further step of activating and expanding the immune cells.

26) A method according to any one of claims 18 to 25, wherein said method includes a further step of purifying the resulting immune cells by excluding the cells presenting said marker antigen at their surface.

27) A method according to any one of claims 18 to 26, wherein said method includes a previous step of procuring the immune cells from a donor.

28) A method according to any one of claims 18 to 26, wherein said method includes a previous step of procuring the immune cells from a patient who is affected by the development of said pathological cells.

29) A method according to any one of claims 18 to 28, wherein said immune cell is derived from a primary stem cell, iPS or hES cell.

30) A method according to claim 29, wherein said immune cell is derived from iPS cell derived from said patient affected by the development of said pathological cells.

31) A method according to anyone of claim 18 to 30, wherein step a) is performed using a RNA-guided endonuclease.

32) A method according to claim 31, wherein the RNA-guided endonuclease is Cas9.

33) A method according to claim 32, wherein RNA-guided endonuclease is split into at least 2 polypeptides, one comprising RuvC and another comprising HNH.

34) A method according to anyone of claim 18 to 33, wherein said endonuclease is expressed from transfected mRNA.

35) A method according to any one of claims 18 to 34, wherein said method includes a further step of inactivating a gene encoding a component of the T-cell receptor (TCR).

36) A method according to claim 35, wherein said component of the T-cell receptor is TCR α .

37) A method according to any one of claims 18 to 36, wherein said method includes a further step of inactivating a gene encoding a component of HLA.

38) A method according to any one of claims 18 to 36, wherein said method includes a further step of inactivating a gene encoding β 2m.

39) A method according to any one of claims 18 to 38, wherein said method includes a further step of inactivating a gene encoding an immune checkpoint protein selected from CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2 and GUCY1B3.

40) A method according to claim 39, wherein said gene locus is involved into the expression of PD1 or CTLA-4 genes.

41) A method according to any one of claims 18 to 40, wherein said method includes a further step of inactivating a gene conferring sensitivity of the immune cells to chemotherapy or immunosuppressive drugs.

42) The method according to claim 41, wherein said further gene encodes CD52.

43) The method according to claim 41, wherein said further gene is hypoxanthine-guanine phosphoribosyltransferase (HPRT).

44) The method according to claim 41, wherein said further gene encodes a glucocorticoid receptor (GR).

45) The method according to claim 41, wherein said further gene is involved in the DCK regulatory pathway, in particular DCK expression.

46) A method according to any one of claims 18 to 45, wherein said immune cells in step a) are derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

47) The method according to claim 46, wherein said T-cells are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.

48) A method according to any one of claims 18 to 47, wherein said transformed immune cells are expanded *in-vitro*.

49) A method according to any one of claims 18 to 47, wherein said transformed immune cells are expanded *in-vivo*.

50) A method according to any one of claims 18 to 49, wherein said pathological cells are selected from malignant cells or infected cells.

51) A method according to any one of claims 18 to 50, for preparing immune cells to be used as a medicament.

52) A method according to claim 51 for preparing immune cells for treating a cancer, an immune disease or an infection in a patient in need thereof.

53) A method according to claim 52, wherein said medicament is used for the treatment of CD38⁺ hematological malignancy.

54) A method according to claim 53, wherein said medicament is used for the treatment of B-cell non-Hodgkins Lymphoma (NHL), multiple myeloma (MM), acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (B-cell ALL) and/or chronic lymphocytic leukaemia (CLL), more particularly multiple myeloma (MM).

55) A method according to claim 54, wherein said medicament is used for the treatment of multiple myeloma (MM).

56) A method according to claim 55, wherein said medicament is used for the treatment of a MM subtype which is refractory/relapsed multiple myeloma (RRMM).

57) A method according to claim 54, wherein said medicament is used for the treatment of acute lymphoblastic leukemia (ALL).

58) A method according to claim 54, wherein said medicament is used for the treatment of a B-cell non-Hodgkin's lymphoma (NHL) patients such as mantle cell leukemia (MCL).

59) A method according to any one of claims 18 to 53, wherein said pathological cells are solid tumor cells.

60) An engineered immune cell (preferably T cell), which expresses an anti-CD38 specific chimeric antigen receptor (anti-CD38 CAR) according to any one of claim 1 to 17, said immune cell having its endogenous CD38 gene genetically inactivated or mutated.

61) An engineered immune cell according to claim 60, wherein said immune cell has its endogenous CD38 and TCR genes genetically inactivated or mutated.

62) An engineered immune cell according to claim 60 or claim 61, wherein said immune cell has its endogenous CD38, TCR and dCK genes genetically inactivated or mutated.

63) An engineered immune cell according to any one of claim 60 to 62, wherein said CD38 gene and additional gene(s) are inactivated by knock-out (KO) using specific rare-cutting endonuclease(s).

64) An engineered immune cell obtainable according to the method of any one of claims 18 to 59.

65) An engineered immune cell according to claim 60 resulting into the phenotype [CAR CD38]⁺[CD38]⁻

66) A population of immune cells comprising at least two engineered immune cells according at any one of claim 60 to 65.

67) A method for treating a patient comprising:

(a) Diagnosing said patient for the presence of pathological cells presenting specific CD38-antigen markers in common with immune cells;

(b) Preparing a population of engineered immune cells according to anyone of claim 60 to 65 or according to the method of any one of claims 18 to 59; and

(c) Administrating said engineered immune cells to said patient diagnosed for said pathological cells.

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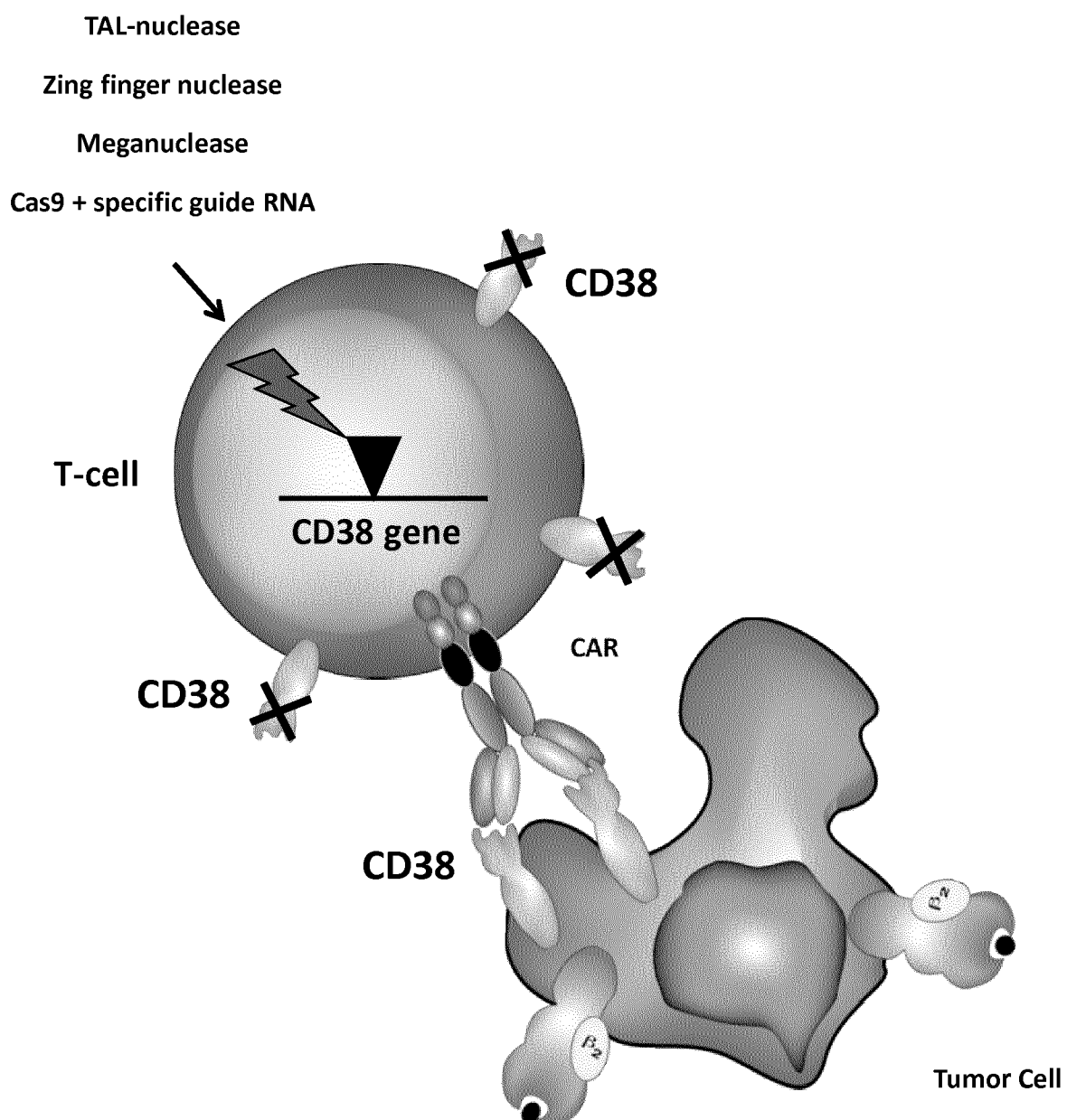
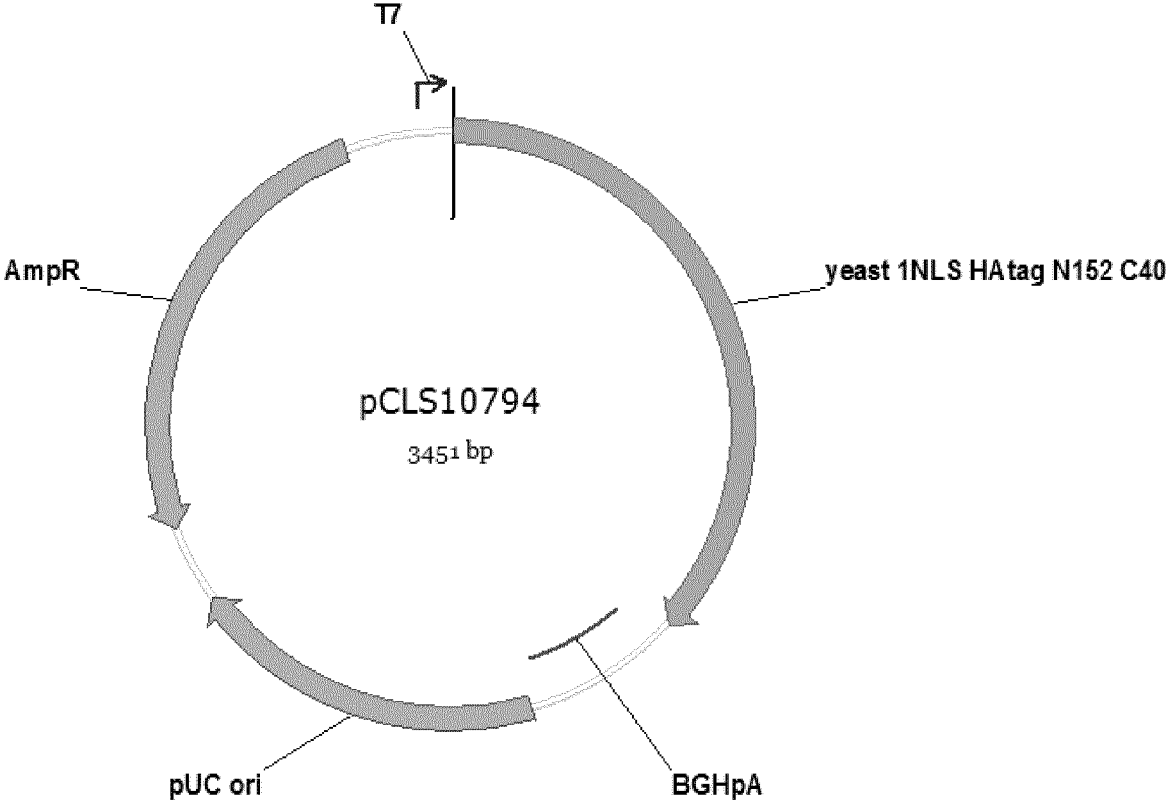


Figure 1

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A



B

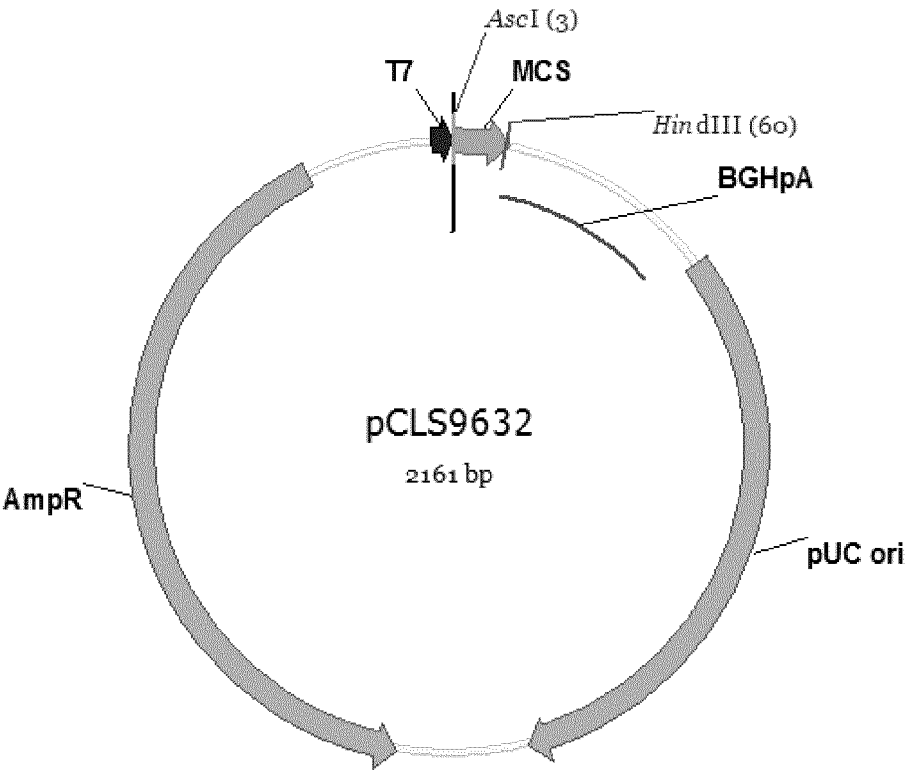
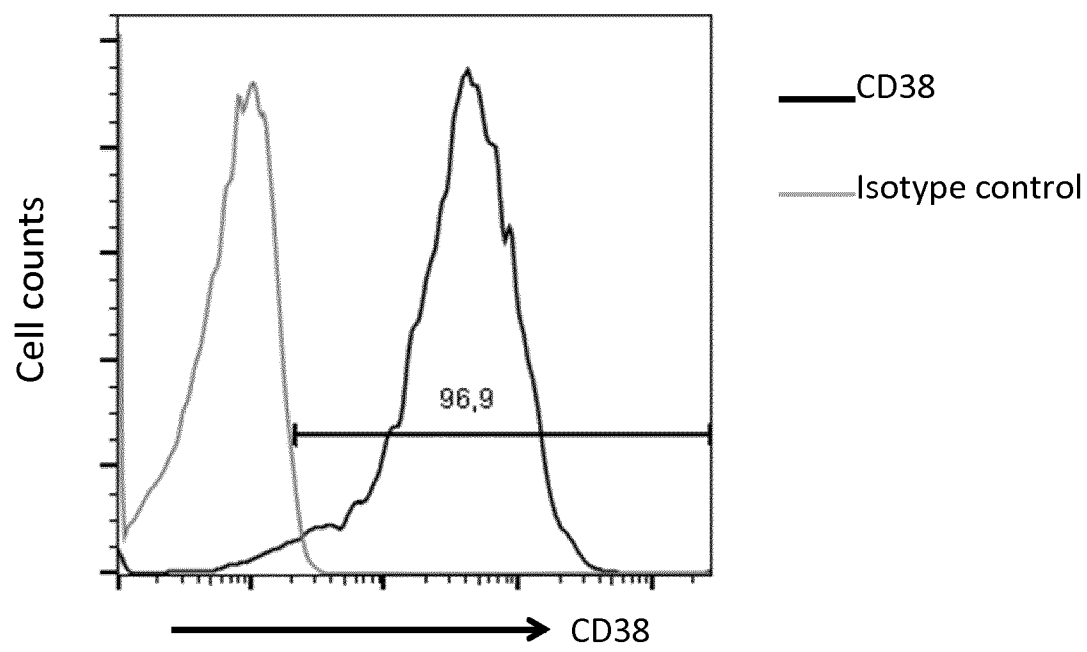


Figure 2

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A



B

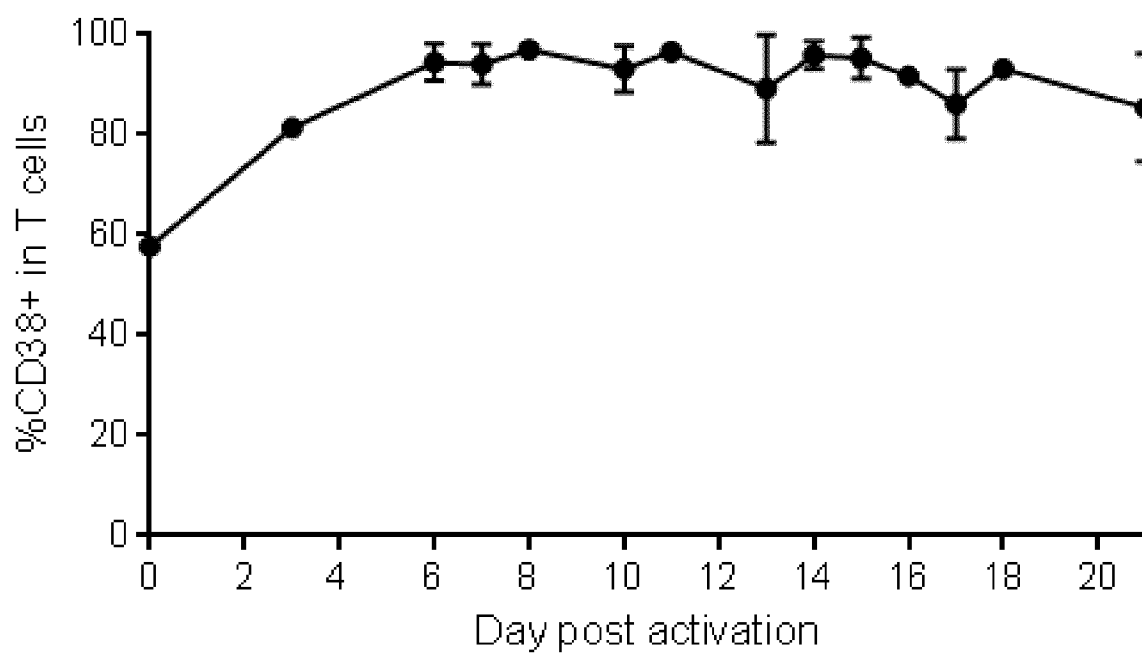
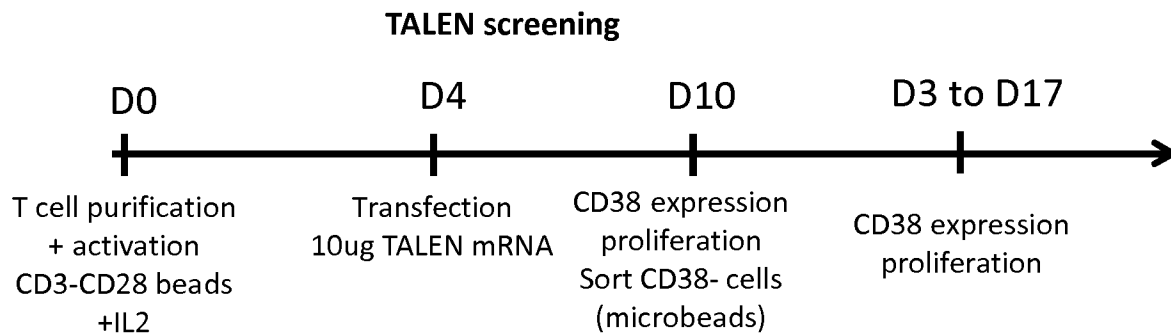
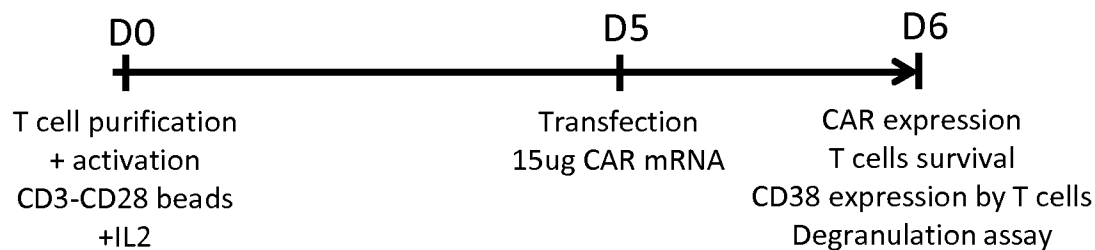


Figure 3

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A**B**

CAR mRNA transfection in WT T cells at Day 5 after T cell activation

**C**

CAR mRNA transfection at day 12 after T cell activation in purified CD38 deficient T cells

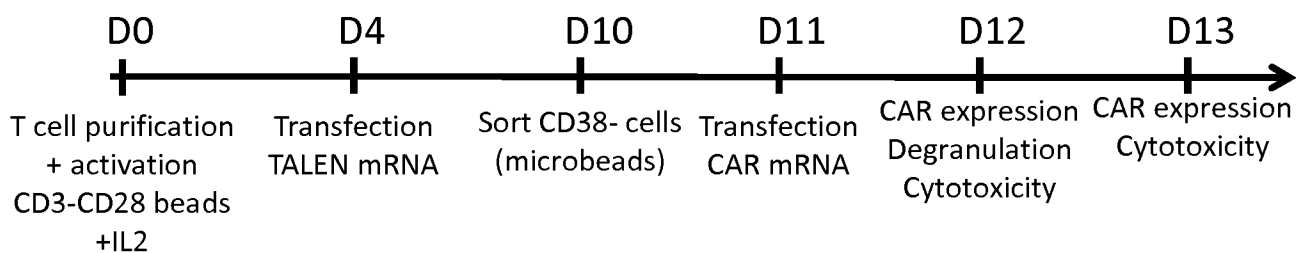


Figure 4

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	TACCGGTTGA	CGCTCAAGTC	GGGCCACAGG	CCCCGTGTTG	GAGAGAGCGC	CGAGAGATCC	TCTCGGTTG	AGACAGAAC	GCACTCATAG	GACAGG	CT
101	TGCTGTGCT	GATCTCTGCG	GTGGTGTCC	CGAGGTGCG	CGAACT	GTGG	AGCGGTCCG	GCACACCA	GCCTTTCC	GAGACGTC	TGGCGGATG
	AGAGACACCA	CCAGAGAGCG	CACCAAGAG	GCTCACACCG	GGTGGT	CAAC	TGGCAGGCC	CGTGTGTT	CGCGAAGGG	CTCTGGCAGG	ACCGCGCTAC
201	CGTCAGTAC	ACTGAATTC	ATCCTGAGT	GAGGTGGTT	GCGCACTAG	GCGCACCGGT	GGGCACTGCG	GGGACACAG	GGCCCCGCG	GCAAGGAGC	
	GCAGTATG	TGACTTAG	TAGACTCA	CTCCACCCA	CCGCTGATTC	CGGTGGCCA	CCGTAAGCG	CCCTGTGTG	CCGGGGCGCG	CGTCCCTCG	
301	CGCCCGATC	GCC									
	GCGGCGCTAG	CGG									

CD38 exon 1 :from AA n°1 to n°233

CD38-1: 1st frame (from AA n°9 to n°58)

CD38-2: 2nd frame (from AA n°98 to n°146)

CD38ex1_T2: 3rd frame (from AA n°229 to n°276)

Figure 5

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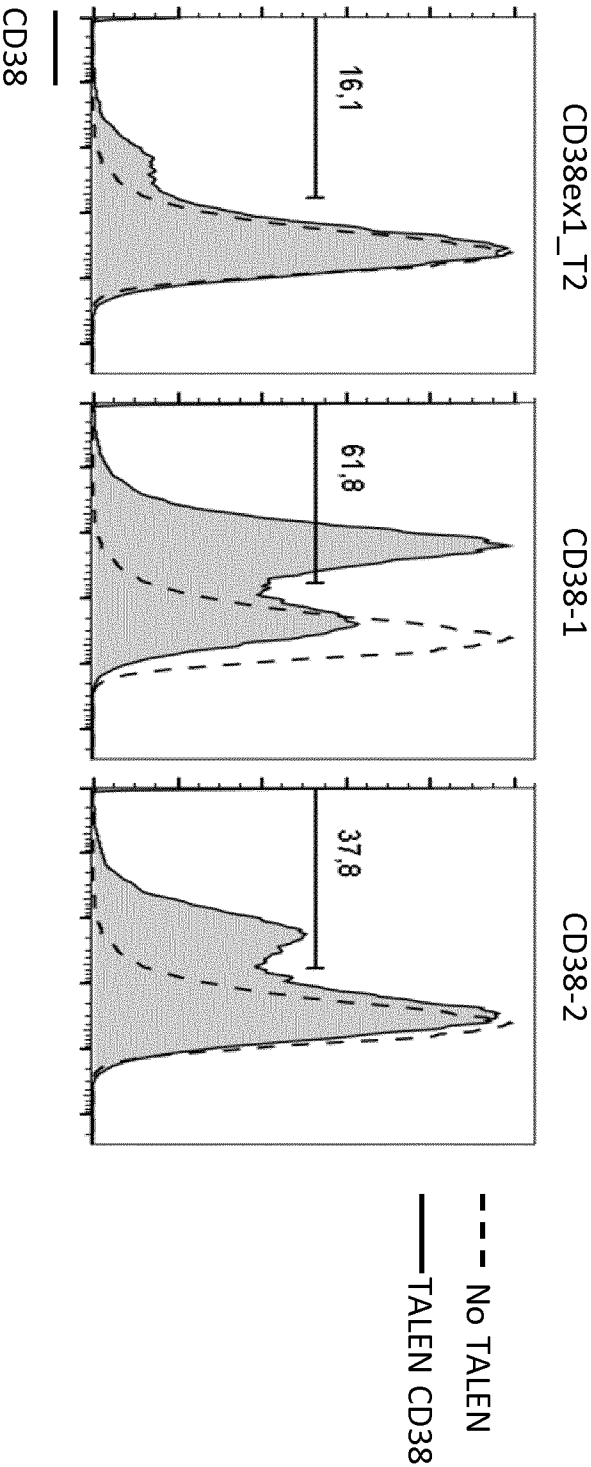


Figure 6

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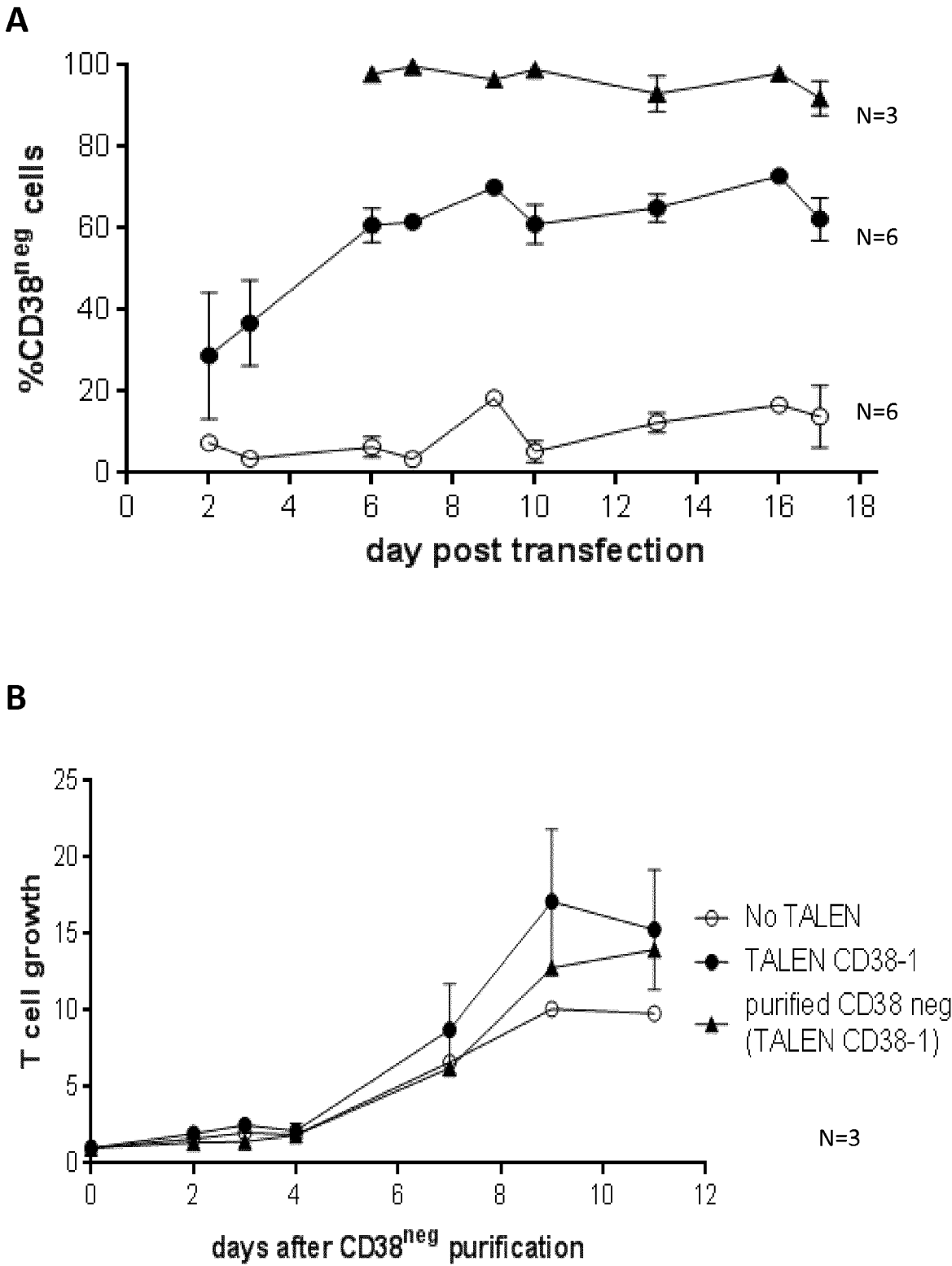


Figure 7

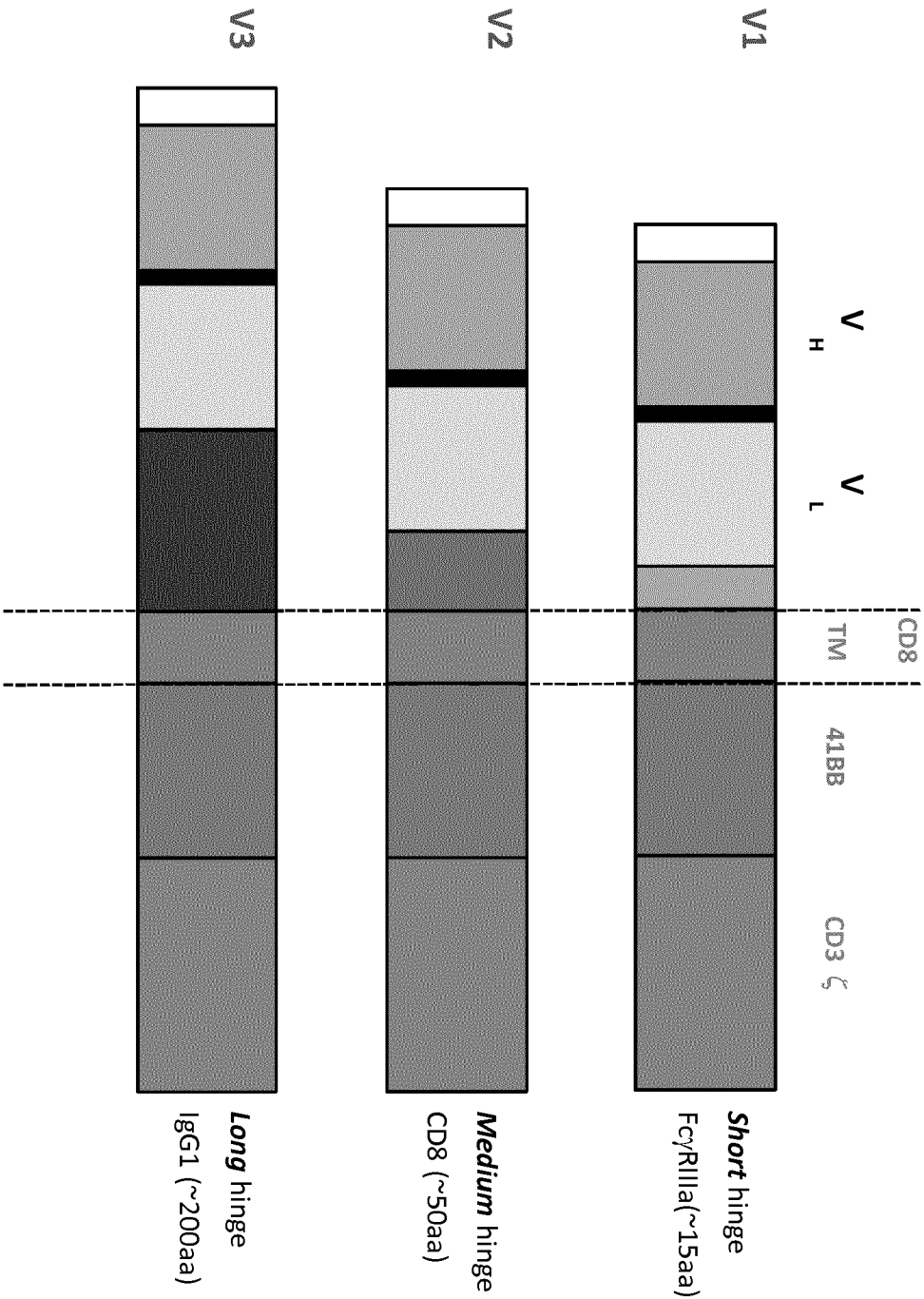


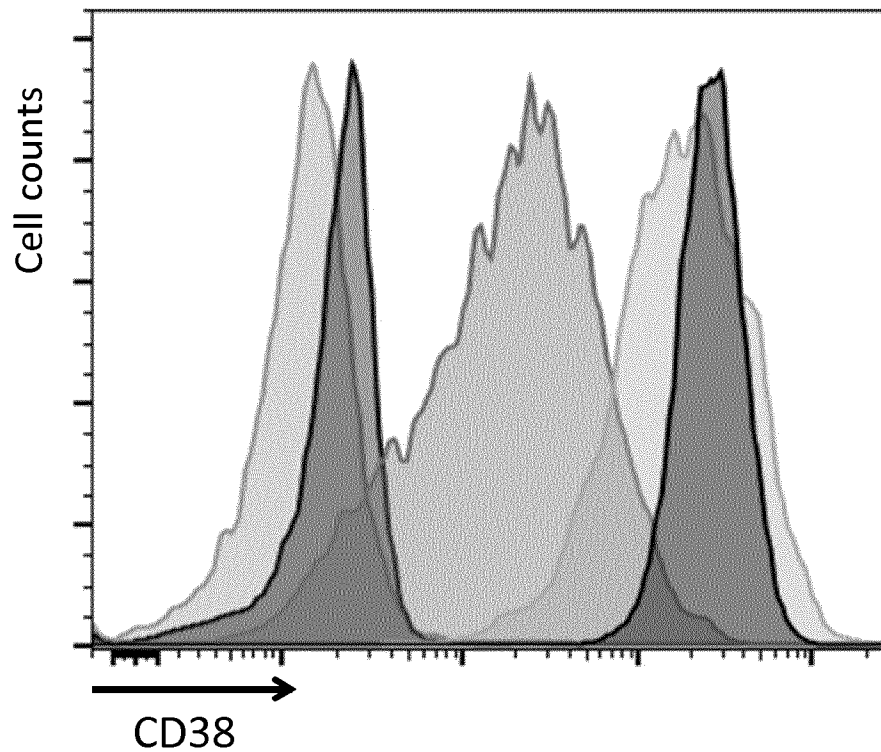
Figure 8

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Plasmid Name	Backbone	Sequence Name	Kd (nM)
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pCLS27541	pCLS9632	10F7-V2	
pCLS27542	pCLS9632	10F7-V3	
pCLS27543	pCLS9632	13F11-V1	0,8
pCLS27544	pCLS9632	13F11-V2	
pCLS27545	pCLS9632	13F11-V3	
pCLS27546	pCLS9632	16B5-V1	3,2
pCLS27547	pCLS9632	16B5-V2	
pCLS27548	pCLS9632	16B5-V3	
pCLS27549	pCLS9632	25A10-V1	0,25
pCLS27550	pCLS9632	25A10-V2	
pCLS27551	pCLS9632	25A10-V3	
pCLS27552	pCLS9632	27B6-V1	2,2
pCLS27553	pCLS9632	27B6-V2	
pCLS27554	pCLS9632	27B6-V3	
pCLS27555	pCLS9632	28F5-V1	unknown
pCLS27556	pCLS9632	28F5-V2	
pCLS27557	pCLS9632	28F5-V3	
pCLS27558	pCLS9632	29B4-V1	0,52
pCLS27559	pCLS9632	29B4-V2	
pCLS27560	pCLS9632	29B4-V3	
pCLS27561	pCLS9632	GMB005-V1	119
pCLS27562	pCLS9632	GMB005-V2	
pCLS27563	pCLS9632	GMB005-V3	

Figure 9

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A

Cell lines from the left to the right (peaks):

- K562
- U266 CD38-;
- U266 CD38+;
- Daudi
- MOLP8

B

Cell lines	Disease	% CD38+	molecules CD38/ cells
MOLP8	MM	97,34	482346
Daudi	Burkitt's lymphoma	97,73	259889
U266 CD38+	MM	84,335	48216
U266 CD38-	MM	2,39	230
K562	CML	0	0

Figure 10

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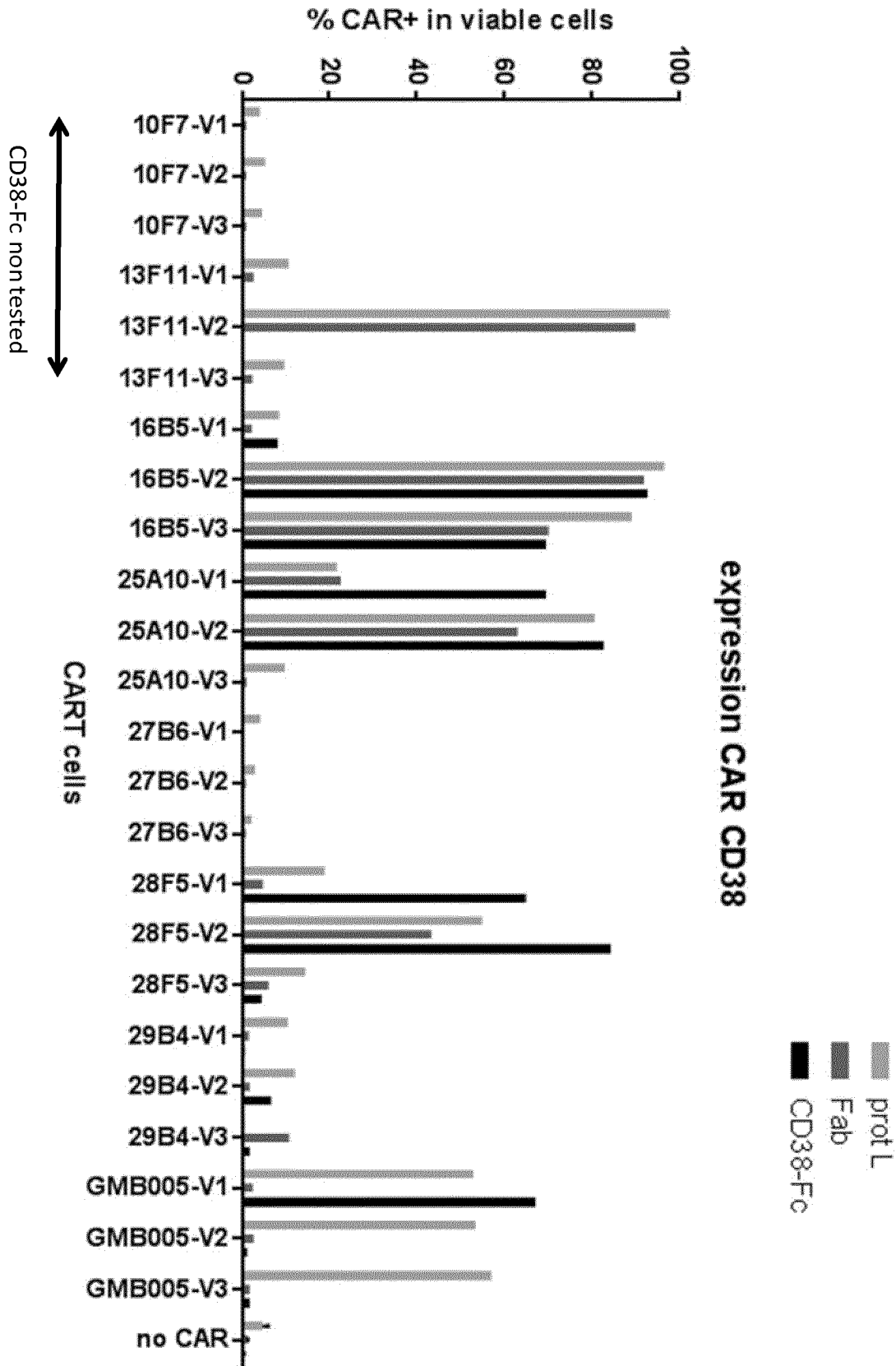
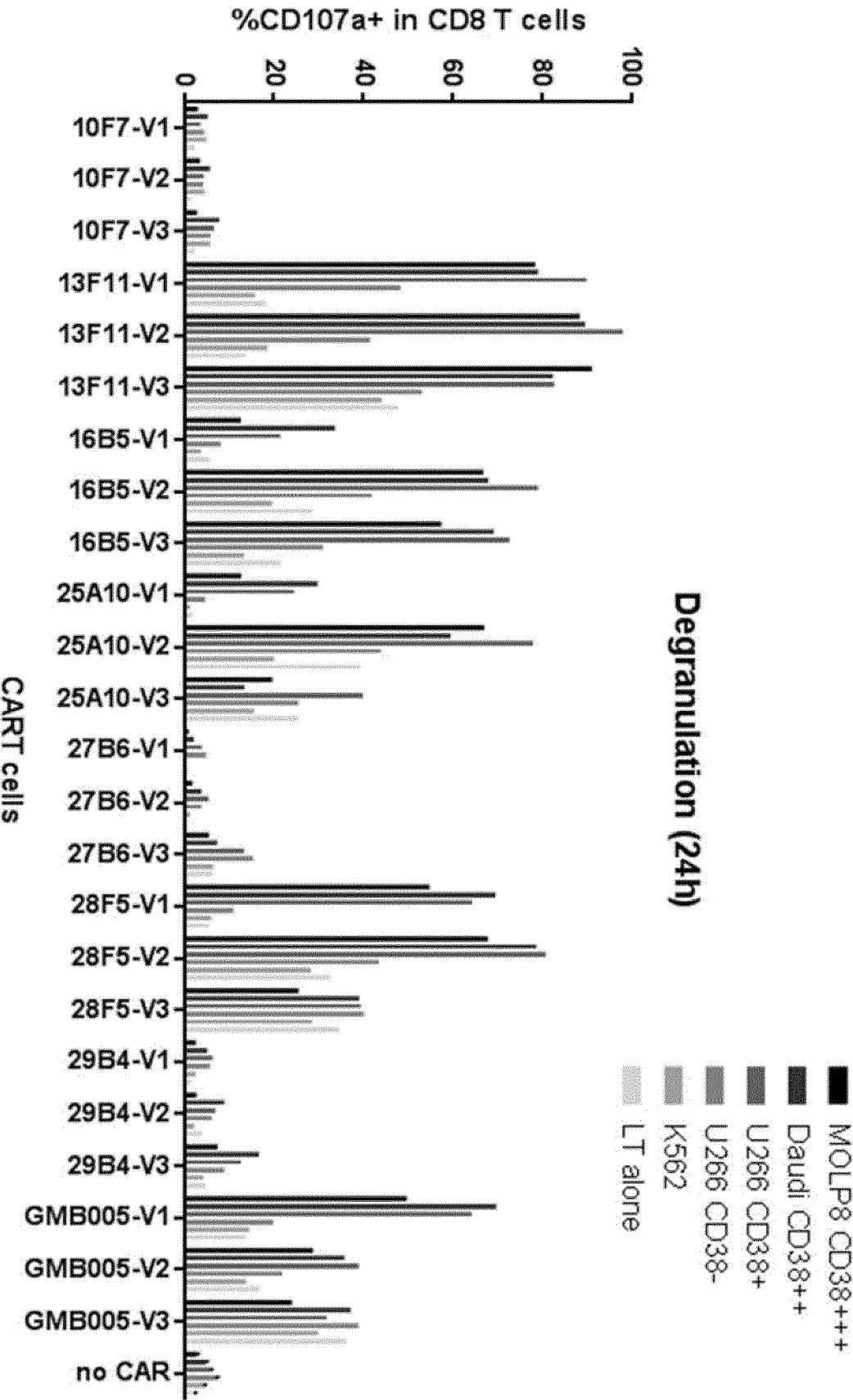


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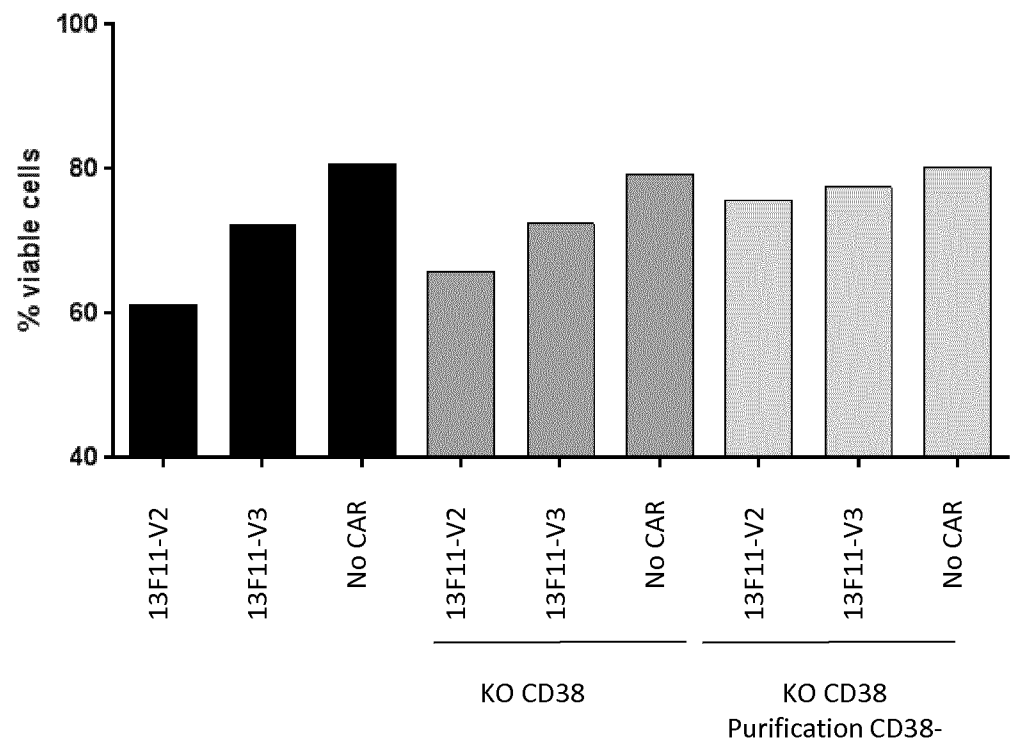
12/34

Figure 12



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A



B

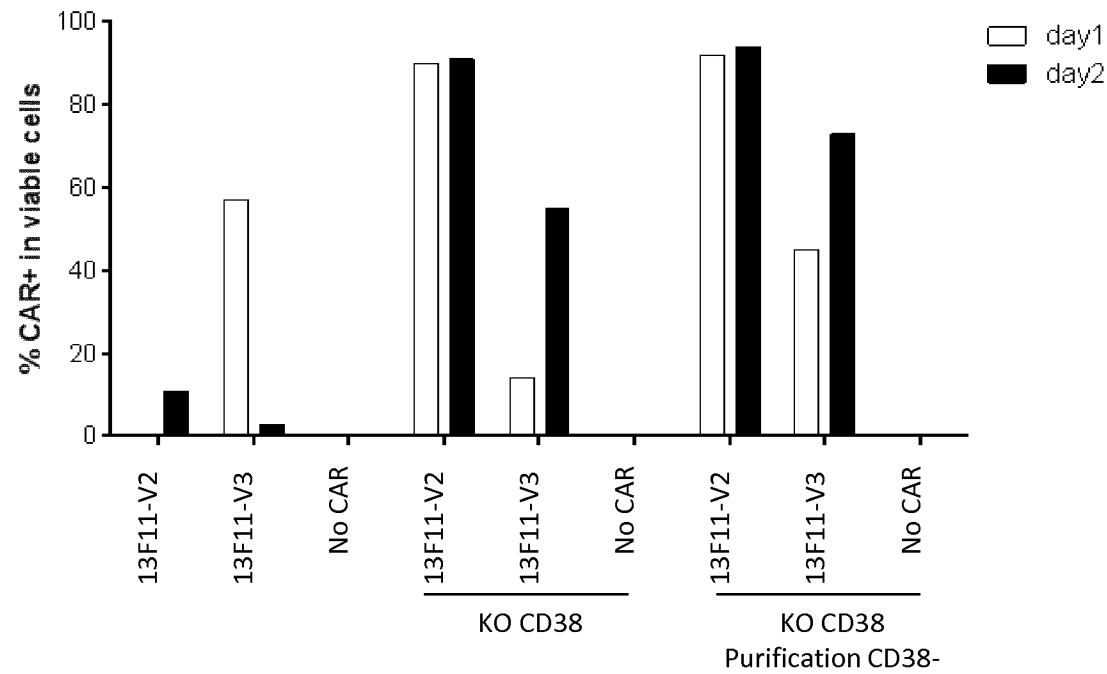
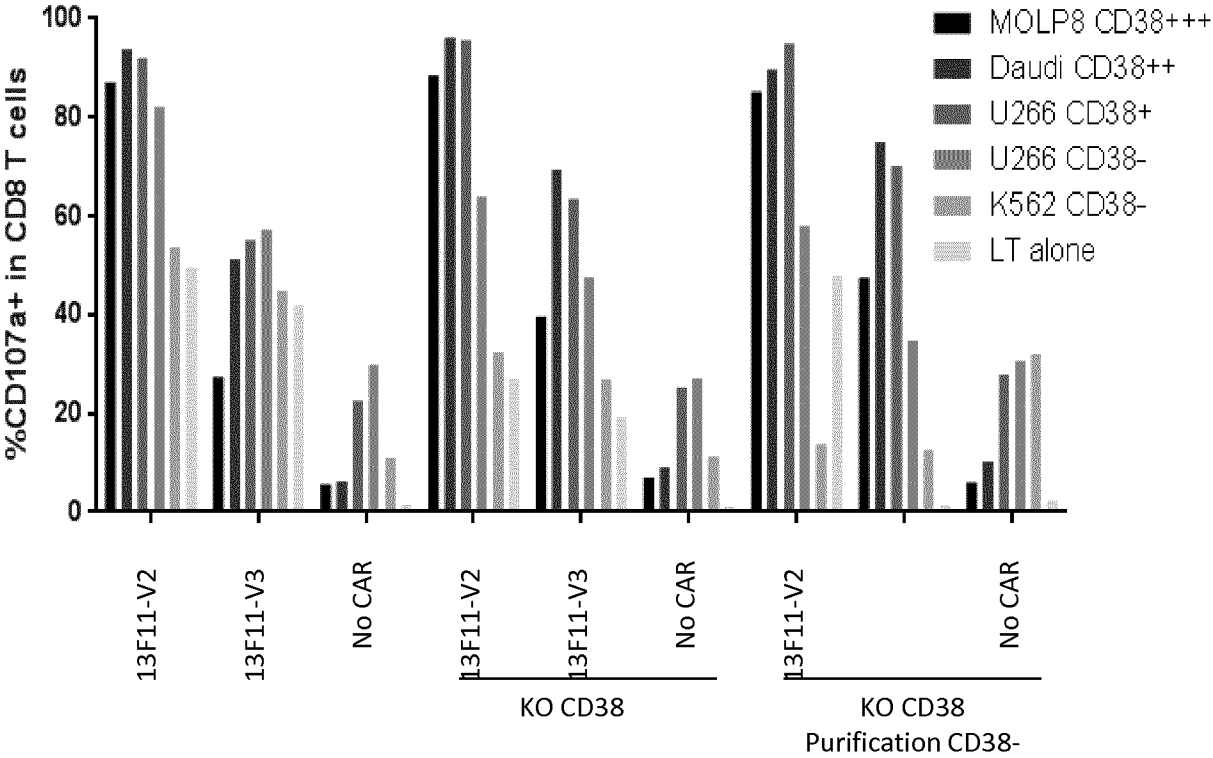


Figure 13

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A



B

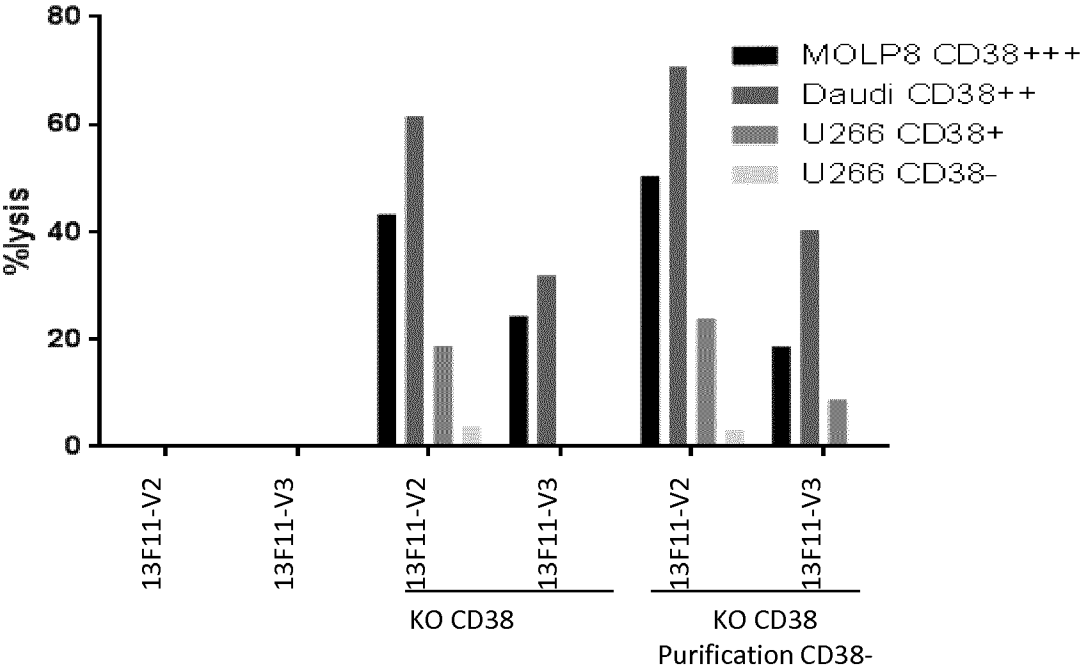


Figure 14

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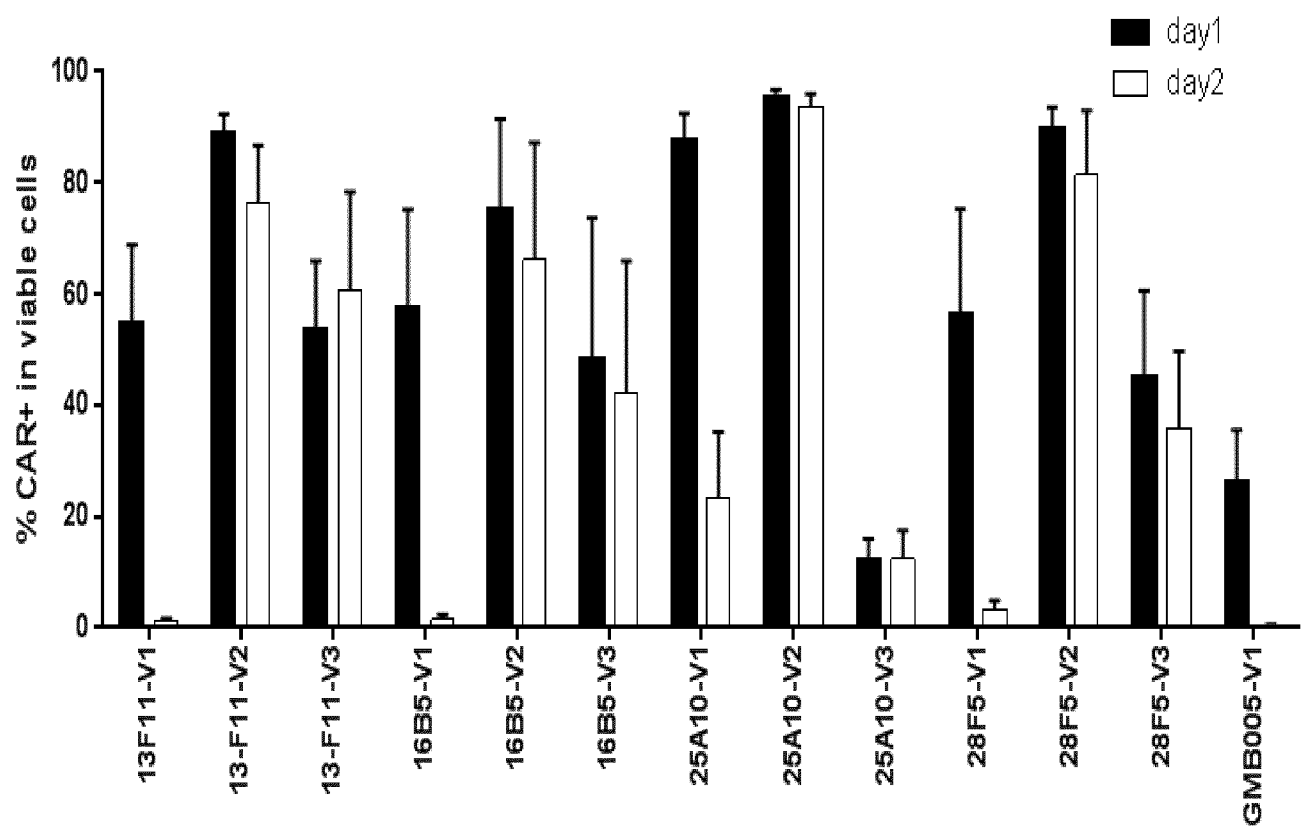


Figure 15

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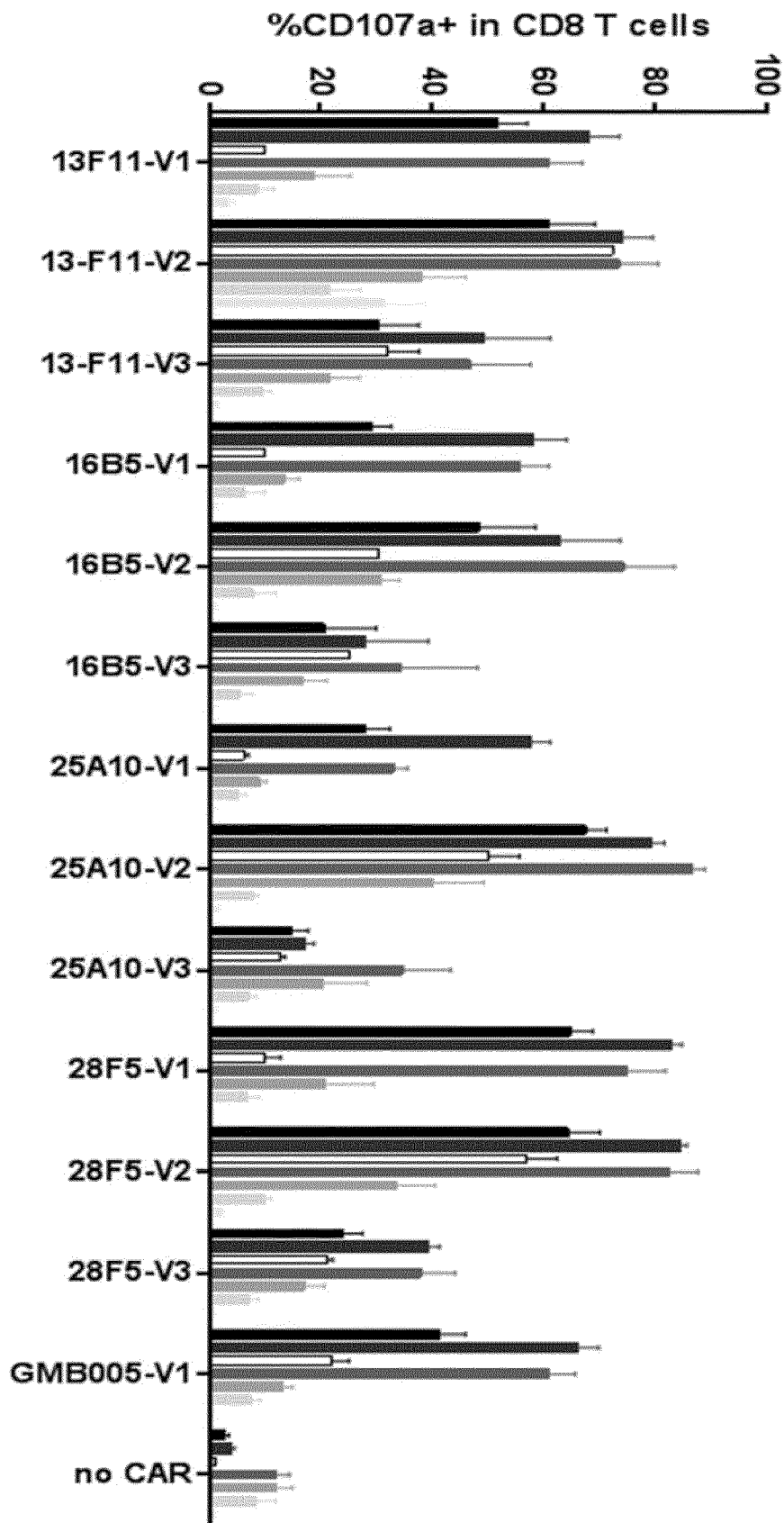


Figure 16

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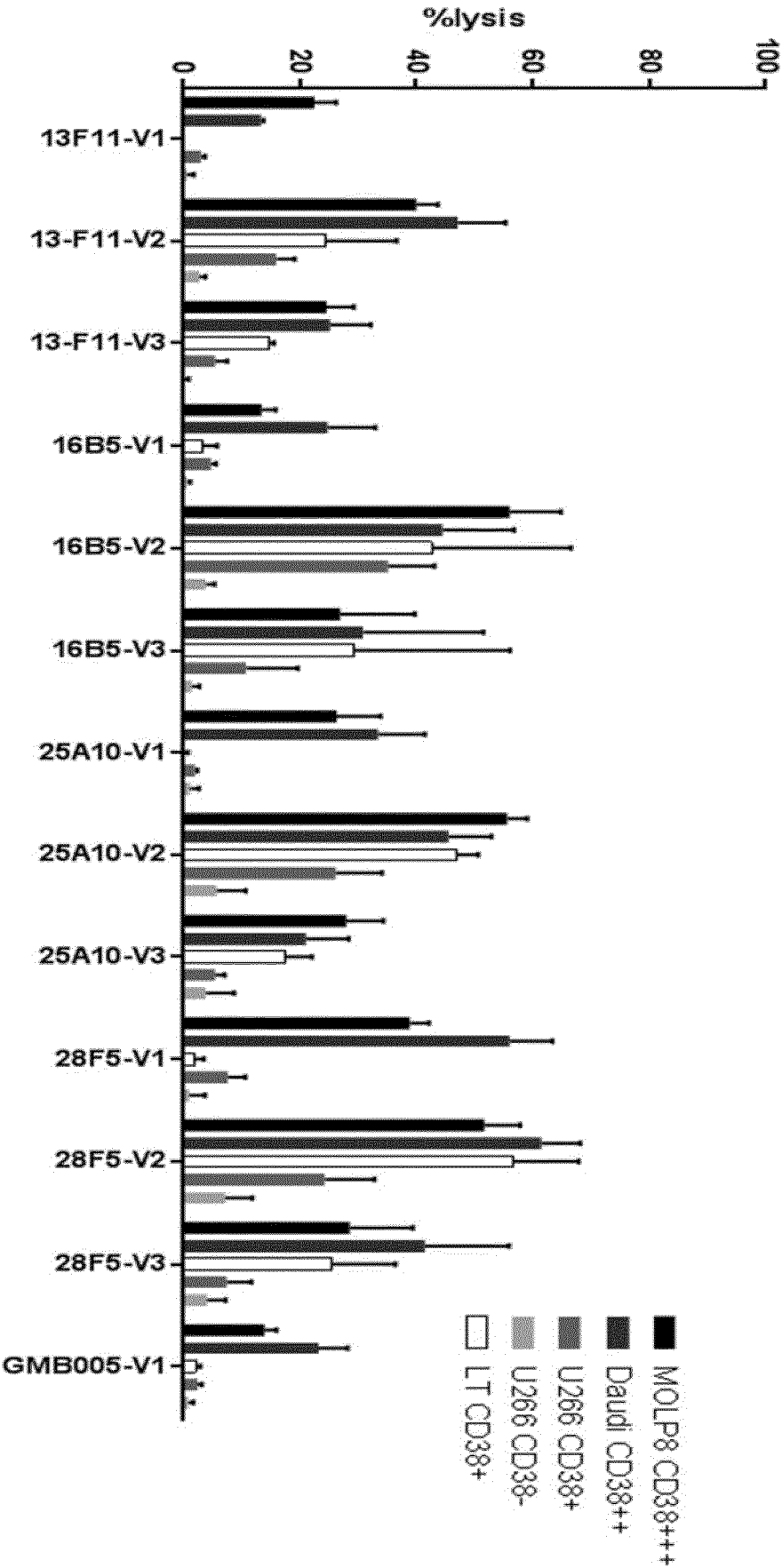


Figure 17

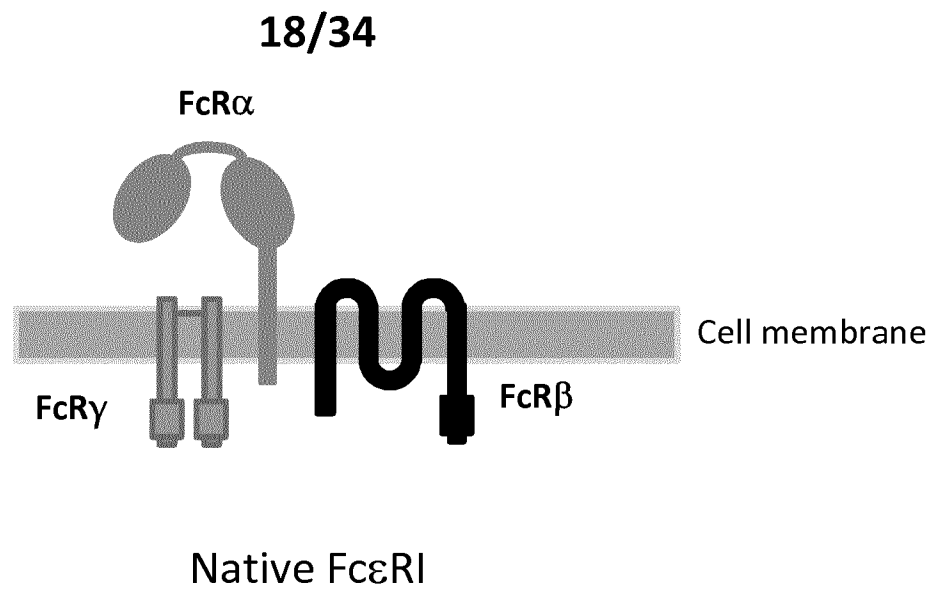
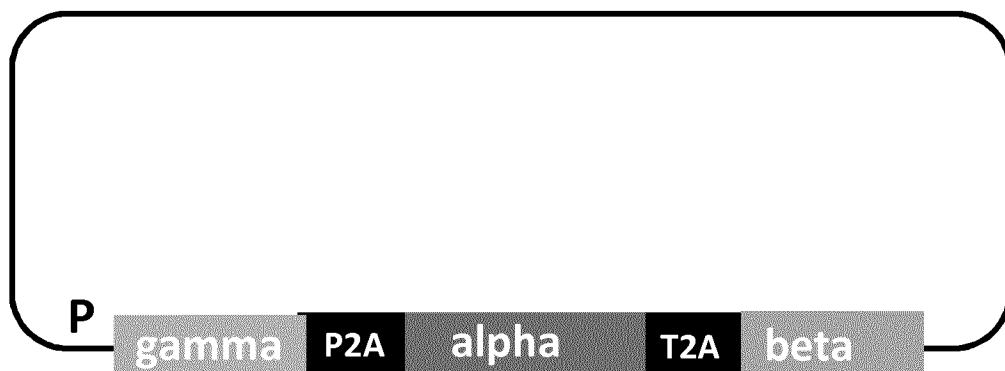


Figure 18



Structure of the polycistronic mcCAR construct

Figure 19

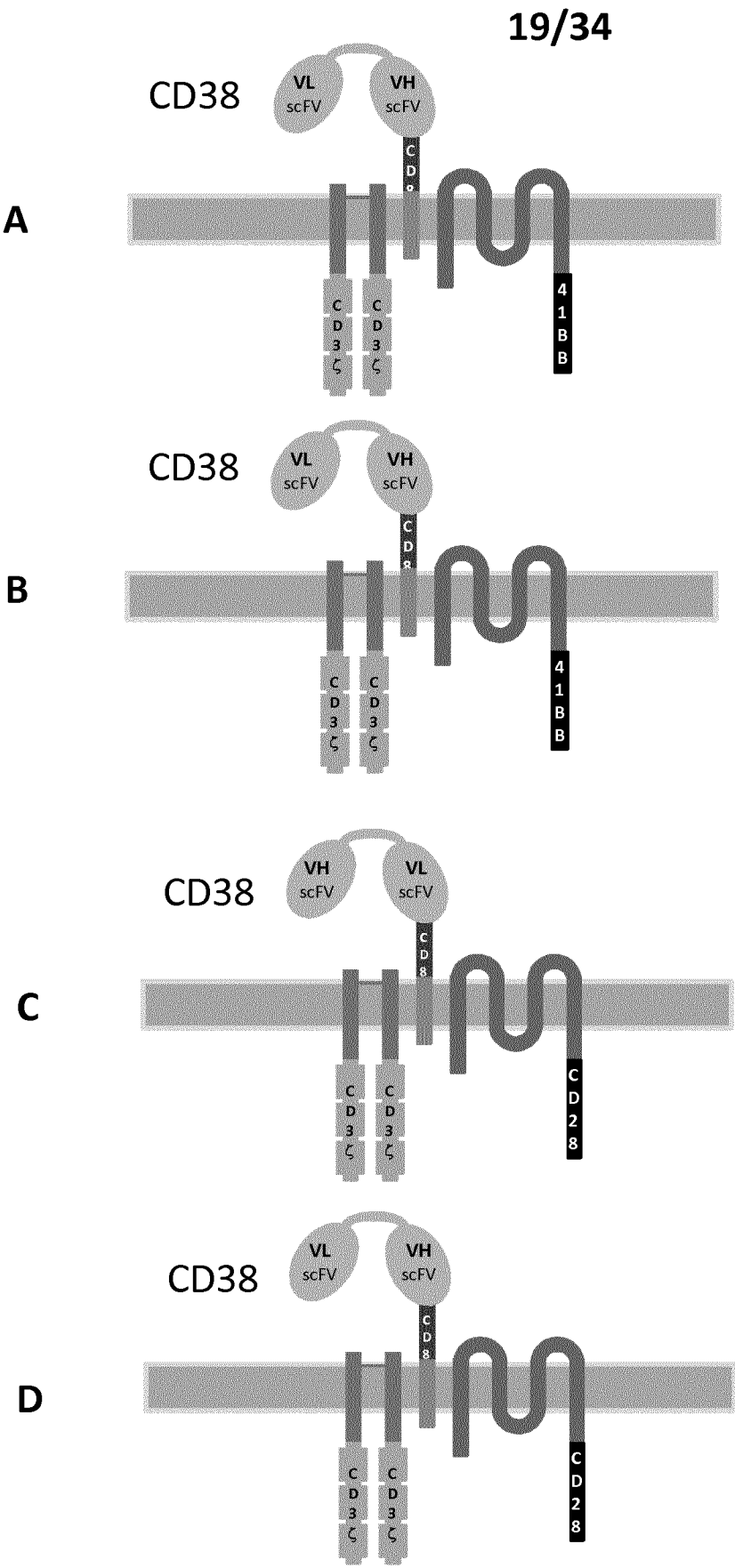


Figure 20

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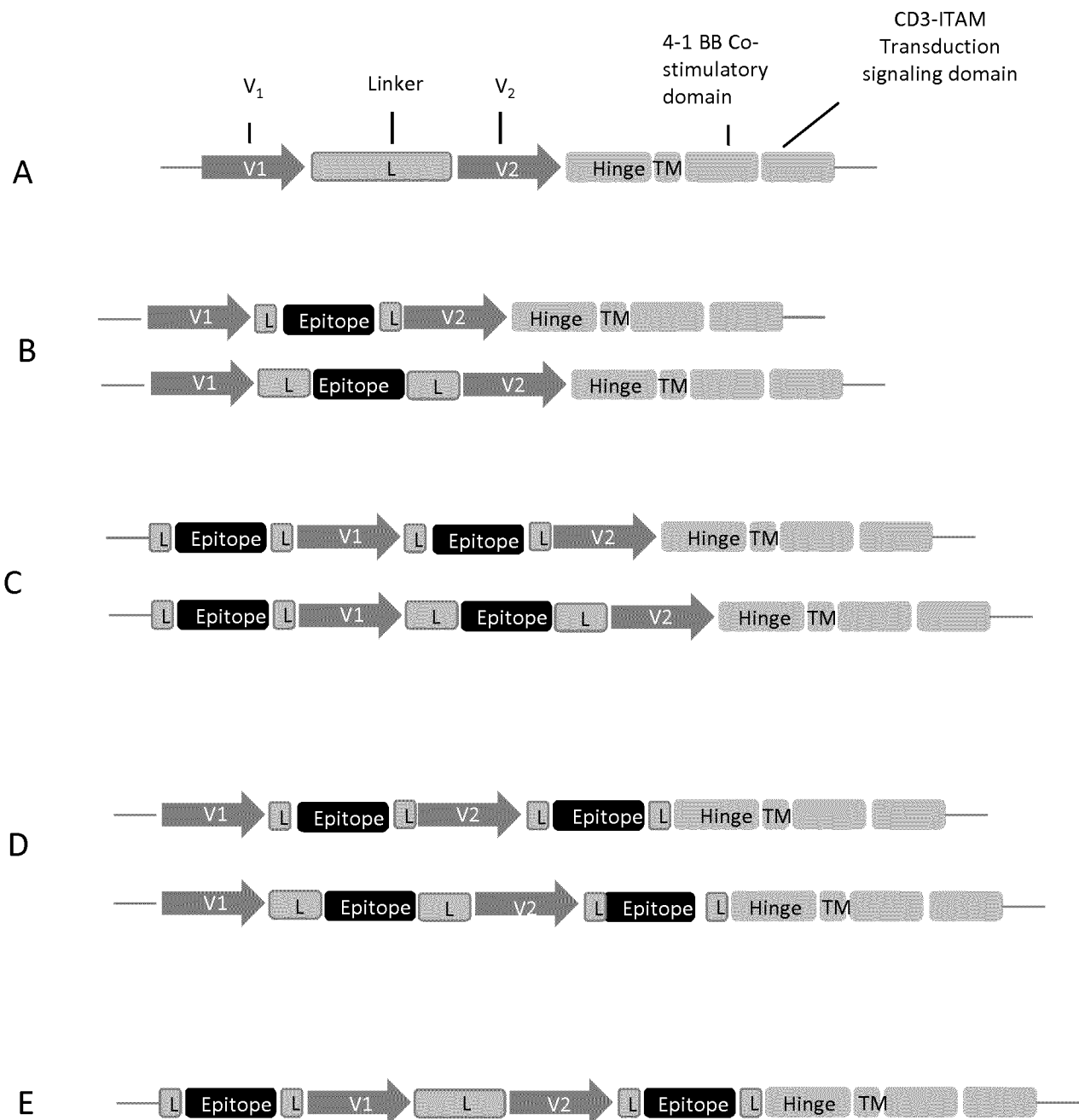


Figure 21A

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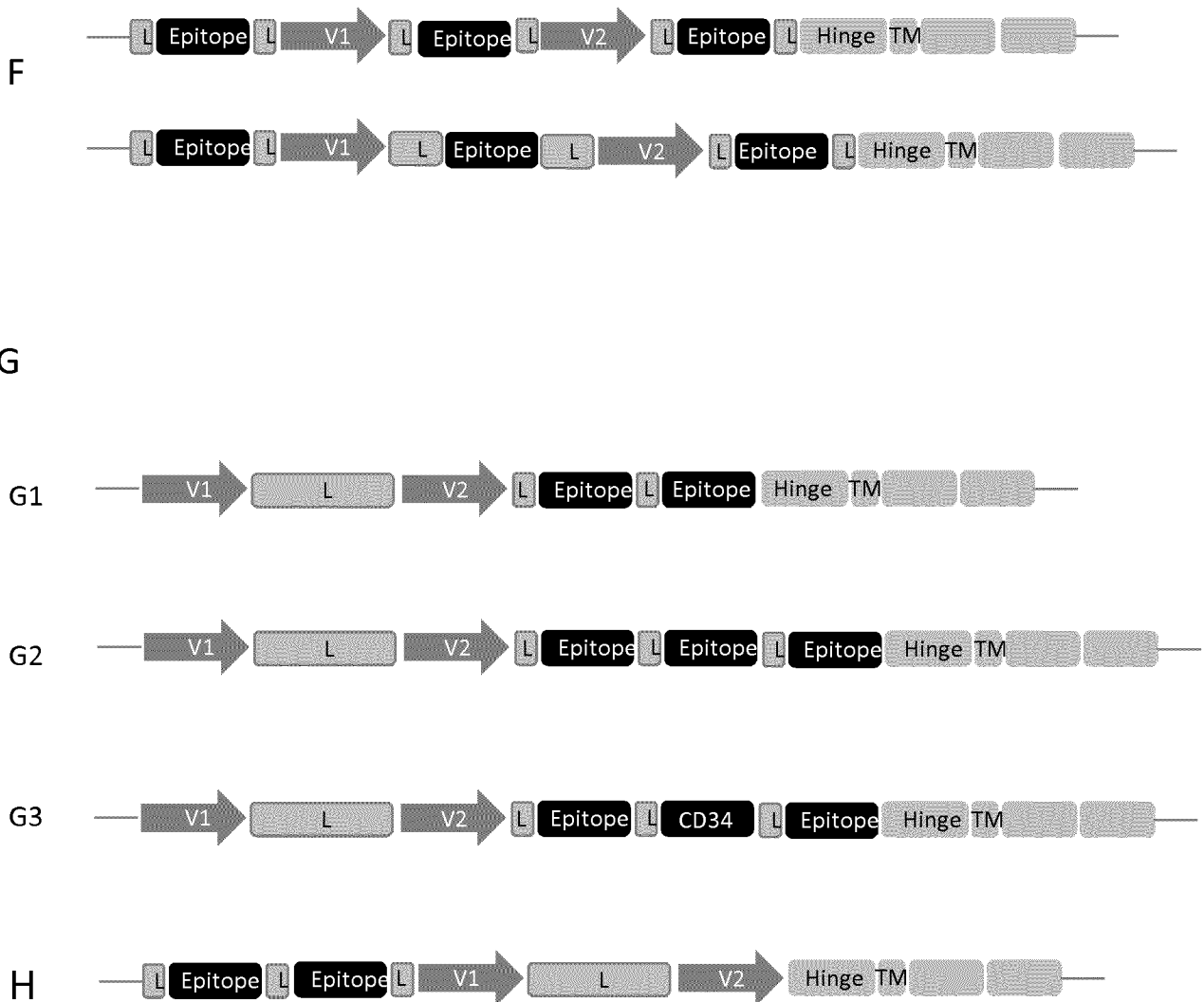


Figure 21B

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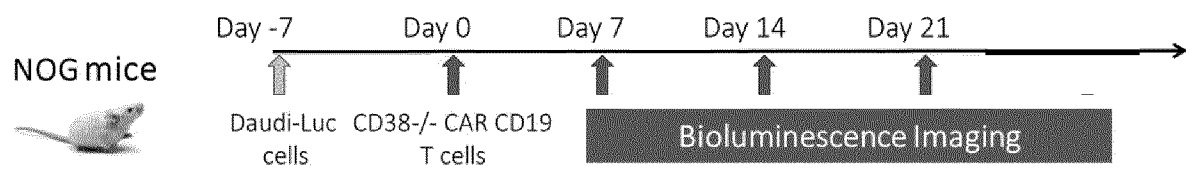


Figure 22A

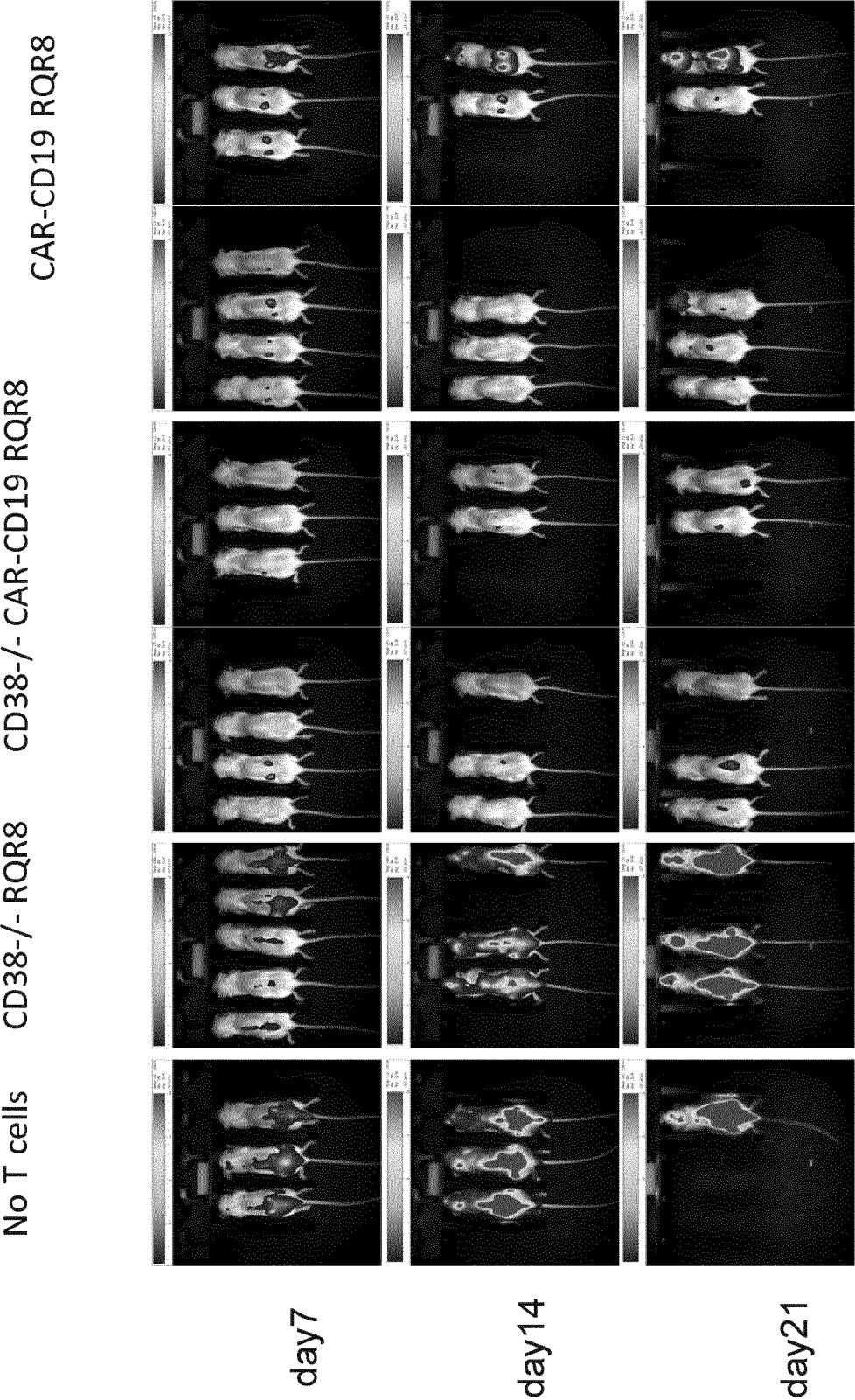


Figure 22B

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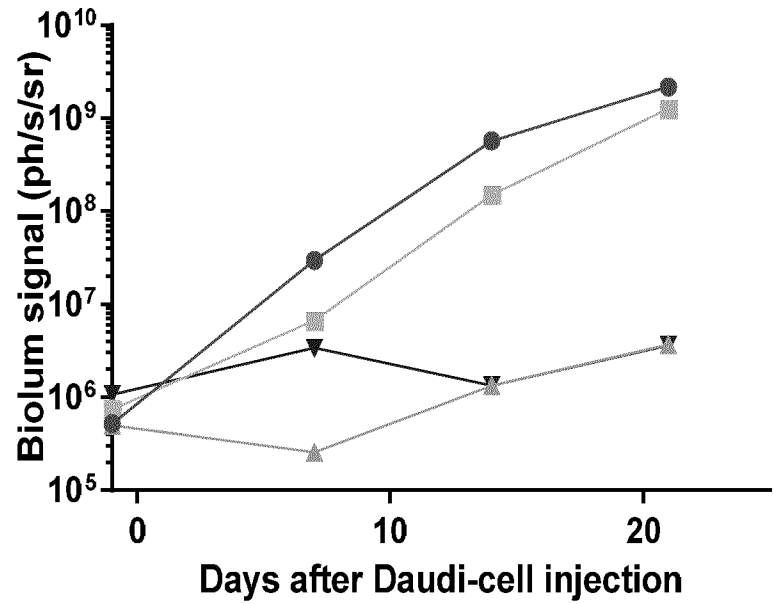


Figure 22C

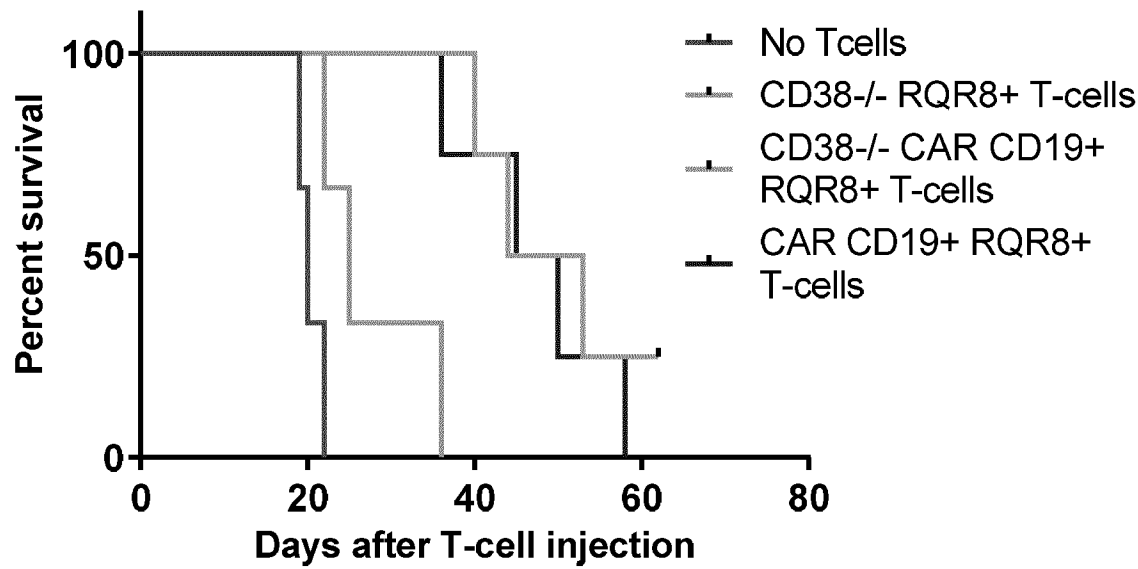


Figure 22D

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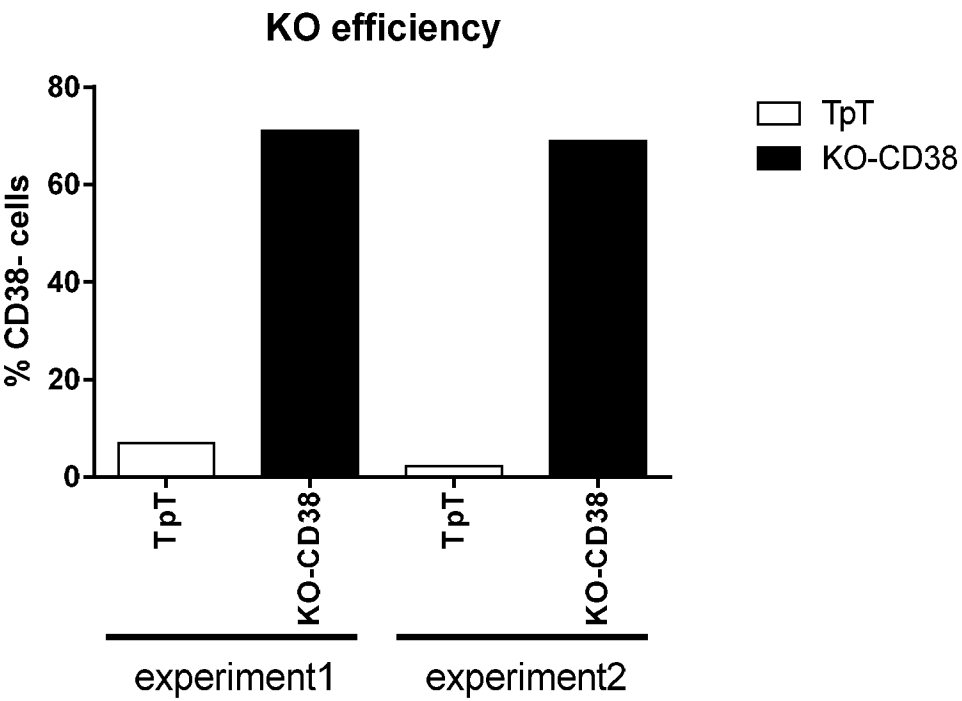


Figure 23

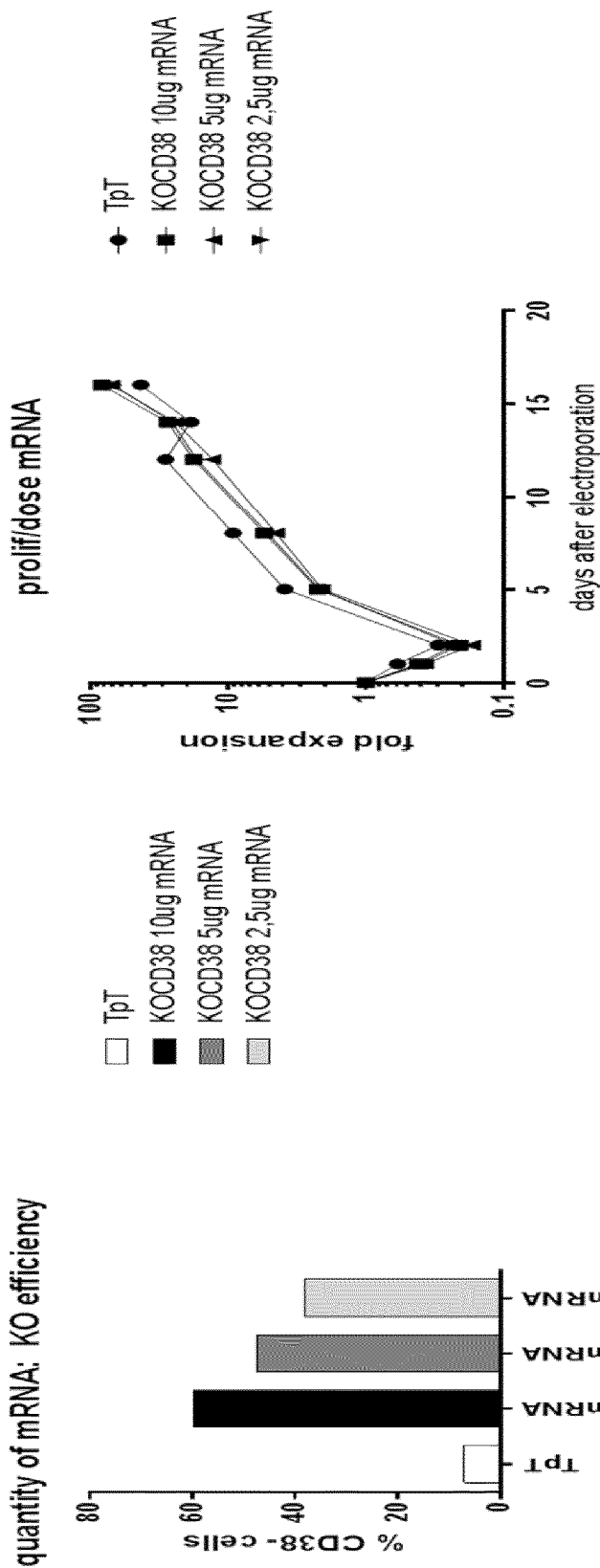


Figure 24B

Figure 24A

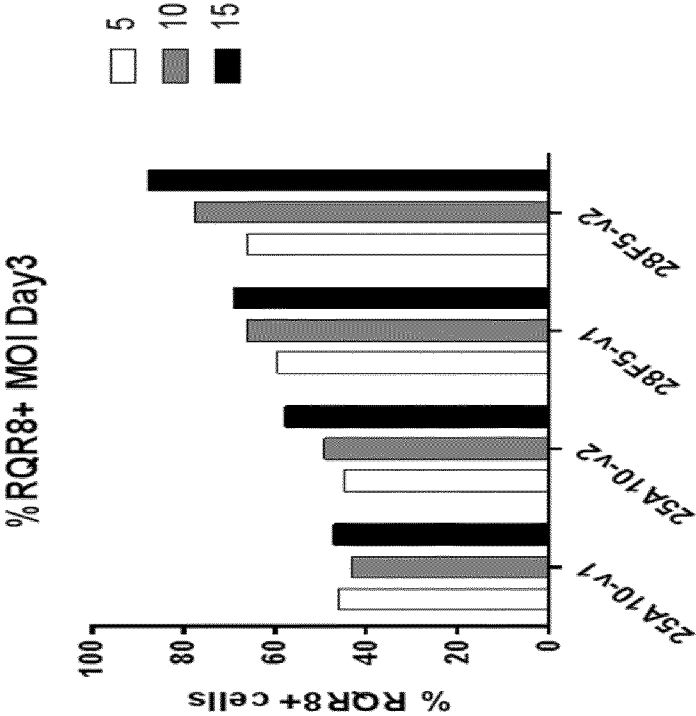


Figure 25B

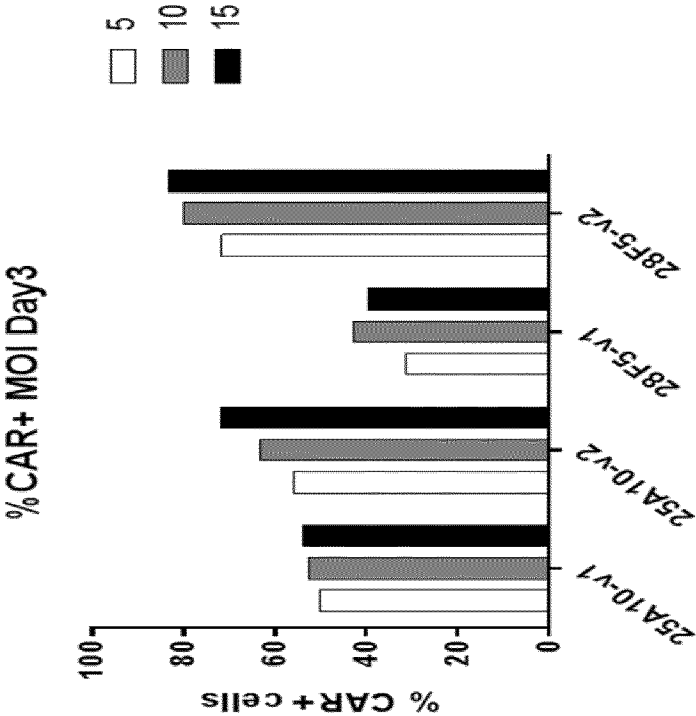


Figure 25A

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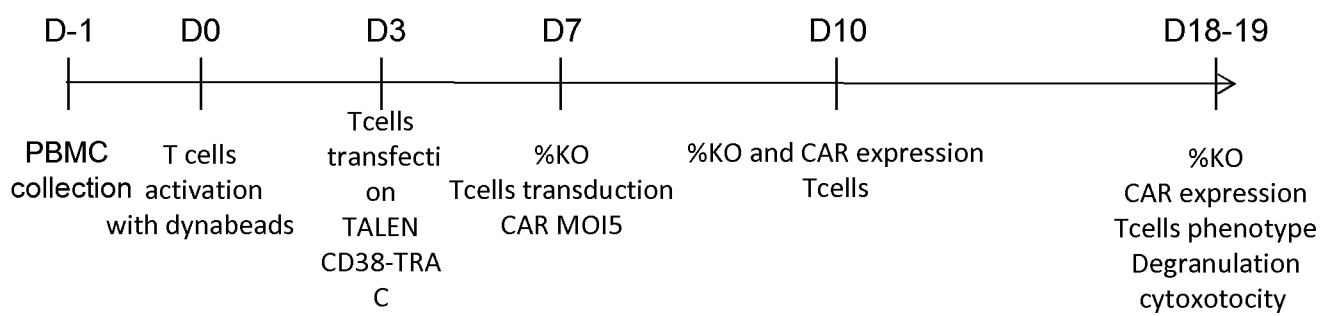


Figure 26

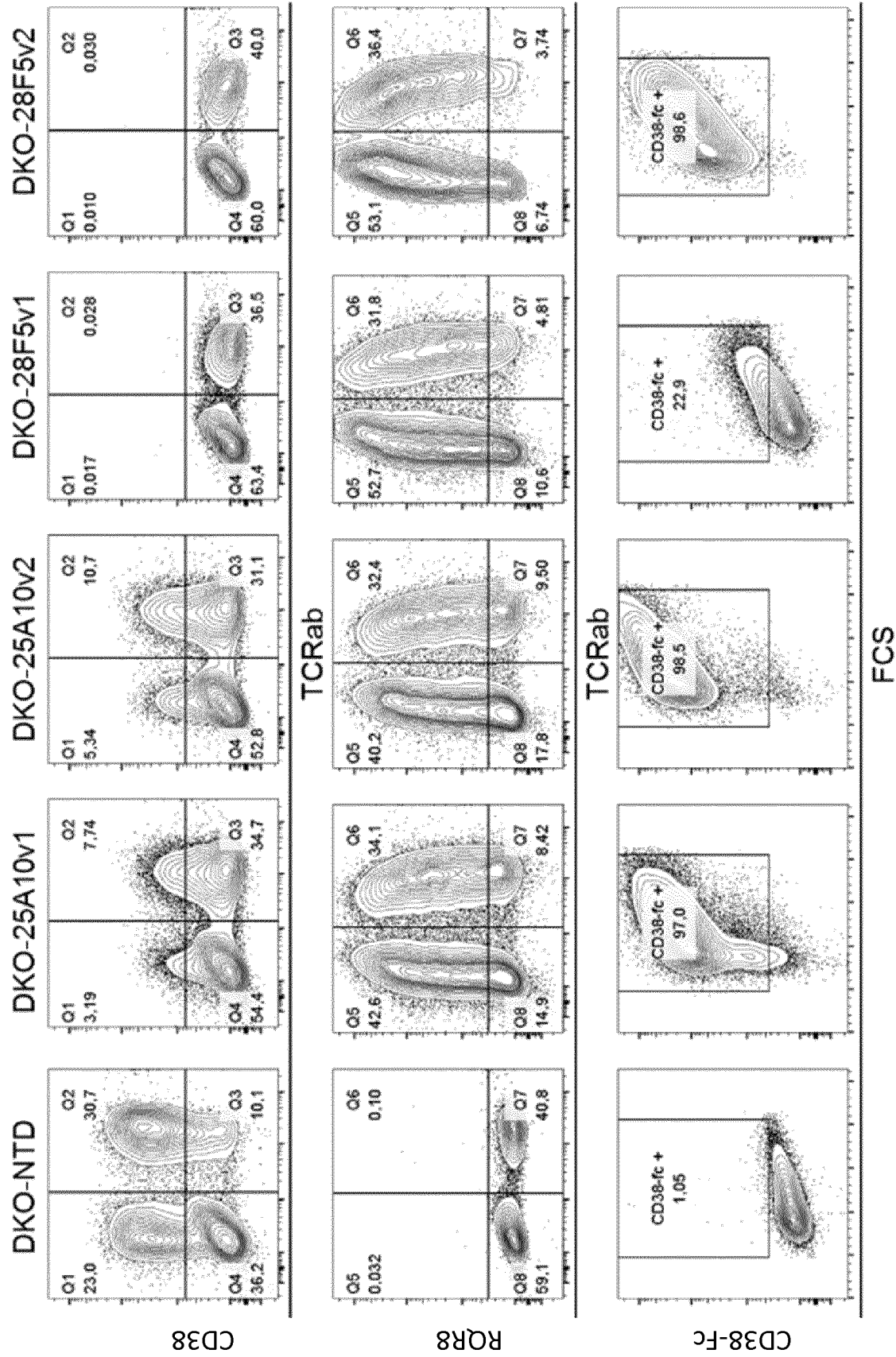


Figure 27A

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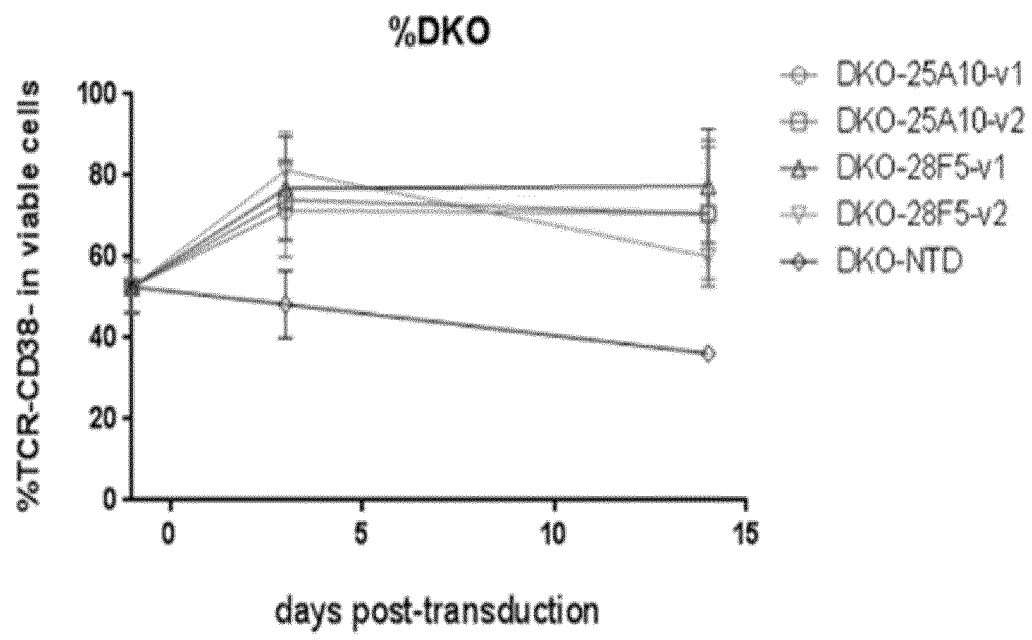


Figure 27B

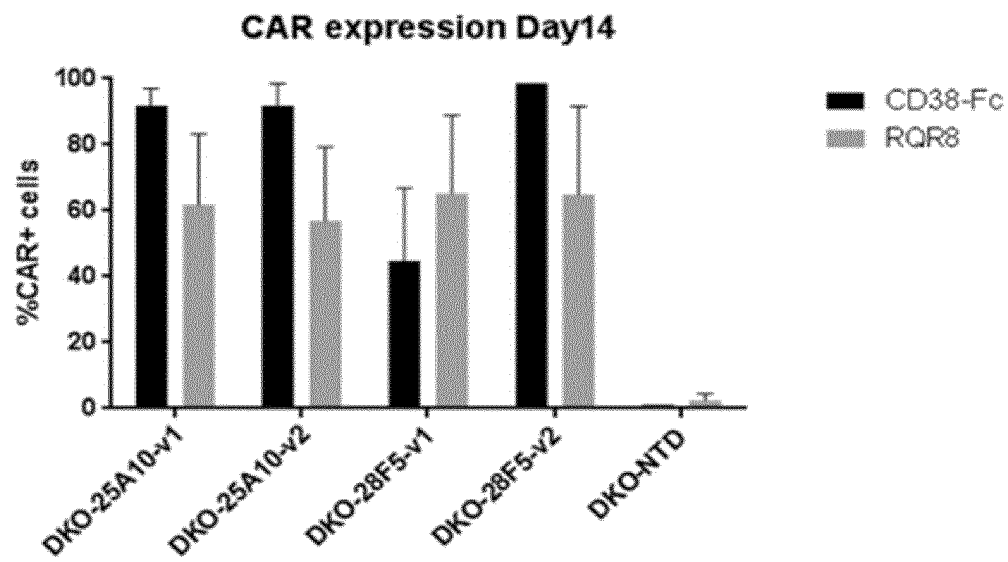


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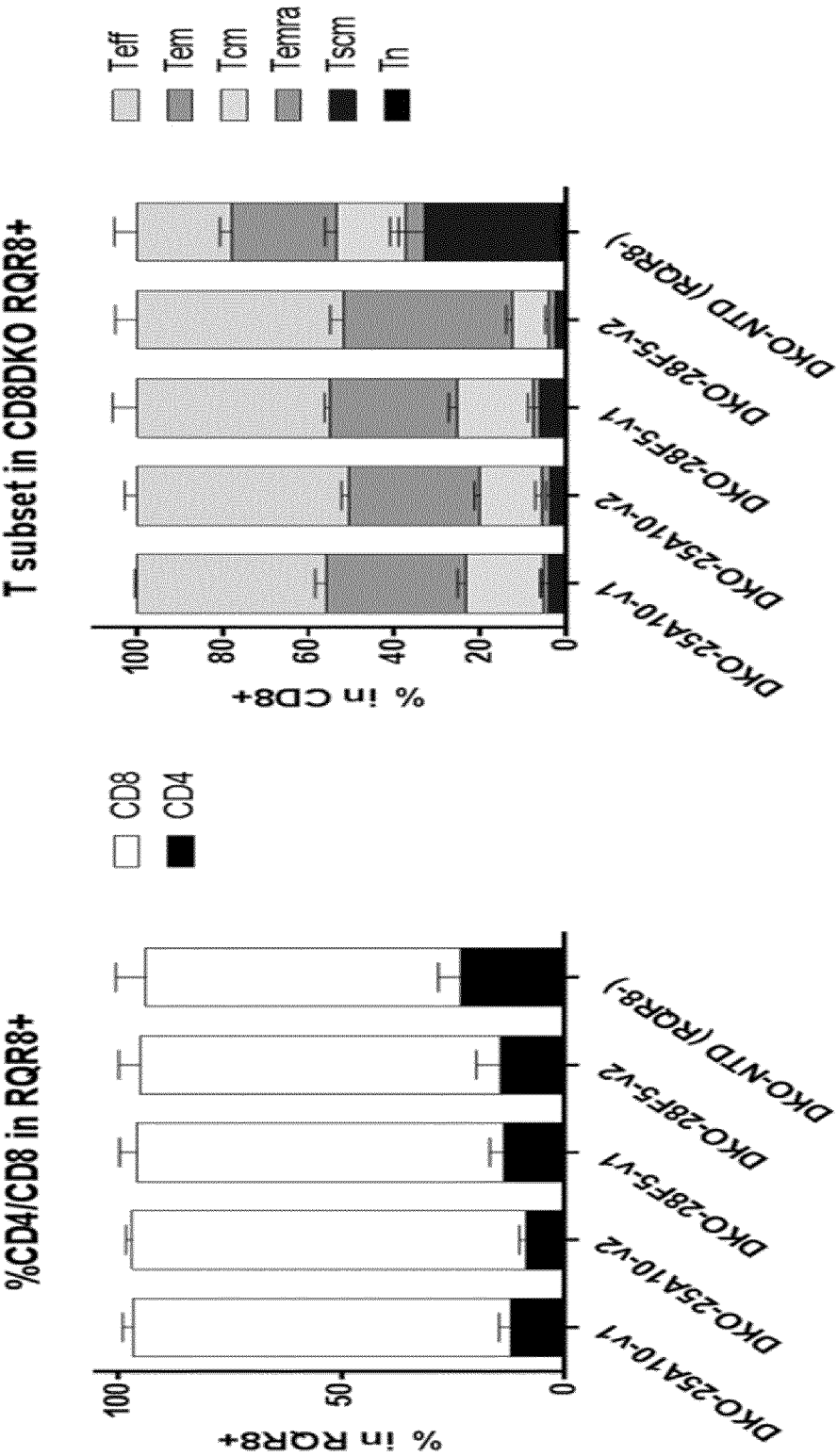


Figure 28A

Figure 28B

32/34

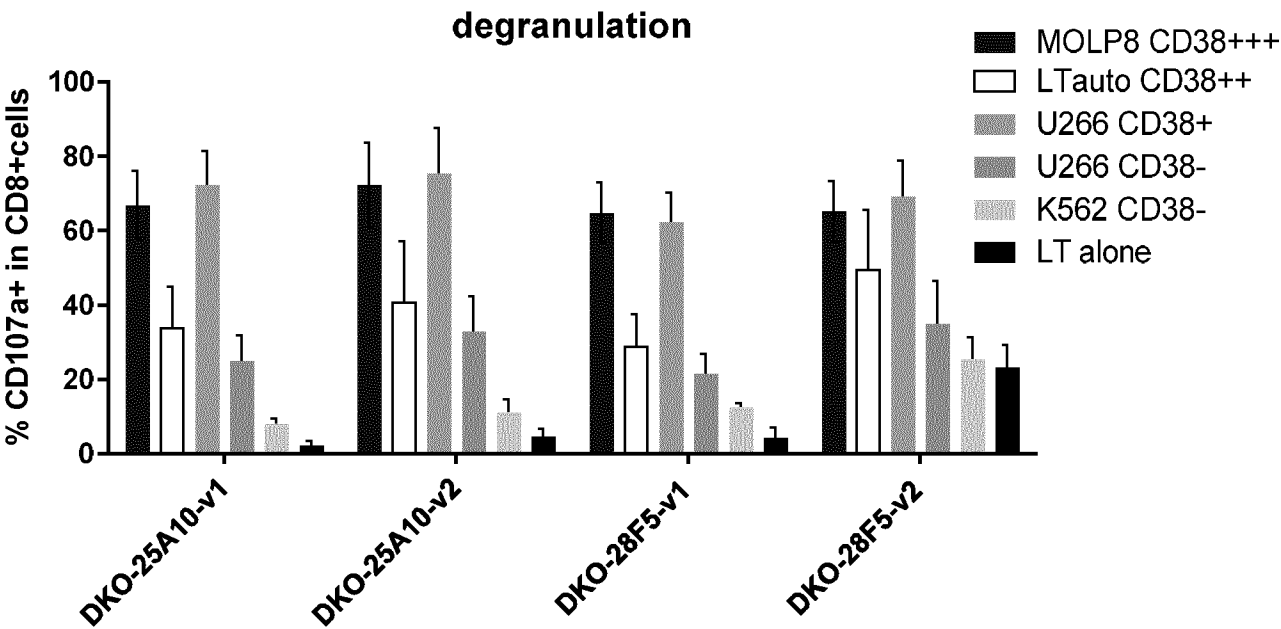


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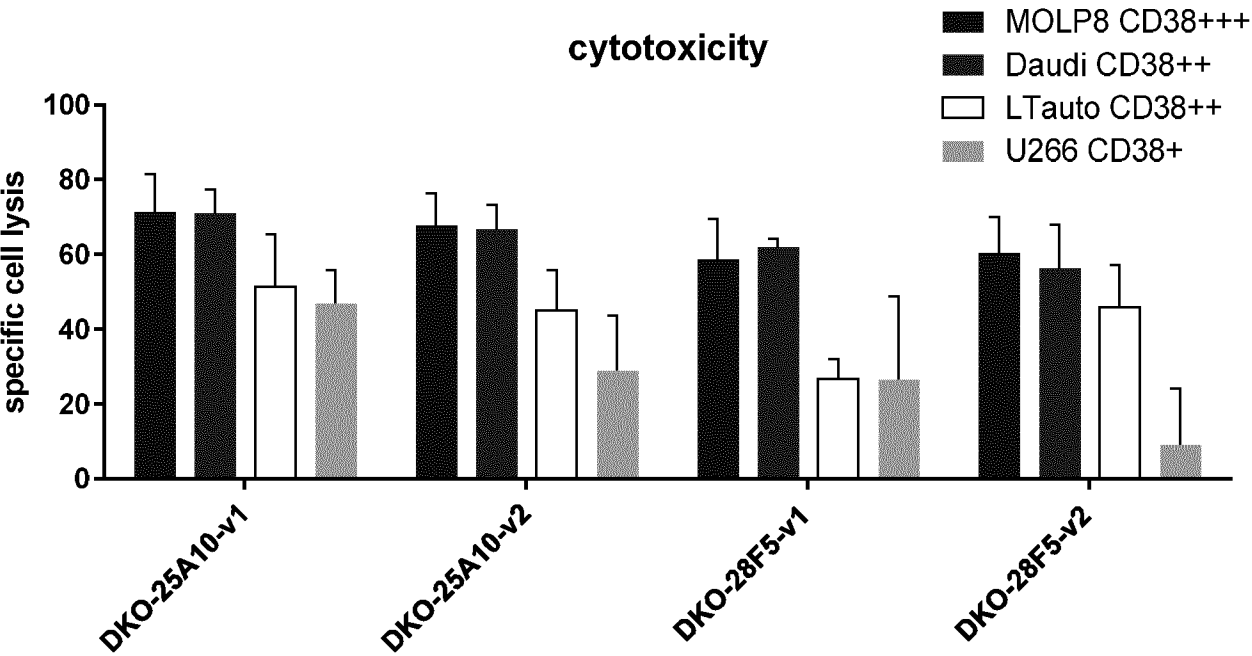


Figure 29B

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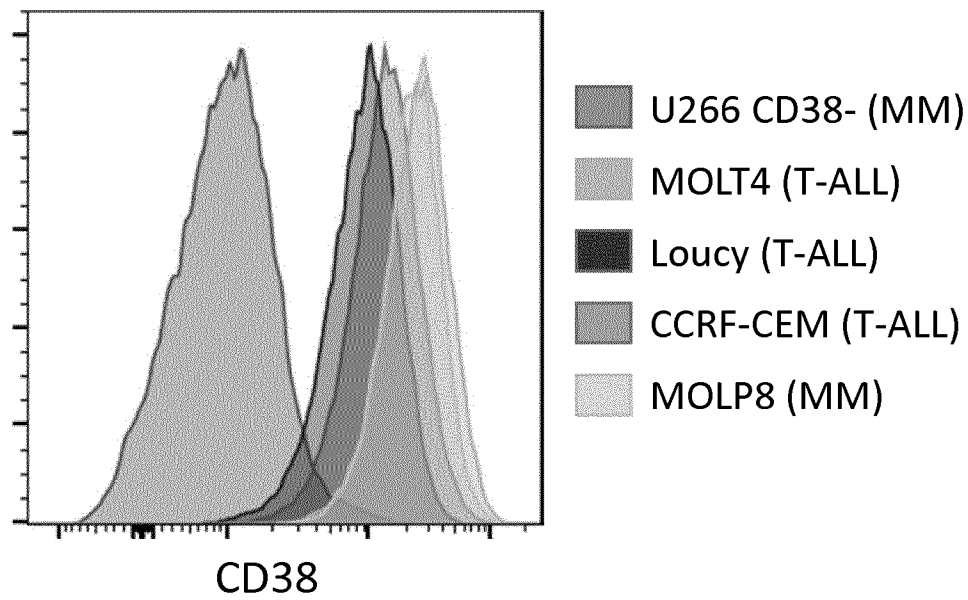


Figure 30

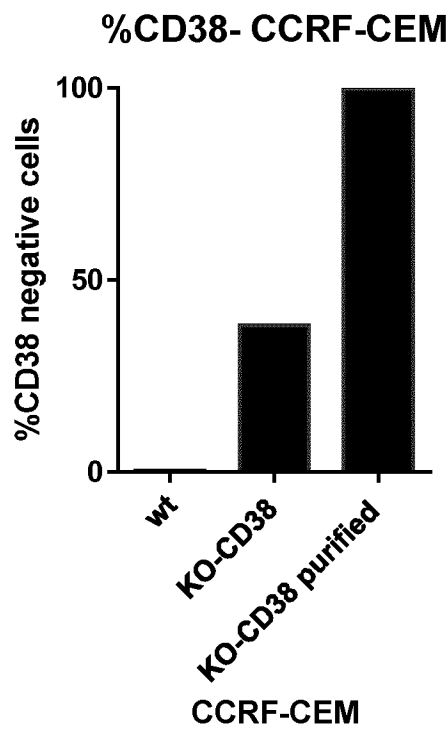
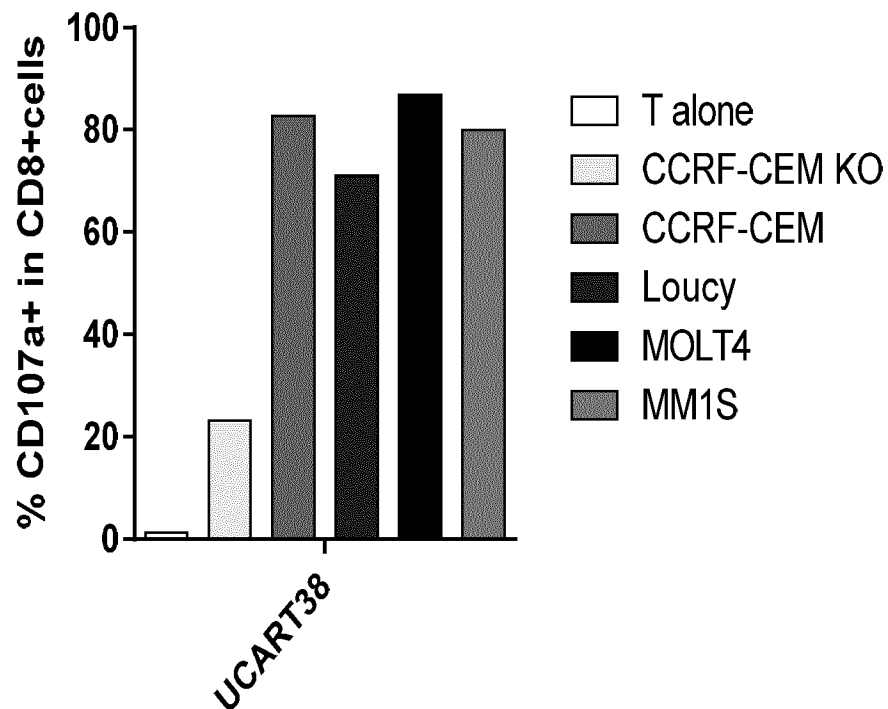
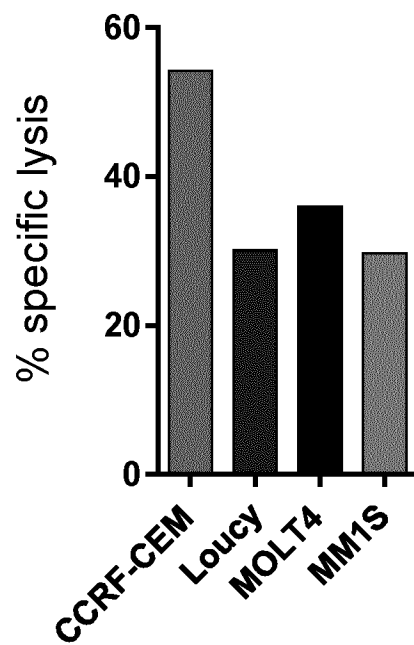


Figure 31

34/34**Figure 32A****Figure 32B**

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<151> 2015-08-11

<150> PCT/EP2016/051467

<151> 2016-01-25

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35 40 45Ala Gln His His Glu Ala Leu Val Gly His Gly Phe Thr His Ala His
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130 135 140

Glu Ala Val His Ala Trp Arg Asn Ala Leu Thr Gly Ala Pro Leu Asn
145 150 155 160

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
165 170 175

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180 185 190

His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Ile Gly
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Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val Leu Cys
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245 250 255

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260 265 270

Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
275 280 285

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
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Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
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Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
545 550 555 560

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
565 570 575

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
580 585 590

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
595 600 605

Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val
610 615 620

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
625 630 635 640

eo1f-seq1.txt

Gln Val Val Ala Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu
645 650 655

Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
660 665 670

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
675 680 685

Leu Glu Ser Ile Val Ala Gln Leu Ser Arg Pro Asp Pro Ala Leu Ala
690 695 700

Ala Leu Thr Asn Asp His Leu Val Ala Leu Ala Cys Leu Gly Gly Arg
705 710 715 720

Pro Ala Leu Asp Ala Val Lys Lys Gly Leu Gly Asp Pro Ile Ser Arg
725 730 735

Ser Gln Leu Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg
740 745 750

His Lys Leu Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile
755 760 765

Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu
770 775 780

Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser
785 790 795 800

Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr
805 810 815

Gly Val Ile Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro
820 825 830

Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr
835 840 845

Arg Asn Lys His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser
850 855 860

Ser Val Thr Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly
865 870 875 880

Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn
885 890 895

Gly Ala Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile
900 905 910

eo1f-seq1.txt

Lys Ala Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn
915 920 925

Gly Glu Ile Asn Phe Ala Ala Asp
930 935

<210> 3

<211> 942

<212> PRT

<213> artificial sequence

<220>

<223> CD38ex1-T2 Right TALEN

<400> 3

Met Gly Asp Pro Lys Lys Lys Arg Lys Val Ile Asp Lys Glu Thr Ala
1 5 10 15

Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Ile Asp Ile Ala Asp
20 25 30

Leu Arg Thr Leu Gly Tyr Ser Gln Gln Gln Gln Glu Lys Ile Lys Pro
35 40 45

Lys Val Arg Ser Thr Val Ala Gln His His Glu Ala Leu Val Gly His
50 55 60

Gly Phe Thr His Ala His Ile Val Ala Leu Ser Gln His Pro Ala Ala
65 70 75 80

Leu Gly Thr Val Ala Val Lys Tyr Gln Asp Met Ile Ala Ala Leu Pro
85 90 95

Glu Ala Thr His Glu Ala Ile Val Gly Val Gly Lys Gln Trp Ser Gly
100 105 110

Ala Arg Ala Leu Glu Ala Leu Leu Thr Val Ala Gly Glu Leu Arg Gly
115 120 125

Pro Pro Leu Gln Leu Asp Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg
130 135 140

Gly Gly Val Thr Ala Val Glu Ala Val His Ala Trp Arg Asn Ala Leu
145 150 155 160

Thr Gly Ala Pro Leu Asn Leu Thr Pro Gln Gln Val Val Ala Ile Ala
165 170 175

Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
180 185 190

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala

195

200

205

Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
 210 215 220

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
 225 230 235 240

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
 245 250 255

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
 260 265 270

Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu
 275 280 285

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
 290 295 300

Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala
 305 310 315 320

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
 325 330 335

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
 340 345 350

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
 355 360 365

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly
 370 375 380

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
 385 390 395 400

Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His
 405 410 415

Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
 420 425 430

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
 435 440 445

Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu
 450 455 460

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala

465 470 475 480

Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
485 490 495

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
500 505 510

Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val
515 520 525

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
530 535 540

Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu
545 550 555 560

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
565 570 575

Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala
580 585 590

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
595 600 605

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
610 615 620

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
625 630 635 640

His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly
645 650 655

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
660 665 670

Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
675 680 685

Gly Gly Gly Arg Pro Ala Leu Glu Ser Ile Val Ala Gln Leu Ser Arg
690 695 700

Pro Asp Pro Ala Leu Ala Ala Leu Thr Asn Asp His Leu Val Ala Leu
705 710 715 720

Ala Cys Leu Gly Gly Arg Pro Ala Leu Asp Ala Val Lys Lys Gly Leu
725 730 735

Gly Asp Pro Ile Ser Arg Ser Gln Leu Val Lys Ser Glu Leu Glu Glu
Page 7

740

745

750

Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr Val Pro His Glu Tyr
 755 760 765
 Ile Glu Leu Ile Glu Ile Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu
 770 775 780
 Glu Met Lys Val Met Glu Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly
 785 790 795 800
 Lys His Leu Gly Gly Ser Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val
 805 810 815
 Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp Thr Lys Ala Tyr Ser
 820 825 830
 Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr
 835 840 845
 Val Glu Glu Asn Gln Thr Arg Asn Lys His Ile Asn Pro Asn Glu Trp
 850 855 860
 Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe Lys Phe Leu Phe Val
 865 870 875 880
 Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn
 885 890 895
 His Ile Thr Asn Cys Asn Gly Ala Val Leu Ser Val Glu Glu Leu Leu
 900 905 910
 Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu Thr Leu Glu Glu Val
 915 920 925
 Arg Arg Lys Phe Asn Asn Gly Glu Ile Asn Phe Ala Ala Asp
 930 935 940

<210> 4

<211> 49

<212> DNA

<213> homo sapiens

<220>

<223> CD38-1 TALEN target

<400> 4

tgcgagttca gcccgggtgtc cggggacaaa ccctgctgcc ggctctcta

49

<210> 5

eo1f-seq1.txt

<211> 970
 <212> PRT
 <213> artificial sequence

<220>
 <223> CD38-1 Left TALEN

<400> 5
 Met Gly Asp Pro Lys Lys Lys Arg Lys Val Ile Asp Tyr Pro Tyr Asp
 1 5 10 15
 Val Pro Asp Tyr Ala Ile Asp Ile Ala Asp Leu Arg Thr Leu Gly Tyr
 20 25 30
 Ser Gln Gln Gln Gln Glu Lys Ile Lys Pro Lys Val Arg Ser Thr Val
 35 40 45
 Ala Gln His His Glu Ala Leu Val Gly His Gly Phe Thr His Ala His
 50 55 60
 Ile Val Ala Leu Ser Gln His Pro Ala Ala Leu Gly Thr Val Ala Val
 65 70 75 80
 Lys Tyr Gln Asp Met Ile Ala Ala Leu Pro Glu Ala Thr His Glu Ala
 85 90 95
 Ile Val Gly Val Gly Lys Gln Trp Ser Gly Ala Arg Ala Leu Glu Ala
 100 105 110
 Leu Leu Thr Val Ala Gly Glu Leu Arg Gly Pro Pro Leu Gln Leu Asp
 115 120 125
 Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg Gly Gly Val Thr Ala Val
 130 135 140
 Glu Ala Val His Ala Trp Arg Asn Ala Leu Thr Gly Ala Pro Leu Asn
 145 150 155 160
 Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys
 165 170 175
 Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
 180 185 190
 His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Asn Gly
 195 200 205
 Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val Leu Cys
 210 215 220
 Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser His
 225 230 235 240

eof-seq1.txt

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

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

Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
275 280 285

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
290 295 300

Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
305 310 315 320

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
325 330 335

Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val
340 345 350

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
355 360 365

Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu
370 375 380

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
385 390 395 400

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

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
420 425 430

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
435 440 445

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
450 455 460

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Ile Gly
465 470 475 480

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
485 490 495

Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
500 505 510

eof-seq1.txt

Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
515 520 525

Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala
530 535 540

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
545 550 555 560

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
565 570 575

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
580 585 590

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
595 600 605

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
610 615 620

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
625 630 635 640

Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu
645 650 655

Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
660 665 670

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala
675 680 685

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
690 695 700

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg
705 710 715 720

Pro Ala Leu Glu Ser Ile Val Ala Gln Leu Ser Arg Pro Asp Pro Ala
725 730 735

Leu Ala Ala Leu Thr Asn Asp His Leu Val Ala Leu Ala Cys Leu Gly
740 745 750

Gly Arg Pro Ala Leu Asp Ala Val Lys Lys Gly Leu Gly Asp Pro Ile
755 760 765

Ser Arg Ser Gln Leu Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu
770 775 780

eo1f-seq1.txt

Leu Arg His Lys Leu Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile
785 790 795 800

Glu Ile Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu Glu Met Lys Val
805 810 815

Met Glu Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly Lys His Leu Gly
820 825 830

Gly Ser Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile
835 840 845

Asp Tyr Gly Val Ile Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn
850 855 860

Leu Pro Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr Val Glu Glu Asn
865 870 875 880

Gln Thr Arg Asn Lys His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr
885 890 895

Pro Ser Ser Val Thr Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe
900 905 910

Lys Gly Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn
915 920 925

Cys Asn Gly Ala Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu
930 935 940

Met Ile Lys Ala Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe
945 950 955 960

Asn Asn Gly Glu Ile Asn Phe Ala Ala Asp
965 970

<210> 6

<211> 942

<212> PRT

<213> artificial sequence

<220>

<223> CD38-1_Right TALEN

<400> 6

Met Gly Asp Pro Lys Lys Lys Arg Lys Val Ile Asp Lys Glu Thr Ala
1 5 10 15

Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Ile Asp Ile Ala Asp
20 25 30

Leu Arg Thr Leu Gly Tyr Ser Gln Gln Gln Gln Glu Lys Ile Lys Pro
35 40 45

eo1f-seq1.txt

Lys Val Arg Ser Thr Val Ala Gln His His Glu Ala Leu Val Gly His
 50 55 60
 Gly Phe Thr His Ala His Ile Val Ala Leu Ser Gln His Pro Ala Ala
 65 70 75 80
 Leu Gly Thr Val Ala Val Lys Tyr Gln Asp Met Ile Ala Ala Leu Pro
 85 90 95
 Glu Ala Thr His Glu Ala Ile Val Gly Val Gly Lys Gln Trp Ser Gly
 100 105 110
 Ala Arg Ala Leu Glu Ala Leu Leu Thr Val Ala Gly Glu Leu Arg Gly
 115 120 125
 Pro Pro Leu Gln Leu Asp Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg
 130 135 140
 Gly Gly Val Thr Ala Val Glu Ala Val His Ala Trp Arg Asn Ala Leu
 145 150 155 160
 Thr Gly Ala Pro Leu Asn Leu Thr Pro Gln Gln Val Val Ala Ile Ala
 165 170 175
 Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
 180 185 190
 Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
 195 200 205
 Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
 210 215 220
 Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
 225 230 235 240
 Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
 245 250 255
 Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
 260 265 270
 Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu
 275 280 285
 Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
 290 295 300
 Pro Glu Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala
 305 310 315 320

eo1f-seq1.txt

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
 325 330 335
 Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
 340 345 350
 Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
 355 360 365
 His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser His Asp Gly
 370 375 380
 Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
 385 390 395 400
 Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn
 405 410 415
 Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
 420 425 430
 Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
 435 440 445
 Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu
 450 455 460
 Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
 465 470 475 480
 Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
 485 490 495
 Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
 500 505 510
 Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val
 515 520 525
 Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
 530 535 540
 Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu
 545 550 555 560
 Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
 565 570 575
 Pro Glu Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala
 580 585 590

eo1f-seq1.txt

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
 595 600 605
 Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
 610 615 620
 Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
 625 630 635 640
 His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Gly Gly
 645 650 655
 Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
 660 665 670
 Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
 675 680 685
 Ile Gly Gly Arg Pro Ala Leu Glu Ser Ile Val Ala Gln Leu Ser Arg
 690 695 700
 Pro Asp Pro Ala Leu Ala Ala Leu Thr Asn Asp His Leu Val Ala Leu
 705 710 715 720
 Ala Cys Leu Gly Gly Arg Pro Ala Leu Asp Ala Val Lys Lys Gly Leu
 725 730 735
 Gly Asp Pro Ile Ser Arg Ser Gln Leu Val Lys Ser Glu Leu Glu Glu
 740 745 750
 Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr Val Pro His Glu Tyr
 755 760 765
 Ile Glu Leu Ile Glu Ile Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu
 770 775 780
 Glu Met Lys Val Met Glu Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly
 785 790 795 800
 Lys His Leu Gly Gly Ser Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val
 805 810 815
 Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp Thr Lys Ala Tyr Ser
 820 825 830
 Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr
 835 840 845
 Val Glu Glu Asn Gln Thr Arg Asn Lys His Ile Asn Pro Asn Glu Trp
 850 855 860

eo1f-seq1.txt

Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe Lys Phe Leu Phe Val
865 870 875 880

Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn
885 890 895

His Ile Thr Asn Cys Asn Gly Ala Val Leu Ser Val Glu Glu Leu Leu
900 905 910

Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu Thr Leu Glu Glu Val
915 920 925

Arg Arg Lys Phe Asn Asn Gly Glu Ile Asn Phe Ala Ala Asp
930 935 940

<210> 7

<211> 49

<212> PRT

<213> homo sapiens

<220>

<223> CD38-2_TALEN target

<400> 7

Thr Gly Ala Thr Cys Cys Thr Cys Gly Thr Cys Gly Thr Gly Gly Thr
1 5 10 15

Gly Cys Thr Cys Gly Cys Gly Gly Thr Gly Gly Thr Cys Gly Thr Cys
20 25 30

Cys Cys Gly Ala Gly Gly Thr Gly Gly Cys Gly Cys Cys Ala Gly Cys
35 40 45

Ala

<210> 8

<211> 936

<212> PRT

<213> artificial sequence

<220>

<223> CD38-2 Left TALEN

<400> 8

Met Gly Asp Pro Lys Lys Lys Arg Lys Val Ile Asp Tyr Pro Tyr Asp
1 5 10 15

Val Pro Asp Tyr Ala Ile Asp Ile Ala Asp Leu Arg Thr Leu Gly Tyr
20 25 30

Ser Gln Gln Gln Gln Glu Lys Ile Lys Pro Lys Val Arg Ser Thr Val
35 40 45

eo1f-seq1.txt

Ala Gln His His Glu Ala Leu Val Gly His Gly Phe Thr His Ala His
50 55 60

Ile Val Ala Leu Ser Gln His Pro Ala Ala Leu Gly Thr Val Ala Val
65 70 75 80

Lys Tyr Gln Asp Met Ile Ala Ala Leu Pro Glu Ala Thr His Glu Ala
85 90 95

Ile Val Gly Val Gly Lys Gln Trp Ser Gly Ala Arg Ala Leu Glu Ala
100 105 110

Leu Leu Thr Val Ala Gly Glu Leu Arg Gly Pro Pro Leu Gln Leu Asp
115 120 125

Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg Gly Gly Val Thr Ala Val
130 135 140

Glu Ala Val His Ala Trp Arg Asn Ala Leu Thr Gly Ala Pro Leu Asn
145 150 155 160

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys
165 170 175

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
180 185 190

His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Asn Gly
195 200 205

Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val Leu Cys
210 215 220

Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
225 230 235 240

Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
245 250 255

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

Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
275 280 285

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
290 295 300

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
305 310 315 320

eo1f-seq1.txt

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
 325 330 335
 Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
 340 345 350
 Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
 355 360 365
 Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu
 370 375 380
 Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
 385 390 395 400
 Pro Gln Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala
 405 410 415
 Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
 420 425 430
 Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
 435 440 445
 Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
 450 455 460
 His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly
 465 470 475 480
 Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
 485 490 495
 Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser His
 500 505 510
 Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
 515 520 525
 Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala
 530 535 540
 Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
 545 550 555 560
 Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
 565 570 575
 Ile Ala Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
 580 585 590

eo1f-seq1.txt

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
 595 600 605
 Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val
 610 615 620
 Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
 625 630 635 640
 Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu
 645 650 655
 Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
 660 665 670
 Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
 675 680 685
 Leu Glu Ser Ile Val Ala Gln Leu Ser Arg Pro Asp Pro Ala Leu Ala
 690 695 700
 Ala Leu Thr Asn Asp His Leu Val Ala Leu Ala Cys Leu Gly Gly Arg
 705 710 715 720
 Pro Ala Leu Asp Ala Val Lys Lys Gly Leu Gly Asp Pro Ile Ser Arg
 725 730 735
 Ser Gln Leu Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg
 740 745 750
 His Lys Leu Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile
 755 760 765
 Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu
 770 775 780
 Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser
 785 790 795 800
 Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr
 805 810 815
 Gly Val Ile Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro
 820 825 830
 Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr
 835 840 845
 Arg Asn Lys His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser
 850 855 860

eo1f-seq1.txt

Ser Val Thr Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly
865 870 875 880

Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn
885 890 895

Gly Ala Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile
900 905 910

Lys Ala Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn
915 920 925

Gly Glu Ile Asn Phe Ala Ala Asp
930 935

<210> 9

<211> 942

<212> PRT

<213> artificial sequence

<220>

<223> CD38-2 Right TALEN

<400> 9

Met Gly Asp Pro Lys Lys Lys Arg Lys Val Ile Asp Lys Glu Thr Ala
1 5 10 15

Ala Ala Lys Phe Glu Arg Gln His Met Asp Ser Ile Asp Ile Ala Asp
20 25 30

Leu Arg Thr Leu Gly Tyr Ser Gln Gln Gln Gln Glu Lys Ile Lys Pro
35 40 45

Lys Val Arg Ser Thr Val Ala Gln His His Glu Ala Leu Val Gly His
50 55 60

Gly Phe Thr His Ala His Ile Val Ala Leu Ser Gln His Pro Ala Ala
65 70 75 80

Leu Gly Thr Val Ala Val Lys Tyr Gln Asp Met Ile Ala Ala Leu Pro
85 90 95

Glu Ala Thr His Glu Ala Ile Val Gly Val Gly Lys Gln Trp Ser Gly
100 105 110

Ala Arg Ala Leu Glu Ala Leu Leu Thr Val Ala Gly Glu Leu Arg Gly
115 120 125

Pro Pro Leu Gln Leu Asp Thr Gly Gln Leu Leu Lys Ile Ala Lys Arg
130 135 140

eof-seq1.txt

Gly 145 Gly Val Thr Ala Val 150 Glu Ala Val His Ala 155 Trp Arg Asn Ala Leu 160
 Thr Gly Ala Pro Leu 165 Asn Leu Thr Pro Gln 170 Gln Val Val Ala Ile 175 Ala
 Ser His Asp Gly 180 Gly Lys Gln Ala Leu 185 Glu Thr Val Gln Arg 190 Leu Leu
 Pro Val Leu 195 Cys Gln Ala His Gly 200 Leu Thr Pro Gln 205 Gln Val Val Ala
 Ile 210 Ala Ser Asn Asn Gly 215 Gly Lys Gln Ala Leu Glu 220 Thr Val Gln Arg
 Leu 225 Leu Pro Val Leu Cys 230 Gln Ala His Gly Leu 235 Thr Pro Glu Gln Val 240
 Val Ala Ile Ala Ser 245 Asn Ile Gly Gly Lys 250 Gln Ala Leu Glu Thr Val 255
 Gln Arg Leu Leu 260 Pro Val Leu Cys Gln 265 Ala His Gly Leu Thr 270 Pro Glu
 Gln Val Val 275 Ala Ile Ala Ser Asn 280 Asn Gly Gly Lys Gln 285 Ala Leu Glu
 Thr Val 290 Gln Arg Leu Leu Pro 295 Val Leu Cys Gln Ala 300 His Gly Leu Thr
 Pro 305 Glu Gln Val Val Ala 310 Ile Ala Ser Asn 315 Asn Gly Gly Lys Gln Ala 320
 Leu Glu Thr Val Gln 325 Arg Leu Leu Pro Val 330 Leu Cys Gln Ala His Gly 335
 Leu Thr Pro Glu 340 Gln Val Val Ala Ile 345 Ala Ser Asn Gly Gly 350 Gly Lys
 Gln Ala Leu 355 Glu Thr Val Gln Arg Leu Leu Pro Val Leu 365 Cys Gln Ala
 His Gly 370 Leu Thr Pro Gln Gln 375 Val Val Ala Ile Ala 380 Ser Asn Asn Gly
 Gly 385 Lys Gln Ala Leu Glu 390 Thr Val Gln Arg Leu 395 Leu Pro Val Leu Cys 400
 Gln Ala His Gly 405 Leu Thr Pro Glu Gln Val 410 Val Ala Ile Ala Ser 415 Asn

eof-seq1.txt

Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
420 425 430

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
435 440 445

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu
450 455 460

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
465 470 475 480

Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
485 490 495

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val
500 505 510

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
515 520 525

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
530 535 540

Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu
545 550 555 560

Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr
565 570 575

Pro Glu Gln Val Val Ala Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala
580 585 590

Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly
595 600 605

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
610 615 620

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
625 630 635 640

His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly
645 650 655

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
660 665 670

Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn
675 680 685

eof-seq1.txt

Ile Gly Gly Arg Pro Ala Leu Glu Ser Ile Val Ala Gln Leu Ser Arg
690 695 700

Pro Asp Pro Ala Leu Ala Ala Leu Thr Asn Asp His Leu Val Ala Leu
705 710 715 720

Ala Cys Leu Gly Gly Arg Pro Ala Leu Asp Ala Val Lys Lys Gly Leu
725 730 735

Gly Asp Pro Ile Ser Arg Ser Gln Leu Val Lys Ser Glu Leu Glu Glu
740 745 750

Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr Val Pro His Glu Tyr
755 760 765

Ile Glu Leu Ile Glu Ile Ala Arg Asn Ser Thr Gln Asp Arg Ile Leu
770 775 780

Glu Met Lys Val Met Glu Phe Phe Met Lys Val Tyr Gly Tyr Arg Gly
785 790 795 800

Lys His Leu Gly Gly Ser Arg Lys Pro Asp Gly Ala Ile Tyr Thr Val
805 810 815

Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp Thr Lys Ala Tyr Ser
820 825 830

Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp Glu Met Gln Arg Tyr
835 840 845

Val Glu Glu Asn Gln Thr Arg Asn Lys His Ile Asn Pro Asn Glu Trp
850 855 860

Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe Lys Phe Leu Phe Val
865 870 875 880

Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln Leu Thr Arg Leu Asn
885 890 895

His Ile Thr Asn Cys Asn Gly Ala Val Leu Ser Val Glu Glu Leu Leu
900 905 910

Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu Thr Leu Glu Glu Val
915 920 925

Arg Arg Lys Phe Asn Asn Gly Glu Ile Asn Phe Ala Ala Asp
930 935 940

<210> 10

<211> 113

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 25A10

<400> 10

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

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Gly Asn Gln Arg Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro 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 Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp Tyr Asp Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 11

<211> 12

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 25A10

<400> 11

Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
1 5 10

<210> 12

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 25A10

<400> 12

Trp Ala Ser
1

<210> 13

eo1f-seq1.txt

<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> CDR3 of VL chain for 25A10

<400> 13
Gln Asn Asp Tyr Asp Tyr Pro Tyr Thr
1 5

<210> 14

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

<220>
<223> VH chain for 25A10

<400> 14
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15

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

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

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

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

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

Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser
115

<210> 15

<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> CDR1 of VH chain for 25A10

<400> 15
Gly Phe Asn Ile Lys Asp Ser Leu
1 5

<210> 16

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 25A10

<400> 16

Ile Asp Pro Glu Asp Asp Lys Thr
1 5

<210> 17

<211> 11

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 25A10

<400> 17

Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr
1 5 10

<210> 18

<211> 107

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 29B4

<400> 18

Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15Asp Arg Val Asn Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala
20 25 30Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
35 40 45Tyr Trp Ala Ser Thr Arg His Ala Gly Val Pro Asp Arg Phe Thr Gly
50 55 60Ser Gly Ser Gly Thr Asp Tyr Ala Leu Thr Ile Ser Ser Val Gln Ala
65 70 75 80Glu Asp Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg
85 90 95Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 19

<211> 6
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDR1 of VL chain for 29B4

<400> 19
 Gln Asp Val Asn Thr Ala
 1 5

<210> 20

<211> 3
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDR2 of VL chain for 29B4

<400> 20
 Trp Ala Ser
 1

<210> 21

<211> 9
 <212> PRT
 <213> artificial sequence

<220>
 <223> CDR3 of VL chain for 29B4

<400> 21
 Gln Gln His Tyr Ser Thr Pro Arg Thr
 1 5

<210> 22

<211> 122
 <212> PRT
 <213> artificial sequence

<220>
 <223> VH chain for 29B4

<400> 22
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

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

Phe Met Tyr Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45

Ala Ile Ile Ser Asp Gly Gly Ile Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Asn Leu Tyr
 65 70 75 80

eo1f-seq1.txt

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

Ala Arg Asp Gly Arg Asp Asp Tyr Asp Gly Trp Tyr Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 23

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 29B4

<400> 23

Gly Phe Thr Phe Ser Asp Tyr Phe
1 5

<210> 24

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 29B4

<400> 24

Ile Ser Asp Gly Gly Ile Tyr Thr
1 5

<210> 25

<211> 15

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 29B4

<400> 25

Ala Arg Asp Gly Arg Asp Asp Tyr Asp Gly Trp Tyr Phe Asp Val
1 5 10 15

<210> 26

<211> 111

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 16B5

<400> 26

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

eo1f-seq1.txt

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

Gly Thr Thr Phe Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

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

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

Pro Val Glu Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Lys
85 90 95

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

<210> 27

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 16B5

<400> 27

Glu Ser Val Asp Asn Tyr Gly Thr Thr Phe
1 5 10

<210> 28

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 16B5

<400> 28

Leu Ala Ser
1

<210> 29

<211> 9

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VL chain for 16B5

<400> 29

Gln Gln Asn Lys Glu Asp Pro Trp Thr
1 5

eo1f-seq1.txt

<210> 30

<211> 121

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for 16B5

<400> 30

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser 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

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

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

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

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

Ala Arg Gly Gly Ile Tyr Tyr Tyr Gly Ser Ser Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Thr Leu Thr Val Ser Ser
115 120

<210> 31

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 16B5

<400> 31

Gly Tyr Thr Phe Thr Ser Tyr Asn
1 5

<210> 32

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 16B5

<400> 32

Ile Tyr Pro Gly Asn Gly Gly Thr
1 5

eo1f-seq1.txt

<210> 33

<211> 14

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 16B5

<400> 33

Ala Arg Gly Gly Ile Tyr Tyr Tyr Gly Ser Ser Leu Asp Tyr
1 5 10

<210> 34

<211> 111

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 10F7

<400> 34

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

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

Gly Asn Thr Phe Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro
35 40 45

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

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

Pro Val Glu Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Asn
85 90 95

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

<210> 35

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 10F7

<400> 35

Glu Ser Val Asp Ser Tyr Gly Asn Thr Phe
1 5 10

<210> 36

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 10F7

<400> 36

Leu Ala Ser

1

<210> 37

<211> 9

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VL chain for 10F7

<400> 37

Gln Gln Asn Asn Glu Asp Pro Trp Thr

1

5

<210> 38

<211> 120

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for 10F7

<400> 38

Gln Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser 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

Asn Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile

35

40

45

Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn Tyr Asn Gln Lys Phe

50

55

60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser Ser Ser Thr Ala Tyr

65

70

75

80

Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys

85

90

95

Ala Arg Gly Gly Gln Leu Gly Arg Pro Trp Phe Ala Tyr Trp Gly Gln

100

105

110

Gly Thr Leu Val Thr Val Ser Ala

115

120

<210> 39

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 10F7

<400> 39

Gly Tyr Thr Phe Thr Ser Tyr Asn
1 5

<210> 40

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 10F7

<400> 40

Ile Tyr Pro Gly Asn Gly Gly Thr
1 5

<210> 41

<211> 13

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 10F7

<400> 41

Ala Arg Gly Gly Gln Leu Gly Arg Pro Trp Phe Ala Tyr
1 5 10

<210> 42

<211> 106

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 27B6

<400> 42

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

Glu Lys Val Thr Met Thr Cys Ser Thr Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

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

eo1f-seq1.txt

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

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

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 43

<211> 5

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 27B6

<400> 43

Ser Ser Val Ser Tyr
1 5

<210> 44

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 27B6

<400> 44

Asp Thr Ser
1

<210> 45

<211> 9

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VL chain for 27B6

<400> 45

Gln Gln Trp Ser Ser Tyr Pro Pro Thr
1 5

<210> 46

<211> 122

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for 27B6

<400> 46

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

eo1f-seq1.txt

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

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

Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr Asn Ala Ala Phe Ile
50 55 60

Ser Arg Leu Lys Ile Ser Lys Asp Asp Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Ser Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Arg His Ser Pro Leu Val Ser Thr Pro Asp Trp Tyr Phe Asp Val Trp
100 105 110

Gly Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 47

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 27B6

<400> 47

Gly Phe Ser Leu Thr Ser Tyr Asn
1 5

<210> 48

<211> 7

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 27B6

<400> 48

Ile Trp Ser Gly Gly Ser Thr
1 5

<210> 49

<211> 16

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 27B6

<400> 49

eo1f-seq1.txt

Ala Arg His Ser Pro Leu Val Ser Thr Pro Asp Trp Tyr Phe Asp Val
1 5 10 15

<210> 50

<211> 107

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 13F11

<400> 50

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

Glu Lys Val Thr Ile Arg Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp
20 25 30

Met Asn Trp Tyr Gln Gln Lys Pro Gly Glu Pro Pro Lys Val Leu Ile
35 40 45

Ser Glu Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser
50 55 60

Ser Gly Tyr Gly Thr Asp Phe Val Phe Thr Ile Glu Asn Thr Leu Ser
65 70 75 80

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

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 51

<211> 6

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 13F11

<400> 51

Thr Asp Ile Asp Asp Asp
1 5

<210> 52

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 13F11

<400> 52

Glu Gly Asn
1

eo1f-seq1.txt

<210> 53

<211> 9

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VL chain for 13F11

<400> 53

Leu Gln Ser Asn Asn Met Pro Tyr Thr
1 5

<210> 54

<211> 123

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for 13F11

<400> 54

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

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

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu Glu Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

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

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 55

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 13F11

<400> 55

Gly Tyr Thr Phe Lys Lys Tyr Gly
1 5

<210> 56

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 13F11

<400> 56

Ile Asn Thr Asn Thr Gly Glu Pro
1 5

<210> 57

<211> 16

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 13F11

<400> 57

Ala Arg Trp Tyr Tyr Gly Ser Thr Pro Ser Ser Tyr Thr Met Asp Tyr
1 5 10 15

<210> 58

<211> 108

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for 28F5

<400> 58

Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Thr Ser Ser Leu Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp
35 40 45

Ile Tyr Ser Thr Ser His Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
50 55 60

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

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Leu Ser Pro
85 90 95

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

eo1f-seq1.txt

<210> 59

<211> 7

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for 28F5

<400> 59

Ser Ser Leu Ser Ser Ser Tyr
1 5

<210> 60

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for 28F5

<400> 60

Ser Thr Ser
1

<210> 61

<211> 9

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VL chain for 28F5

<400> 61

His Gln Tyr His Leu Ser Pro Tyr Thr
1 5

<210> 62

<211> 126

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for 28F5

<400> 62

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

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

Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

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

eo1f-seq1.txt

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu Val Ser Tyr Tyr Ala
100 105 110

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120 125

<210> 63

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for 28F5

<400> 63

Gly Tyr Thr Phe Thr Lys Tyr Gly
1 5

<210> 64

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for 28F5

<400> 64

Ile Asn Thr Asn Ser Gly Glu Pro
1 5

<210> 65

<211> 19

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for 28F5

<400> 65

Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu Val Ser Tyr Tyr Ala
1 5 10 15

Met Asp Tyr

<210> 66

<211> 107

<212> PRT

<213> artificial sequence

<220>

<223> VL chain for GMB005

<400> 66

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

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
20 25 30

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

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 67

<211> 6

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VL chain for GMB005

<400> 67

Gln Ser Val Ser Ser Tyr
1 5

<210> 68

<211> 3

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VL chain for GMB005

<400> 68

Asp Ala Ser
1

<210> 69

<211> 9

<212> PRT

<213> artificial sequence

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<220>

<223> CDR3 of VL chain for GMB005

<400> 69

Gln Gln Arg Ser Asn Trp Pro Pro Thr
1 5

<210> 70

<211> 124

<212> PRT

<213> artificial sequence

<220>

<223> VH chain for GMB005

<400> 70

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

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Asn Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val
50 55 60

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

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

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser
115 120

<210> 71

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR1 of VH chain for GMB005

<400> 71

Gly Phe Thr Phe Asn Ser Phe Ala
1 5

<210> 72

<211> 8

<212> PRT

<213> artificial sequence

<220>

<223> CDR2 of VH chain for GMB005

<400> 72

Ile Ser Gly Ser Gly Gly Gly Thr
1 5

<210> 73

<211> 15

<212> PRT

<213> artificial sequence

<220>

<223> CDR3 of VH chain for GMB005

<400> 73

Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro Val Phe Asp Tyr
1 5 10 15

<210> 74

<211> 21

<212> PRT

<213> artificial sequence

<220>

<223> CD8a;-Signal peptide (SP)

<400> 74

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
20

<210> 75

<211> 15

<212> PRT

<213> artificial sequence

<220>

<223> GS linker

<400> 75

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

<210> 76

<211> 16

<212> PRT

<213> homo sapiens

<220>

<223> FCRIIIa; hinge

<400> 76

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln
1 5 10 15

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<210> 77

<211> 45

<212> PRT

<213> homo sapiens

<220>

<223> CD8a; hinge

<400> 77

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
35 40 45

<210> 78

<211> 231

<212> PRT

<213> homo sapiens

<220>

<223> IgG1 hinge

<400> 78

Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
20 25 30

Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val
35 40 45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
50 55 60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65 70 75 80

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
85 90 95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
100 105 110

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
115 120 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
130 135 140

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Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145 150 155 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
165 170 175

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
180 185 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
195 200 205

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
210 215 220

Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 79

<211> 24

<212> PRT

<213> homo sapiens

<220>

<223> CD8a; TM domain

<400> 79

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> 80

<211> 42

<212> PRT

<213> homo sapiens

<220>

<223> 4-1 BB co-stimulatory domain

<400> 80

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> 81

eo1f-seq1.txt

<211> 112
<212> PRT
<213> homo sapiens

<220>
<223> CD3ζ activation domain

<400> 81
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> 82
<211> 461
<212> PRT
<213> artificial sequence

<220>
<223> 25A10-V1 CAR

<400> 82
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 Ala Glu Leu
20 25 30

Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
35 40 45

Asn Ile Lys Asp Ser Leu Ile His Trp Val Lys Gln Arg Pro Glu Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Asp Lys Thr Lys
65 70 75 80

Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
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Ser Asn Thr Ala Tyr Leu Gln Leu Ser Thr Leu Thr Ser Glu Asp Thr
 100 105 110
 Ala Ile Tyr Tyr Cys Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr
 115 120 125
 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
 130 135 140
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln
 145 150 155 160
 Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser
 165 170 175
 Cys Lys Ser Ser Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
 180 185 190
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro 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 Ala
 225 230 235 240
 Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr
 245 250 255
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 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> 83

<211> 490

<212> PRT

<213> artificial sequence

<220>

<223> 25A10-V2 CAR

<400> 83

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 Ala Glu Leu
 20 25 30

Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 35 40 45

Asn Ile Lys Asp Ser Leu Ile His Trp Val Lys Gln Arg Pro Glu Gln
 50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Asp Lys Thr Lys
 65 70 75 80

Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
 85 90 95

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Thr Leu Thr Ser Glu Asp Thr
 100 105 110

Ala Ile Tyr Tyr Cys Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr

115

120

125

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
 130 135 140
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln
 145 150 155 160
 Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser
 165 170 175
 Cys Lys Ser Ser Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
 180 185 190
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro 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 Ala
 225 230 235 240
 Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr
 245 250 255
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 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

eo1f-seq1.txt

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> 84

<211> 676

<212> PRT

<213> artificial sequence

<220>

<223> 25A10-V3 CAR

<400> 84

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 Ala Glu Leu
20 25 30

Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
35 40 45

Asn Ile Lys Asp Ser Leu Ile His Trp Val Lys Gln Arg Pro Glu Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Asp Lys Thr Lys
65 70 75 80

Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Thr Leu Thr Ser Glu Asp Thr
100 105 110

Ala Ile Tyr Tyr Cys Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr
115 120 125

eo1f-seq1.txt

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln
145 150 155 160

Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
180 185 190

Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro 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 Ala
225 230 235 240

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 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

eo1f-seq1.txt

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

eo1f-seq1.txt

Leu Pro Pro Arg
675

<210> 85

<211> 459

<212> PRT

<213> artificial sequence

<220>

<223> 29B4-V1 CAR

<400> 85

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 Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe
35 40 45

Thr Phe Ser Asp Tyr Phe Met Tyr Trp Val Arg Gln Thr Pro Glu Lys
50 55 60

Arg Leu Glu Trp Val Ala Ile Ile Ser Asp Gly Gly Ile Tyr Thr Tyr
65 70 75 80

Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
85 90 95

Lys Asn Asn Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr
100 105 110

Ala Met Tyr Tyr Cys Ala Arg Asp Gly Arg Asp Asp Tyr Asp Gly Trp
115 120 125

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
145 150 155 160

Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly Asp Arg
165 170 175

Val Asn Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Val Ala
180 185 190

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp
195 200 205

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

210

215

Ser Gly Thr Asp Tyr Ala Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
225 230 235 240

Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg Thr Phe
245 250 255

Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile
260 265 270

Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu
275 280 285

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

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

Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
325 330 335

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

Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr
355 360 365

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
370 375 380

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
385 390 395 400

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

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

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

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
450 455

<210> 86

<211> 488

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> 29B4-V2 CAR

<400> 86

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 Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe
35 40 45

Thr Phe Ser Asp Tyr Phe Met Tyr Trp Val Arg Gln Thr Pro Glu Lys
50 55 60

Arg Leu Glu Trp Val Ala Ile Ile Ser Asp Gly Gly Ile Tyr Thr Tyr
65 70 75 80

Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
85 90 95

Lys Asn Asn Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr
100 105 110

Ala Met Tyr Tyr Cys Ala Arg Asp Gly Arg Asp Asp Tyr Asp Gly Trp
115 120 125

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Asp Ile
145 150 155 160

Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly Asp Arg
165 170 175

Val Asn Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Val Ala
180 185 190

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp
195 200 205

Ala Ser Thr Arg His Ala Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
210 215 220

Ser Gly Thr Asp Tyr Ala Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
225 230 235 240

Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg Thr Phe
245 250 255

eo1f-seq1.txt

Gly Gly Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg
260 265 270

Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg
275 280 285

Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly
290 295 300

Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr
305 310 315 320

Cys Gly Val Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg
325 330 335

Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro
340 345 350

Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu
355 360 365

Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala
370 375 380

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
385 390 395 400

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
405 410 415

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
420 425 430

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
435 440 445

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
450 455 460

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
465 470 475 480

His Met Gln Ala Leu Pro Pro Arg
485

<210> 87

<211> 674

<212> PRT

<213> artificial sequence

<220>

eo1f-seq1.txt

<223> 29B4-V3 CAR

<400> 87

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 Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe
35 40 45

Thr Phe Ser Asp Tyr Phe Met Tyr Trp Val Arg Gln Thr Pro Glu Lys
50 55 60

Arg Leu Glu Trp Val Ala Ile Ile Ser Asp Gly Gly Ile Tyr Thr Tyr
65 70 75 80

Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
85 90 95

Lys Asn Asn Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr
100 105 110

Ala Met Tyr Tyr Cys Ala Arg Asp Gly Arg Asp Asp Tyr Asp Gly Trp
115 120 125

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
145 150 155 160

Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly Asp Arg
165 170 175

Val Asn Ile Thr Cys Lys Ala Ser Gln Asp Val Asn Thr Ala Val Ala
180 185 190

Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp
195 200 205

Ala Ser Thr Arg His Ala Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
210 215 220

Ser Gly Thr Asp Tyr Ala Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
225 230 235 240

Leu Ala Leu Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Arg Thr Phe
245 250 255

Gly Gly Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp Lys

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
275 280 285

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg
290 295 300

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
305 310 315 320

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
325 330 335

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
340 345 350

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
355 360 365

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
370 375 380

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
385 390 395 400

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
405 410 415

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
420 425 430

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
435 440 445

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
450 455 460

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
465 470 475 480

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490 495

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
500 505 510

Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu
515 520 525

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu

eo1f-seq1.txt
540

530

535

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
545 550 555 560

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
565 570 575

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
580 585 590

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
595 600 605

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
610 615 620

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
625 630 635 640

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
645 650 655

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
660 665 670

Pro Arg

<210> 88

<211> 462

<212> PRT

<213> artificial sequence

<220>

<223> 16B5-V1 CAR

<400> 88

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Leu His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

eo1f-seq1.txt

Tyr Asn Gln Lys Phe₈₅ Lys Gly Lys Ala Thr₉₀ Leu Thr Ala Asp Thr₉₅ Ser
 Ser Ser Thr Ala₁₀₀ Tyr Met Gln Ile Ser₁₀₅ Ser Leu Thr Ser Glu₁₁₀ Asp Ser
 Ala Val Tyr₁₁₅ Phe Cys Ala Arg Gly₁₂₀ Gly Ile Tyr Tyr Tyr₁₂₅ Gly Ser Ser
 Leu Asp₁₃₀ Tyr Trp Gly Gln Gly₁₃₅ Thr Thr Leu Thr Val₁₄₀ Ser Ser Gly Gly
 Gly₁₄₅ Gly Ser Gly Gly Gly₁₅₀ Gly Ser Gly Gly Gly₁₅₅ Gly Ser Asn Ile Val₁₆₀
 Leu Thr Gln Ser Pro₁₆₅ Ala Ser Leu Ala Val₁₇₀ Ser Leu Gly Gln Arg₁₇₅ Ala
 Thr Ile Ser Cys₁₈₀ Arg Ala Ser Glu Ser₁₈₅ Val Asp Asn Tyr Gly₁₉₀ Thr Thr
 Phe Met Tyr₁₉₅ Trp Tyr Gln Gln Lys₂₀₀ Pro Gly Gln Pro Pro₂₀₅ Lys Leu Leu
 Ile Tyr₂₁₀ Leu Ala Ser Asn Leu₂₁₅ Glu Ser Gly Val Pro₂₂₀ Ala Arg Phe Ser
 Gly₂₂₅ Ser Gly Ser Gly Thr₂₃₀ Asp Phe Thr Leu Thr₂₃₅ Ile Asp Pro Val Glu₂₄₀
 Ala Asp Asp Ala Ala₂₄₅ Thr Tyr Tyr Cys Gly₂₅₀ Gln Asn Lys Glu Asp₂₅₅ Pro
 Trp Thr Phe Gly₂₆₀ Gly Gly Thr Lys Leu₂₆₅ Glu Ile Lys Gly Leu₂₇₀ Ala Val
 Ser Thr Ile₂₇₅ Ser Ser Phe Phe Pro₂₈₀ Pro Gly Tyr Gln Ile₂₈₅ Tyr Ile Trp
 Ala Pro₂₉₀ Leu Ala Gly Thr Cys₂₉₅ Gly Val Leu Leu Leu₃₀₀ Ser Leu Val Ile
 Thr₃₀₅ Leu Tyr Cys Lys Arg₃₁₀ Gly Arg Lys Lys Leu₃₁₅ Leu Tyr Ile Phe Lys₃₂₀
 Gln Pro Phe Met Arg₃₂₅ Pro Val Gln Thr Thr₃₃₀ Gln Glu Glu Asp Gly₃₃₅ Cys
 Ser Cys Arg Phe₃₄₀ Pro Glu Glu Glu Glu₃₄₅ Gly Gly Cys Glu Leu₃₅₀ Arg Val

eo1f-seq1.txt

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

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

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

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

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

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

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

<210> 89

<211> 491

<212> PRT

<213> artificial sequence

<220>

<223> 16B5-V2 CAR

<400> 89

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Leu His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Ser Thr Ala Tyr Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Phe Cys Ala Arg Gly Gly Ile Tyr Tyr Tyr Gly Ser Ser

115

120

125

Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly
 130 135 140
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val
 145 150 155 160
 Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala
 165 170 175
 Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr Gly Thr Thr
 180 185 190
 Phe Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu
 195 200 205
 Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser
 210 215 220
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu
 225 230 235 240
 Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Lys Glu Asp Pro
 245 250 255
 Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro
 260 265 270
 Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
 275 280 285
 Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
 290 295 300
 Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
 305 310 315 320
 Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
 325 330 335
 Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
 340 345 350
 Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
 355 360 365
 Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
 370 375 380
 Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr

eo1f-seq1.txt

385 390 395 400

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
405 410 415

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
420 425 430

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
435 440 445

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
450 455 460

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
465 470 475 480

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 90

<211> 677

<212> PRT

<213> artificial sequence

<220>

<223> 16B5-V3 CAR

<400> 90

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Leu His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Ser Thr Ala Tyr Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Phe Cys Ala Arg Gly Gly Ile Tyr Tyr Tyr Gly Ser Ser
115 120 125

eo1f-seq1.txt

Leu Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly
 130 135 140
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val
 145 150 155 160
 Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala
 165 170 175
 Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr Gly Thr Thr
 180 185 190
 Phe Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu
 195 200 205
 Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser
 210 215 220
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu
 225 230 235 240
 Ala Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Lys Glu Asp Pro
 245 250 255
 Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser
 260 265 270
 Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
 275 280 285
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 290 295 300
 Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 305 310 315 320
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 325 330 335
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 340 345 350
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 355 360 365
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
 370 375 380
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 385 390 395 400

eo1f-seq1.txt

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 405 410 415
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 420 425 430
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 435 440 445
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 450 455 460
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 465 470 475 480
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 485 490 495
 Pro Gly Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val
 500 505 510
 Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys
 515 520 525
 Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
 530 535 540
 Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
 545 550 555 560
 Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
 565 570 575
 Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
 580 585 590
 Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
 595 600 605
 Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
 610 615 620
 Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
 625 630 635 640
 Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
 645 650 655
 Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
 660 665 670

eo1f-seq1.txt

Ala Leu Pro Pro Arg
675

<210> 91

<211> 461

<212> PRT

<213> artificial sequence

<220>

<223> 10F7-V1 CAR

<400> 91

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Ser Thr Ala Tyr Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Phe Cys Ala Arg Gly Gly Gln Leu Gly Arg Pro Trp Phe
115 120 125

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Pro Gly Gln Arg Ala Thr
165 170 175

Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Asn Thr Phe
180 185 190

Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Val Arg Phe Ser Gly

210

215

Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu Ala
225 230 235 240

Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Trp
245 250 255

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys 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> 92

<211> 490

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> 10F7-V2 CAR

<400> 92

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Ser Thr Ala Tyr Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Phe Cys Ala Arg Gly Gly Gln Leu Gly Arg Pro Trp Phe
115 120 125

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Pro Gly Gln Arg Ala Thr
165 170 175

Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Asn Thr Phe
180 185 190

Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Val Arg Phe Ser Gly
210 215 220

Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu Ala
225 230 235 240

Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Trp
245 250 255

eo1f-seq1.txt

Thr Phe Gly Gly Thr Lys Val Glu Ile Lys 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 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> 93

<211> 676

<212> PRT

<213> artificial sequence

<220>

eo1f-seq1.txt

<223> 10F7-V3 CAR

<400> 93

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 Ala Tyr Leu Gln Gln Ser Gly Ala Glu Leu
20 25 30

Val Arg Ser Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Asn Met His Trp Val Lys Gln Thr Pro Gly Gln
50 55 60

Gly Leu Glu Trp Ile Gly Tyr Ile Tyr Pro Gly Asn Gly Gly Thr Asn
65 70 75 80

Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Ser Thr Ala Tyr Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Phe Cys Ala Arg Gly Gly Gln Leu Gly Arg Pro Trp Phe
115 120 125

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Gly Gly Gly
130 135 140

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Ile Val Leu
145 150 155 160

Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Pro Gly Gln Arg Ala Thr
165 170 175

Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Ser Tyr Gly Asn Thr Phe
180 185 190

Met Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Val Arg Phe Ser Gly
210 215 220

Ser Gly Ser Arg Thr Asp Phe Thr Leu Thr Ile Asp Pro Val Glu Ala
225 230 235 240

Asp Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Asn Asn Glu Asp Pro Trp
245 250 255

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Glu Pro Lys Ser Pro
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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
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530

535

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> 94

<211> 458

<212> PRT

<213> artificial sequence

<220>

<223> 27B6-V1 CAR

<400> 94

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 Lys Gln Ser Gly Pro Gly Leu
20 25 30

Val Gln Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe
35 40 45

Ser Leu Thr Ser Tyr Asn Ile Asp Trp Val Arg Gln Ser Pro Gly Lys
50 55 60

Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr
65 70 75 80

eo1f-seq1.txt

Asn Ala Ala Phe Ile Ser Arg Leu Lys Ile Ser Lys Asp Asp Ser Lys
85 90 95

Ser Gln Val Phe Phe Lys Met Asn Ser Leu Gln Ser Asp Asp Thr Ala
100 105 110

Ile Tyr Tyr Cys Ala Arg His Ser Pro Leu Val Ser Thr Pro Asp Trp
115 120 125

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
130 135 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Ile
145 150 155 160

Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys
165 170 175

Val Thr Met Thr Cys Ser Thr Ser Ser Val Ser Tyr Met His Trp
180 185 190

Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr
195 200 205

Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser
210 215 220

Gly Thr Ser Tyr Ser Leu Thr Ile Asn Asn Met Glu Ala Glu Asp Ala
225 230 235 240

Ala Thr Tyr Ser Cys Gln Gln Trp Ser Ser Tyr Pro Pro Thr Phe Gly
245 250 255

Gly Gly Thr Lys Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile Ser
260 265 270

Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala
275 280 285

Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys
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

eo1f-seq1.txt

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> 95

<211> 487

<212> PRT

<213> artificial sequence

<220>

<223> 27B6-V2 CAR

<400> 95

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 Lys Gln Ser Gly Pro Gly Leu
20 25 30

Val Gln Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe
35 40 45

Ser Leu Thr Ser Tyr Asn Ile Asp Trp Val Arg Gln Ser Pro Gly Lys
50 55 60

Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr
65 70 75 80

Asn Ala Ala Phe Ile Ser Arg Leu Lys Ile Ser Lys Asp Asp Ser Lys
85 90 95

Ser Gln Val Phe Phe Lys Met Asn Ser Leu Gln Ser Asp Asp Thr Ala
100 105 110

Ile Tyr Tyr Cys Ala Arg His Ser Pro Leu Val Ser Thr Pro Asp Trp

115

120

125

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
 130 135 140
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile
 145 150 155 160
 Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys
 165 170 175
 Val Thr Met Thr Cys Ser Thr Ser Ser Val Ser Tyr Met His Trp
 180 185 190
 Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr
 195 200 205
 Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser
 210 215 220
 Gly Thr Ser Tyr Ser Leu Thr Ile Asn Asn Met Glu Ala Glu Asp Ala
 225 230 235 240
 Ala Thr Tyr Ser Cys Gln Gln Trp Ser Ser Tyr Pro Pro Thr Phe Gly
 245 250 255
 Gly Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro
 260 265 270
 Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro
 275 280 285
 Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu
 290 295 300
 Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
 305 310 315 320
 Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys 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

eo1f-seq1.txt

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 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> 96

<211> 673

<212> PRT

<213> artificial sequence

<220>

<223> 27B6-V3 CAR

<400> 96

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 Lys Gln Ser Gly Pro Gly Leu
20 25 30

Val Gln Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe
35 40 45

Ser Leu Thr Ser Tyr Asn Ile Asp Trp Val Arg Gln Ser Pro Gly Lys
50 55 60

Gly Leu Glu Trp Leu Gly Val Ile Trp Ser Gly Gly Ser Thr Asp Tyr
65 70 75 80

Asn Ala Ala Phe Ile Ser Arg Leu Lys Ile Ser Lys Asp Asp Ser Lys
85 90 95

Ser Gln Val Phe Phe Lys Met Asn Ser Leu Gln Ser Asp Asp Thr Ala
100 105 110

Ile Tyr Tyr Cys Ala Arg His Ser Pro Leu Val Ser Thr Pro Asp Trp
115 120 125

eo1f-seq1.txt

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser Gly
 130 135 140
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile
 145 150 155 160
 Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly Glu Lys
 165 170 175
 Val Thr Met Thr Cys Ser Thr Ser Ser Val Ser Tyr Met His Trp
 180 185 190
 Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr Asp Thr
 195 200 205
 Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser
 210 215 220
 Gly Thr Ser Tyr Ser Leu Thr Ile Asn Asn Met Glu Ala Glu Asp Ala
 225 230 235 240
 Ala Thr Tyr Ser Cys Gln Gln Trp Ser Ser Tyr Pro Pro Thr Phe Gly
 245 250 255
 Gly Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr
 260 265 270
 His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val
 275 280 285
 Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr
 290 295 300
 Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 305 310 315 320
 Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 325 330 335
 Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 340 345 350
 Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
 355 360 365
 Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
 370 375 380
 Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
 385 390 395 400

eo1f-seq1.txt

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
405 410 415

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
420 425 430

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
435 440 445

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
450 455 460

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
465 470 475 480

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile
485 490 495

Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser
500 505 510

Leu Val Ile Thr Leu Tyr Cys 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

eo1f-seq1.txt

Arg

<210> 97

<211> 460

<212> PRT

<213> artificial sequence

<220>

<223> 13F11-V1 CAR

<400> 97

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Lys Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Trp Tyr Tyr Gly Ser Thr Pro Ser Ser
115 120 125

Tyr Thr Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
145 150 155 160

Thr Thr Val Thr Gln Ser Pro Ala Ser Leu Ser Val Ala Thr Gly Glu
165 170 175

Lys Val Thr Ile Arg Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp Met
180 185 190

Asn Trp Tyr Gln Gln Lys Pro Gly Glu Pro Pro Lys Val Leu Ile Ser
195 200 205

Glu Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser Ser
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Gly Tyr Gly Thr Asp Phe Val Phe Thr Ile Glu Asn Thr Leu Ser Glu
225 230 235 240
Asp Val Ala Asp Tyr Tyr Cys Leu Gln Ser Asn Asn Met Pro Tyr Thr
245 250 255
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Leu Ala Val Ser Thr
260 265 270
Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro
275 280 285
Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu
290 295 300
Tyr Cys 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> 98

<211> 489

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> 13F11-V2 CAR

<400> 98

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Lys Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Trp Tyr Tyr Gly Ser Thr Pro Ser Ser
115 120 125

Tyr Thr Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
145 150 155 160

Thr Thr Val Thr Gln Ser Pro Ala Ser Leu Ser Val Ala Thr Gly Glu
165 170 175

Lys Val Thr Ile Arg Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp Met
180 185 190

Asn Trp Tyr Gln Gln Lys Pro Gly Glu Pro Pro Lys Val Leu Ile Ser
195 200 205

Glu Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser Ser
210 215 220

Gly Tyr Gly Thr Asp Phe Val Phe Thr Ile Glu Asn Thr Leu Ser Glu
225 230 235 240

Asp Val Ala Asp Tyr Tyr Cys Leu Gln Ser Asn Asn Met Pro Tyr Thr
245 250 255

eo1f-seq1.txt

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro
260 265 270

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu
275 280 285

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg
290 295 300

Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly
305 310 315 320

Thr Cys Gly Val Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys 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> 99

<211> 675

<212> PRT

<213> artificial sequence

<220>

eo1f-seq1.txt

<223> 13F11-V3 CAR

<400> 99

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Lys Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Trp Tyr Tyr Gly Ser Thr Pro Ser Ser
115 120 125

Tyr Thr Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
145 150 155 160

Thr Thr Val Thr Gln Ser Pro Ala Ser Leu Ser Val Ala Thr Gly Glu
165 170 175

Lys Val Thr Ile Arg Cys Ile Thr Ser Thr Asp Ile Asp Asp Asp Met
180 185 190

Asn Trp Tyr Gln Gln Lys Pro Gly Glu Pro Pro Lys Val Leu Ile Ser
195 200 205

Glu Gly Asn Thr Leu Arg Pro Gly Val Pro Ser Arg Phe Ser Ser Ser
210 215 220

Gly Tyr Gly Thr Asp Phe Val Phe Thr Ile Glu Asn Thr Leu Ser Glu
225 230 235 240

Asp Val Ala Asp Tyr Tyr Cys Leu Gln Ser Asn Asn Met Pro Tyr Thr
245 250 255

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
 275 280 285

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala
 290 295 300

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 305 310 315 320

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 325 330 335

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 340 345 350

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 355 360 365

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 370 375 380

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 385 390 395 400

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 405 410 415

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 420 425 430

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 435 440 445

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 450 455 460

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 465 470 475 480

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 485 490 495

Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
 500 505 510

Leu Ser Leu Val Ile Thr Leu Tyr Cys 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

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> 100

<211> 464

<212> PRT

<213> artificial sequence

<220>

<223> 28F5-V1 CAR

<400> 100

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Ser Gly Glu Pro Thr
65 70 75 80

eo1f-seq1.txt

Tyr Ala Glu Glu Phe₈₅ Lys Gly Arg Phe₉₀ Ala Phe Ser Leu Glu₉₅ Thr Ser
 Ala Ser Thr Ala₁₀₀ Tyr Leu Gln Ile Asn₁₀₅ Asn Leu Lys Asn Glu₁₁₀ Asp Thr
 Ala Thr Tyr₁₁₅ Phe Cys Ala Arg Gly₁₂₀ Ala Tyr Tyr Arg Tyr₁₂₅ Asp Gly Glu
 Val₁₃₀ Ser Tyr Tyr Ala Met Asp₁₃₅ Tyr Trp Gly Gln Gly₁₄₀ Thr Ser Val Thr
 Val₁₄₅ Ser Ser Gly Gly₁₅₀ Gly Ser Gly Gly₁₅₅ Gly Ser Gly Gly₁₆₀
 Gly Ser Gln Ile₁₆₅ Ile Leu Thr Gln Ser Pro₁₇₀ Ala Ile Met Ser Ala₁₇₅ Ser
 Leu Gly Glu Arg₁₈₀ Val Thr Met Thr Cys₁₈₅ Thr Ala Thr Ser Ser₁₉₀ Leu Ser
 Ser Ser Tyr₁₉₅ Leu His Trp Tyr Gln₂₀₀ Gln Lys Pro Gly Ser₂₀₅ Ser Pro Lys
 Leu Trp₂₁₀ Ile Tyr Ser Thr Ser₂₁₅ His Leu Ala Ser Gly₂₂₀ Val Pro Ala Arg
 Phe₂₂₅ Ser Gly Gly Gly₂₃₀ Ser Gly Thr Ser Tyr Ser₂₃₅ Leu Thr Ile Ser Ser₂₄₀
 Met Glu Ala Glu Asp₂₄₅ Ala Ala Thr Tyr Tyr₂₅₀ Cys His Gln Tyr His₂₅₅ Leu
 Ser Pro Tyr Thr₂₆₀ Phe Gly Gly Gly Thr₂₆₅ Lys Leu Glu Ile Lys₂₇₀ Gly Leu
 Ala Val Ser₂₇₅ Thr Ile Ser Ser Phe₂₈₀ Phe Pro Pro Gly Tyr₂₈₅ Gln Ile Tyr
 Ile Trp₂₉₀ Ala Pro Leu Ala Gly₂₉₅ Thr Cys Gly Val Leu₃₀₀ Leu Leu Ser Leu
 Val₃₀₅ Ile Thr Leu Tyr Cys₃₁₀ Lys Arg Gly Arg Lys₃₁₅ Lys Leu Leu Tyr Ile₃₂₀
 Phe Lys Gln Pro Phe₃₂₅ Met Arg Pro Val Gln₃₃₀ Thr Thr Gln Glu Glu₃₃₅ Asp
 Gly Cys Ser Cys₃₄₀ Arg Phe Pro Glu Glu₃₄₅ Glu Glu Gly Gly Cys₃₅₀ Glu Leu

eo1f-seq1.txt

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> 101

<211> 493

<212> PRT

<213> artificial sequence

<220>

<223> 28F5-V2 CAR

<400> 101

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Ser Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu

115

120

125

Val Ser Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
 130 135 140
 Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
 145 150 155 160
 Gly Ser Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
 165 170 175
 Leu Gly Glu Arg Val Thr Met Thr Cys Thr Ala Thr Ser Ser Leu Ser
 180 185 190
 Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
 195 200 205
 Leu Trp Ile Tyr Ser Thr Ser His Leu Ala Ser Gly Val Pro Ala Arg
 210 215 220
 Phe Ser Gly Gly Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser
 225 230 235 240
 Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Leu
 245 250 255
 Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Thr Thr
 260 265 270
 Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln
 275 280 285
 Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala
 290 295 300
 Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala
 305 310 315 320
 Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Ser Leu Val Ile Thr
 325 330 335
 Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
 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

eo1f-seq1.txt

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> 102

<211> 679

<212> PRT

<213> artificial sequence

<220>

<223> 28F5-V3 CAR

<400> 102

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
 20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
 35 40 45

Thr Phe Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys
 50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Ser Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
 85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
 100 105 110

Ala Thr Tyr Phe Cys Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu
 115 120 125

eo1f-seq1.txt

Val Ser Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
130 135 140

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
145 150 155 160

Gly Ser Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
165 170 175

Leu Gly Glu Arg Val Thr Met Thr Cys Thr Ala Thr Ser Ser Leu Ser
180 185 190

Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
195 200 205

Leu Trp Ile Tyr Ser Thr Ser His Leu Ala Ser Gly Val Pro Ala Arg
210 215 220

Phe Ser Gly Gly Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser
225 230 235 240

Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Leu
245 250 255

Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Glu Pro
260 265 270

Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro
275 280 285

Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
290 295 300

Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
305 310 315 320

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
325 330 335

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
340 345 350

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
355 360 365

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
370 375 380

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
385 390 395 400

eo1f-seq1.txt

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
405 410 415

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

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
435 440 445

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
450 455 460

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
465 470 475 480

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
485 490 495

Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys
500 505 510

Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys 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

eo1f-seq1.txt

Met Gln Ala Leu Pro Pro Arg
675

<210> 103

<211> 461

<212> PRT

<213> artificial sequence

<220>

<223> GMB005-V1 CAR

<400> 103

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 Leu Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe
35 40 45

Thr Phe Asn Ser Phe Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys
50 55 60

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

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
85 90 95

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
100 105 110

Ala Val Tyr Phe Cys Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro
115 120 125

Val Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
165 170 175

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
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215

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
225 230 235 240

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
245 250 255

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 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> 104

<211> 490

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> GMB005-V2 CAR

<400> 104

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 Leu Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe
35 40 45

Thr Phe Asn Ser Phe Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys
50 55 60

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

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
85 90 95

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
100 105 110

Ala Val Tyr Phe Cys Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro
115 120 125

Val Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
165 170 175

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
225 230 235 240

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
245 250 255

eo1f-seq1.txt

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 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 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> 105

<211> 676

<212> PRT

<213> artificial sequence

<220>

eo1f-seq1.txt

<223> GMB005-V3 CAR

<400> 105

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 Leu Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe
35 40 45

Thr Phe Asn Ser Phe Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys
50 55 60

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

Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
85 90 95

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
100 105 110

Ala Val Tyr Phe Cys Ala Lys Asp Lys Ile Leu Trp Phe Gly Glu Pro
115 120 125

Val Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
165 170 175

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr
180 185 190

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
225 230 235 240

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
245 250 255

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 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
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535

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> 106

<211> 18

<212> PRT

<213> homosapiens

<220>

<223> FcεRI γ- signal peptide (SP)

<400> 106

Met Ile Pro Ala Val Val Leu Leu Leu Leu Leu Val Glu Gln Ala
1 5 10 15

Ala Ala

<210> 107

<211> 43

<212> PRT

<213> homosapiens

<220>

<223> Fc Receptor for IgE, gamma chain, without ITAM) (FcεRI γ- DITAM)

<400> 107

eof-seq1.txt

Leu Gly Glu Pro Gln Leu Cys Tyr Ile Leu Asp Ala Ile Leu Phe Leu
1 5 10 15

Tyr Gly Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile Gln Val
20 25 30

Arg Lys Ala Ala Ile Thr Ser Tyr Glu Lys Ser
35 40

<210> 108

<211> 22

<212> PRT

<213> artificial sequence

<220>

<223> GSG-P2A ribosomal skip peptide (GSG-P2A)

<400> 108

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
1 5 10 15

Glu Glu Asn Pro Gly Pro
20

<210> 109

<211> 21

<212> PRT

<213> artificial sequence

<220>

<223> GSG-T2A ribosomal skip peptide (GSG-T2A)

<400> 109

Gly Ser Gly Glu Gly Arg Gly Ser Leu Leu Thr Cys Gly Asp Val Glu
1 5 10 15

Glu Asn Pro Gly Pro
20

<210> 110

<211> 25

<212> PRT

<213> homosapiens

<220>

<223> FcεRI a- signal peptide (SP)

<400> 110

Met Ala Pro Ala Met Glu Ser Pro Thr Leu Leu Cys Val Ala Leu Leu
1 5 10 15

Phe Phe Ala Pro Asp Gly Val Leu Ala
20 25

<210> 111

eo1f-seq1.txt

<211> 52
<212> PRT
<213> homosapiens

<220>
<223> Fc Receptor for IgE, alpha chain, transmembrane and intracellular domain (FcεRI a-TM-IC)

<400> 111
Phe Phe Ile Pro Leu Leu Val Val Ile Leu Phe Ala Val Asp Thr Gly
1 5 10 15

Leu Phe Ile Ser Thr Gln Gln Gln Val Thr Phe Leu Leu Lys Ile Lys
20 25 30

Arg Thr Arg Lys Gly Phe Arg Leu Leu Asn Pro His Pro Lys Pro Asn
35 40 45

Pro Lys Asn Asn
50

<210> 112
<211> 215
<212> PRT
<213> homosapiens

<220>
<223> Receptor for IgE, beta chain, without ITAM (FcεRIγ-ΔITAM)

<400> 112
Met Asp Thr Glu Ser Asn Arg Arg Ala Asn Leu Ala Leu Pro Gln Glu
1 5 10 15

Pro Ser Ser Val Pro Ala Phe Glu Val Leu Glu Ile Ser Pro Gln Glu
20 25 30

Val Ser Ser Gly Arg Leu Leu Lys Ser Ala Ser Ser Pro Pro Leu His
35 40 45

Thr Trp Leu Thr Val Leu Lys Lys Glu Gln Glu Phe Leu Gly Val Thr
50 55 60

Gln Ile Leu Thr Ala Met Ile Cys Leu Cys Phe Gly Thr Val Val Cys
65 70 75 80

Ser Val Leu Asp Ile Ser His Ile Glu Gly Asp Ile Phe Ser Ser Phe
85 90 95

Lys Ala Gly Tyr Pro Phe Trp Gly Ala Ile Phe Phe Ser Ile Ser Gly
100 105 110

Met Leu Ser Ile Ile Ser Glu Arg Arg Asn Ala Thr Tyr Leu Val Arg
115 120 125

Gly Ser Leu Gly Ala Asn Thr Ala Ser Ser Ile Ala Gly Gly Thr Gly
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135

Ile Thr Ile Leu Ile Ile Asn Leu Lys Lys Ser Leu Ala Tyr Ile His
145 150 155 160

Ile His Ser Cys Gln Lys Phe Phe Glu Thr Lys Cys Phe Met Ala Ser
165 170 175

Phe Ser Thr Glu Ile Val Val Met Met Leu Phe Leu Thr Ile Leu Gly
180 185 190

Leu Gly Ser Ala Val Ser Leu Thr Ile Cys Gly Ala Gly Glu Glu Leu
195 200 205

Lys Gly Asn Lys Val Pro Glu
210 215

<210> 113

<211> 41

<212> PRT

<213> homosapiens

<220>

<223> CD28 co-stimulatory domain (CD28-IC)

<400> 113

Arg Ser Lys Arg Ser Arg Gly Gly His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser
35 40

<210> 114

<211> 10

<212> PRT

<213> artificial sequence

<220>

<223> CD20 mimotope

<400> 114

Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser
1 5 10

<210> 115

<211> 24

<212> PRT

<213> artificial sequence

<220>

<223> Epitope for palivizumab

eo1f-seq1.txt

<400> 115
Asn Ser Glu Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp
1 5 10 15

Gln Lys Lys Leu Met Ser Asn Asn
20

<210> 116

<211> 12

<212> PRT

<213> artificial sequence

<220>

<223> Mimotope 1 for cetuximab

<400> 116
Cys Gln Phe Asp Leu Ser Thr Arg Arg Leu Lys Cys
1 5 10

<210> 117

<211> 12

<212> PRT

<213> artificial sequence

<220>

<223> Mimotope 2 for cetuximab

<400> 117
Cys Gln Tyr Asn Leu Ser Ser Arg Ala Leu Lys Cys
1 5 10

<210> 118

<211> 12

<212> PRT

<213> artificial sequence

<220>

<223> Mimotope 3 for cetuximab

<400> 118
Cys Val Trp Gln Arg Trp Gln Lys Ser Tyr Val Cys
1 5 10

<210> 119

<211> 12

<212> PRT

<213> artificial sequence

<220>

<223> Mimotope 4 for cetuximab

<400> 119
Cys Met Trp Asp Arg Phe Ser Arg Trp Tyr Lys Cys
1 5 10

<210> 120

<211> 25

<212> PRT
 <213> artificial sequence

<220>
 <223> Epitope A for nivolumab

<400> 120
 Ser Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp
 1 5 10 15

Lys Leu Ala Ala Phe Pro Glu Asp Arg
 20 25

<210> 121

<211> 19
 <212> PRT
 <213> artificial sequence

<220>
 <223> Epitope B for nivolumab

<400> 121
 Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala Gln
 1 5 10 15

Ile Lys Glu

<210> 122

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

<220>
 <223> Epitope 1 for CD34

<400> 122
 Thr Phe Ser Asn Val Ser Thr Asn Val Ser Tyr Gln Glu Thr
 1 5 10

<210> 123

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

<220>
 <223> Epitope 2 for CD34

<400> 123
 Asn Thr Asn Ser Ser Val Gln Ser Gln Thr Ser Val Ile Ser
 1 5 10

<210> 124

<211> 533
 <212> PRT
 <213> artificial sequence

<220>

<223> 28F5v2 CAR with 2 CD20 mimotopes

<400> 124

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Ser Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu
115 120 125

Val Ser Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
130 135 140

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
145 150 155 160

Gly Ser Gln Ile Ile Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser
165 170 175

Leu Gly Glu Arg Val Thr Met Thr Cys Thr Ala Thr Ser Ser Leu Ser
180 185 190

Ser Ser Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
195 200 205

Leu Trp Ile Tyr Ser Thr Ser His Leu Ala Ser Gly Val Pro Ala Arg
210 215 220

Phe Ser Gly Gly Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser
225 230 235 240

Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Leu
245 250 255

Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ser Asp
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260

265

270

Pro Gly Ser Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu
 275 280 285

Cys Ser Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu Cys
 290 295 300

Ser Gly Gly Gly Gly Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr
 305 310 315 320

Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala
 325 330 335

Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe
 340 345 350

Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val
 355 360 365

Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys
 370 375 380

Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
 385 390 395 400

Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
 405 410 415

Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
 420 425 430

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
 435 440 445

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
 450 455 460

Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
 465 470 475 480

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
 485 490 495

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
 500 505 510

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
 515 520 525

Ala Leu Pro Pro Arg

530

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<210> 125
<211> 573
<212> PRT
<213> artificial sequence
<220>
<223> 28F5v2 CAR with 2 CD20 mimotopes and 1 CD34 epitope
<400> 125
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 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro
      20      25      30

Ser Leu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile
      35      40      45

Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr Val
50      55      60

Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Gly Met
65      70      75      80

Asn Trp Val Lys Gln Thr Pro Gly Lys Gly Leu Lys Trp Met Gly Trp
      85      90      95

Ile Asn Thr Asn Ser Gly Glu Pro Thr Tyr Ala Glu Glu Phe Lys Gly
100      105      110

Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr Leu Gln
115      120      125

Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
130      135      140

Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu Val Ser Tyr Tyr Ala Met Asp
145      150      155      160

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly
165      170      175

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Ile Leu Thr
180      185      190

Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu Arg Val Thr Met
195      200      205

Thr Cys Thr Ala Thr Ser Ser Leu Ser Ser Ser Tyr Leu His Trp Tyr
210      215      220

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eo1f-seq1.txt

Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp Ile Tyr Ser Thr Ser
 225 230 235 240
 His Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Gly Gly Ser Gly
 245 250 255
 Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala
 260 265 270
 Thr Tyr Tyr Cys His Gln Tyr His Leu Ser Pro Tyr Thr Phe Gly Gly
 275 280 285
 Gly Thr Lys Leu Glu Ile Lys Gly Ser Gly Gly Gly Gly Ser Cys Pro
 290 295 300
 Tyr Ser Asn Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Glu Leu Pro
 305 310 315 320
 Thr Gln Gly Thr Phe Ser Asn Val Ser Thr Asn Val Ser Pro Ala Lys
 325 330 335
 Pro Thr Thr Thr Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Thr Thr
 340 345 350
 Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln
 355 360 365
 Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala
 370 375 380
 Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala
 385 390 395 400
 Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr
 405 410 415
 Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
 420 425 430
 Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
 435 440 445
 Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
 450 455 460
 Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
 465 470 475 480
 Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
 485 490 495

eo1f-seq1.txt

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
500 505 510

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
515 520 525

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
530 535 540

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
545 550 555 560

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
565 570

<210> 126

<211> 504

<212> PRT

<213> artificial sequence

<220>

<223> 28F5v1 CAR with 2 CD20 mimotopes

<400> 126

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 Ile Gln Leu Val Gln Ser Gly Pro Glu Leu
20 25 30

Lys Lys Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Thr Pro Gly Lys
50 55 60

Gly Leu Lys Trp Met Gly Trp Ile Asn Thr Asn Ser Gly Glu Pro Thr
65 70 75 80

Tyr Ala Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser
85 90 95

Ala Ser Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr
100 105 110

Ala Thr Tyr Phe Cys Ala Arg Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu
115 120 125

Val Ser Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
130 135 140

Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
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eo1f-seq1.txt

145		150		155		160
Gly	Ser	Gln	Ile	Ile	Leu	Thr
			165			
					Pro	Ala
					170	
						Ile
						Met
						Ser
						Ala
						175
Leu	Gly	Glu	Arg	Val	Thr	Met
			180			
					Thr	
					Cys	
					185	
						Ala
						Thr
						Ser
						Ser
						190
Ser	Ser	Tyr	Leu	His	Trp	Tyr
		195				
					Gln	Gln
					200	
						Lys
						Pro
						Gly
						Ser
						205
						Ser
						Pro
						Lys
Leu	Trp	Ile	Tyr	Ser	Thr	Ser
	210					
					Ser	
					215	
						His
						Leu
						Ala
						Ser
						Gly
						220
						Val
						Pro
						Ala
						Arg
Phe	Ser	Gly	Gly	Gly	Ser	Gly
225					230	
						Thr
						Ser
						Tyr
						Ser
						235
						Leu
						Thr
						Ile
						Ser
						240
Met	Glu	Ala	Glu	Asp	Ala	Ala
				245		
						Thr
						Tyr
						Tyr
						250
						Cys
						His
						Gln
						Tyr
						His
						255
						Leu
Ser	Pro	Tyr	Thr	Phe	Gly	Gly
			260			
						Gly
						Gly
						Thr
						265
						Lys
						Leu
						Glu
						Ile
						Lys
						270
						Ser
						Asp
Pro	Gly	Ser	Gly	Gly	Gly	Gly
		275				
						Ser
						280
						Cys
						Pro
						Tyr
						Ser
						Asn
						285
						Pro
						Ser
						Leu
						Cys
Cys	Ser	Gly	Gly	Gly	Gly	Ser
	290					
						Cys
						295
						Pro
						Tyr
						Ser
						Asn
						300
						Pro
						Ser
						Leu
						Cys
Ser	Gly	Gly	Gly	Gly	Ser	Gly
305					310	
						Leu
						Ala
						Val
						Ser
						315
						Thr
						Ile
						Ser
						Ser
						Phe
						320
Phe	Pro	Pro	Gly	Tyr	Gln	Ile
				325		
						Tyr
						Ile
						Trp
						330
						Ala
						Pro
						Leu
						Ala
						Gly
						335
						Thr
Cys	Gly	Val	Leu	Leu	Leu	Ser
			340			
						Leu
						Val
						345
						Ile
						Thr
						Leu
						Tyr
						Cys
						350
						Lys
						Arg
Gly	Arg	Lys	Lys	Leu	Leu	Tyr
		355				
						Ile
						360
						Phe
						Lys
						Gln
						Pro
						Phe
						365
						Met
						Arg
						Pro
Val	Gln	Thr	Thr	Gln	Glu	Glu
	370					
						Asp
						Gly
						Cys
						Ser
						Cys
						380
						Arg
						Phe
						Pro
						Glu
Glu	Glu	Glu	Gly	Gly	Cys	Glu
385					390	
						Leu
						Arg
						Val
						Lys
						395
						Phe
						Ser
						Arg
						Ser
						Ala
						400
Asp	Ala	Pro	Ala	Tyr	Gln	Gln
				405		
						Gly
						Gln
						Asn
						410
						Gln
						Leu
						Tyr
						Asn
						Glu
						415
						Leu
Asn	Leu	Gly	Arg	Arg	Glu	Glu
						Tyr
						Asp
						Val
						Leu
						Asp
						Lys
						Arg
						Arg
						Gly

420

425

430

Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu
 435 440 445

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
 450 455 460

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
 465 470 475 480

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
 485 490 495

His Met Gln Ala Leu Pro Pro Arg
 500

<210> 127

<211> 544

<212> PRT

<213> artificial sequence

<220>

<223> 28F5v1 CAR with 2 CD20 mimotopes and 1 CD34 epitope

<400> 127

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 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro
 20 25 30

Ser Leu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile
 35 40 45

Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr Val
 50 55 60

Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Lys Tyr Gly Met
 65 70 75 80

Asn Trp Val Lys Gln Thr Pro Gly Lys Gly Leu Lys Trp Met Gly Trp
 85 90 95

Ile Asn Thr Asn Ser Gly Glu Pro Thr Tyr Ala Glu Glu Phe Lys Gly
 100 105 110

Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr Leu Gln
 115 120 125

Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
 130 135 140

eo1f-seq1.txt

Gly Ala Tyr Tyr Arg Tyr Asp Gly Glu Val Ser Tyr Tyr Ala Met Asp
 145 150 155 160
 Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly
 165 170 175
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Ile Leu Thr
 180 185 190
 Gln Ser Pro Ala Ile Met Ser Ala Ser Leu Gly Glu Arg Val Thr Met
 195 200 205
 Thr Cys Thr Ala Thr Ser Ser Leu Ser Ser Ser Tyr Leu His Trp Tyr
 210 215 220
 Gln Gln Lys Pro Gly Ser Ser Pro Lys Leu Trp Ile Tyr Ser Thr Ser
 225 230 235 240
 His Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Gly Gly Ser Gly
 245 250 255
 Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu Asp Ala Ala
 260 265 270
 Thr Tyr Tyr Cys His Gln Tyr His Leu Ser Pro Tyr Thr Phe Gly Gly
 275 280 285
 Gly Thr Lys Leu Glu Ile Lys Gly Ser Gly Gly Gly Gly Ser Cys Pro
 290 295 300
 Tyr Ser Asn Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Glu Leu Pro
 305 310 315 320
 Thr Gln Gly Thr Phe Ser Asn Val Ser Thr Asn Val Ser Pro Ala Lys
 325 330 335
 Pro Thr Thr Thr Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Gly Leu
 340 345 350
 Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr
 355 360 365
 Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
 370 375 380
 Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
 385 390 395 400
 Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
 405 410 415

eo1f-seq1.txt

Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
420 425 430

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
435 440 445

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
450 455 460

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
465 470 475 480

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
485 490 495

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
500 505 510

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
515 520 525

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
530 535 540

<210> 128

<211> 530

<212> PRT

<213> artificial sequence

<220>

<223> 25A10v2 CAR with 2 CD20 mimotopes

<400> 128

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 Ala Glu Leu
20 25 30

Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
35 40 45

Asn Ile Lys Asp Ser Leu Ile His Trp Val Lys Gln Arg Pro Glu Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Asp Lys Thr Lys
65 70 75 80

Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
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Ser Asn Thr Ala Tyr Leu Gln Leu Ser Thr Leu Thr Ser Glu Asp Thr
 100 105 110
 Ala Ile Tyr Tyr Cys Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr
 115 120 125
 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
 130 135 140
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln
 145 150 155 160
 Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser
 165 170 175
 Cys Lys Ser Ser Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
 180 185 190
 Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro 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 Ala
 225 230 235 240
 Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr
 245 250 255
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ser Asp Pro Gly Ser
 260 265 270
 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly
 275 280 285
 Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly Gly
 290 295 300
 Gly Gly Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
 305 310 315 320
 Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
 325 330 335
 Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
 340 345 350
 Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu

355

360

365

Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu
 370 375 380

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
 385 390 395 400

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
 405 410 415

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
 420 425 430

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
 435 440 445

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
 450 455 460

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
 465 470 475 480

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
 485 490 495

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
 500 505 510

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
 515 520 525

Pro Arg
 530

<210> 129

<211> 569

<212> PRT

<213> artificial sequence

<220>

<223> 25A10v2 CAR with 2 CD20 mimotopes and 1 CD34 epitope

<400> 129

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 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro
 20 25 30

Ser Leu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val
 35 40 45

eo1f-seq1.txt

Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
50 55 60

Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Ser Leu Ile
65 70 75 80

His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp
85 90 95

Ile Asp Pro Glu Asp Asp Lys Thr Lys Tyr Ala Pro Lys Phe Gln Asp
100 105 110

Lys Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln
115 120 125

Leu Ser Thr Leu Thr Ser Glu Asp Thr Ala Ile Tyr Tyr Cys Val Ser
130 135 140

Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr Trp Gly Gln Gly Thr Thr Leu
145 150 155 160

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
165 170 175

Gly Gly Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr
180 185 190

Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu
195 200 205

His Ser Gly Asn Gln Arg Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro
210 215 220

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser
225 230 235 240

Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
245 250 255

Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys
260 265 270

Gln Asn Asp Tyr Asp Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu
275 280 285

Glu Ile Lys Gly Ser Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro
290 295 300

Ser Leu Cys Ser Gly Gly Gly Gly Ser Glu Leu Pro Thr Gln Gly Thr
305 310 315 320

eo1f-seq1.txt

Phe Ser Asn Val Ser Thr Asn Val Ser Pro Ala Lys Pro Thr Thr Thr
325 330 335

Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Thr Thr Thr Pro Ala Pro
340 345 350

Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu
355 360 365

Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg
370 375 380

Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly
385 390 395 400

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
405 410 415

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
420 425 430

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
435 440 445

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
450 455 460

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
465 470 475 480

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
485 490 495

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
500 505 510

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
515 520 525

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
530 535 540

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
545 550 555 560

Leu His Met Gln Ala Leu Pro Pro Arg
565

<210> 130

<211> 501

eo1f-seq1.txt

<212> PRT
<213> artificial sequence

<220>
<223> 25A10v1 CAR with 2 CD20 mimotopes

<400> 130
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 Ala Glu Leu
20 25 30

Val Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
35 40 45

Asn Ile Lys Asp Ser Leu Ile His Trp Val Lys Gln Arg Pro Glu Gln
50 55 60

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Asp Lys Thr Lys
65 70 75 80

Tyr Ala Pro Lys Phe Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser
85 90 95

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Thr Leu Thr Ser Glu Asp Thr
100 105 110

Ala Ile Tyr Tyr Cys Val Ser Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr
115 120 125

Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln
145 150 155 160

Ser Pro Ser Ser Leu Thr Val Thr Ala Gly Glu Lys Val Thr Met Ser
165 170 175

Cys Lys Ser Ser Gln Ser Leu Leu His Ser Gly Asn Gln Arg Asn Tyr
180 185 190

Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln Pro 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 Ala
225 230 235 240

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Ser Asp Pro Gly Ser
 260 265 270
 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly
 275 280 285
 Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser Gly Gly
 290 295 300
 Gly Gly Ser Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro
 305 310 315 320
 Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val
 325 330 335
 Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys
 340 345 350
 Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr
 355 360 365
 Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu
 370 375 380
 Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
 385 390 395 400
 Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
 405 410 415
 Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
 420 425 430
 Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
 435 440 445
 Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
 450 455 460
 Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
 465 470 475 480
 Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
 485 490 495
 Ala Leu Pro Pro Arg
 500

<210> 131

eo1f-seq1.txt

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<211> 541
<212> PRT
<213> artificial sequence

<220>
<223> 25A10v1 CAR with 2 CD20 mimotopes and 1 CD34 epitope

<400> 131
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 Gly Gly Gly Gly Ser Cys Pro Tyr Ser Asn Pro
      20      25

Ser Leu Cys Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val
      35      40      45

Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val
      50      55      60

Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Ser Leu Ile
65      70      75      80

His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp
      85      90      95

Ile Asp Pro Glu Asp Asp Lys Thr Lys Tyr Ala Pro Lys Phe Gln Asp
      100     105     110

Lys Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln
      115     120     125

Leu Ser Thr Leu Thr Ser Glu Asp Thr Ala Ile Tyr Tyr Cys Val Ser
      130     135     140

Arg Tyr Ile Asn Tyr Tyr Phe Ala Tyr Trp Gly Gln Gly Thr Thr Leu
145     150     155     160

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
      165     170     175

Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val
      180     185     190

Thr Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu
      195     200     205

Leu His Ser Gly Asn Gln Arg Asn Tyr Leu Thr Trp Tyr Gln Gln Lys
      210     215     220

Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu
225     230     235     240

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eo1f-seq1.txt

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
245 250 255

Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr
260 265 270

Cys Gln Asn Asp Tyr Asp Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys
275 280 285

Leu Glu Ile Lys Gly Ser Gly Gly Gly Ser Cys Pro Tyr Ser Asn
290 295 300

Pro Ser Leu Cys Ser Gly Gly Gly Gly Ser Glu Leu Pro Thr Gln Gly
305 310 315 320

Thr Phe Ser Asn Val Ser Thr Asn Val Ser Pro Ala Lys Pro Thr Thr
325 330 335

Thr Ala Cys Pro Tyr Ser Asn Pro Ser Leu Cys Gly Leu Ala Val Ser
340 345 350

Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala
355 360 365

Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr
370 375 380

Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
385 390 395 400

Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
405 410 415

Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
420 425 430

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
435 440 445

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
450 455 460

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
465 470 475 480

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
485 490 495

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
500 505 510

eo1f-seq1.txt

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
515 520 525

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
530 535 540

<210> 132

<211> 117

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m2C2

<400> 132

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

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

Tyr Met Phe Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Tyr Ile Ser Asn Gly Asp Gly Asn Thr Tyr Tyr Pro Asp Thr Leu
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn Thr Leu Tyr
65 70 75 80

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

Ala Arg Ser Ile Ser Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 133

<211> 107

<212> PRT

<213> artificial sequence

<220>

<223> VL chain of m2C2

<400> 133

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

Asp Arg Val Ser Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asp Phe
20 25 30

eo1f-seq1.txt

Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Val Ser Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Ser Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Pro
65 70 75 80

Glu Asp Val Gly Val Tyr Tyr Cys Gln Asn Ala His Ser Phe Pro Ser
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 134

<211> 117

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m5G9

<400> 134

Glu Ile His 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 Ser Phe Thr Asp Tyr
20 25 30

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

Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Ala Tyr Tyr Asn Gln Lys Phe
50 55 60

Lys Ala Met Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Phe
65 70 75 80

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

Ala Arg Lys Gly Val Tyr Gly Leu Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 135

<211> 107

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> VL chain of m5G9

<400> 135

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

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

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

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

Ser Gly Ser Gly Ala Gln Tyr Ser Leu Lys Ile Asn Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Gly Thr Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 136

<211> 454

<212> PRT

<213> artificial sequence

<220>

<223> 5G9-V1 CAR

<400> 136

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 Ile His 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

Ser Phe Thr Asp Tyr Asn Ile Tyr Trp Val Lys Gln Ser His Gly Glu
50 55 60

Ser Leu Glu Trp Val Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Ala Tyr
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ala Met Ala Thr Leu Thr Val Asp Lys Ser
85 90 95

Ser Ser Thr Ala Phe Met His Leu Asn Ser Leu Thr Ser Glu Asp Ser
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100

105

110

Ala Val Tyr Tyr Cys Ala Arg Lys Gly Val Tyr Gly Leu Ala Tyr Trp
 115 120 125
 Gly Gln Gly Thr Leu 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 Gln Met Thr Gln Ser
 145 150 155 160
 Pro Ala Ser Leu Ser Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys
 165 170 175
 Arg Ala Ser Glu Ser Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys
 180 185 190
 Gln Gly Lys Ser Pro Gln Leu Leu Val Tyr Ala Ser Thr His Leu Ala
 195 200 205
 Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ala Gln Tyr
 210 215 220
 Ser Leu Lys Ile Asn Ser Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr
 225 230 235 240
 Cys Gln His Phe Trp Gly Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 245 250 255
 Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro
 260 265 270
 Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
 275 280 285
 Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
 290 295 300
 Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
 305 310 315 320
 Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
 325 330 335
 Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
 340 345 350
 Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
 355 360 365
 Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp

370

375

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
385 390 395 400

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
405 410 415

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
420 425 430

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

Gln Ala Leu Pro Pro Arg
450

<210> 137

<211> 483

<212> PRT

<213> artificial sequence

<220>

<223> 5G9-V2 CAR

<400> 137

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 Ile His 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

Ser Phe Thr Asp Tyr Asn Ile Tyr Trp Val Lys Gln Ser His Gly Glu
50 55 60

Ser Leu Glu Trp Val Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Ala Tyr
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ala Met Ala Thr Leu Thr Val Asp Lys Ser
85 90 95

Ser Ser Thr Ala Phe Met His Leu Asn Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Lys Gly Val Tyr Gly Leu Ala Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
130 135 140

eo1f-seq1.txt

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser
 145 150 155 160
 Pro Ala Ser Leu Ser Val Ser Val Gly Glu Thr Val Thr Ile Thr Cys
 165 170 175
 Arg Ala Ser Glu Ser Ile Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys
 180 185 190
 Gln Gly Lys Ser Pro Gln Leu Leu Val Tyr Ala Ser Thr His Leu Ala
 195 200 205
 Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ala Gln Tyr
 210 215 220
 Ser Leu Lys Ile Asn Ser Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr
 225 230 235 240
 Cys Gln His Phe Trp Gly Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 245 250 255
 Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala
 260 265 270
 Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg
 275 280 285
 Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys
 290 295 300
 Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
 305 310 315 320
 Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu
 325 330 335
 Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
 340 345 350
 Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
 355 360 365
 Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
 370 375 380
 Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
 385 390 395 400
 Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
 405 410 415

eo1f-seq1.txt

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
420 425 430

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
435 440 445

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
450 455 460

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
465 470 475 480

Pro Pro Arg

<210> 138

<211> 669

<212> PRT

<213> artificial sequence

<220>

<223> 5G9-V3 CAR

<400> 138

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 Ile His 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

Ser Phe Thr Asp Tyr Asn Ile Tyr Trp Val Lys Gln Ser His Gly Glu
50 55 60

Ser Leu Glu Trp Val Gly Tyr Ile Asp Pro Tyr Asn Gly Gly Ala Tyr
65 70 75 80

Tyr Asn Gln Lys Phe Lys Ala Met Ala Thr Leu Thr Val Asp Lys Ser
85 90 95

Ser Ser Thr Ala Phe Met His Leu Asn Ser Leu Thr Ser Glu Asp Ser
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Lys Gly Val Tyr Gly Leu Ala Tyr Trp
115 120 125

Gly Gln Gly Thr Leu 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 Gln Met Thr Gln Ser
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eo1f-seq1.txt

145		150		155		160
Pro	Ala	Ser	Leu	Ser ₁₆₅	Val	Ser
					Val	Gly
						Glu
						Thr
						Val
						Thr
						Ile
						Thr
						Cys
Arg	Ala	Ser	Glu ₁₈₀	Ser	Ile	Tyr
						Ser
						Asn ₁₈₅
						Leu
						Ala
						Trp
						Tyr
						Gln ₁₉₀
						Gln
						Lys
Gln	Gly	Lys ₁₉₅	Ser	Pro	Gln	Leu
						Leu ₂₀₀
						Val
						Tyr
						Ala
						Ser
						Thr ₂₀₅
						His
						Leu
						Ala
Asp	Gly ₂₁₀	Val	Pro	Ser	Arg	Phe
						Ser
						Gly
						Ser
						Gly
						Ser
						Gly
						Ala
						Gln
						Tyr
Ser	Leu	Lys	Ile	Asn	Ser ₂₃₀	Leu
						Gln
						Ser
						Glu
						Asp ₂₃₅
						Phe
						Gly
						Ser
						Tyr
						Tyr ₂₄₀
Cys	Gln	His	Phe	Trp ₂₄₅	Gly	Thr
						Pro
						Tyr
						Thr ₂₅₀
						Phe
						Gly
						Gly
						Gly
						Thr ₂₅₅
						Lys
Leu	Glu	Ile	Lys ₂₆₀	Glu	Pro	Lys
						Ser
						Pro
						Asp ₂₆₅
						Lys
						Thr
						His
						Thr ₂₇₀
						Cys
						Pro
Pro	Cys	Pro ₂₇₅	Ala	Pro	Pro	Val
						Ala ₂₈₀
						Gly
						Pro
						Ser
						Val
						Phe ₂₈₅
						Leu
						Phe
						Pro
Pro	Lys ₂₉₀	Pro	Lys	Asp	Thr	Leu
						Met
						Ile
						Ala
						Arg
						Thr ₃₀₀
						Pro
						Glu
						Val
						Thr
Cys	Val	Val	Val	Asp	Val ₃₁₀	Ser
						His
						Glu
						Asp
						Pro ₃₁₅
						Glu
						Val
						Lys
						Phe
						Asn ₃₂₀
Trp	Tyr	Val	Asp	Gly ₃₂₅	Val	Glu
						Val
						His
						Asn ₃₃₀
						Ala
						Lys
						Thr
						Lys
						Pro
						Arg
Glu	Glu	Gln	Tyr ₃₄₀	Asn	Ser	Thr
						Tyr
						Arg ₃₄₅
						Val
						Val
						Ser
						Val
						Leu ₃₅₀
						Thr
						Val
Leu	His	Gln ₃₅₅	Asp	Trp	Leu	Asn
						Gly ₃₆₀
						Lys
						Glu
						Tyr
						Lys
						Cys ₃₆₅
						Lys
						Val
						Ser
Asn	Lys ₃₇₀	Ala	Leu	Pro	Ala	Pro
						Ile
						Glu
						Lys
						Thr
						Ile ₃₈₀
						Ser
						Lys
						Ala
						Lys
Gly	Gln	Pro	Arg	Glu	Pro	Gln
						Val
						Tyr
						Thr
						Leu ₃₉₅
						Pro
						Pro
						Ser
						Arg
						Asp ₄₀₀
Glu	Leu	Thr	Lys	Asn ₄₀₅	Gln	Val
						Ser
						Leu
						Thr ₄₁₀
						Cys
						Leu
						Val
						Lys
						Gly ₄₁₅
						Phe
Tyr	Pro	Ser	Asp	Ile	Ala	Val
						Glu
						Trp
						Glu
						Ser
						Asn
						Gly
						Gln
						Pro
						Glu

420

425

430

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 435 440 445

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 450 455 460

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 465 470 475 480

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala
 485 490 495

Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr
 500 505 510

Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
 515 520 525

Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
 530 535 540

Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
 545 550 555 560

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
 565 570 575

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
 580 585 590

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
 595 600 605

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
 610 615 620

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
 625 630 635 640

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
 645 650 655

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
 660 665

<210> 139

<211> 454

<212> PRT

<213> artificial sequence

eo1f-seq1.txt

<220>

<223> 2C2-V1 CAR

<400> 139

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 Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Thr Ser Gly Phe
35 40 45

Thr Phe Ser Asp Tyr Tyr Met Phe Trp Val Arg Gln Thr Pro Glu Lys
50 55 60

Arg Leu Glu Trp Val Ala Tyr Ile Ser Asn Gly Asp Gly Asn Thr Tyr
65 70 75 80

Tyr Pro Asp Thr Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
85 90 95

Asn Asn Thr Leu Tyr Leu Gln Met Ser Arg Leu Lys Ser Glu Asp Thr
100 105 110

Ala Met Tyr Tyr Cys Ala Arg Ser Ile Ser Arg Tyr Phe Asp Val Trp
115 120 125

Gly Ala Gly Thr Thr 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 Val Met Thr Gln Ser
145 150 155 160

Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser Cys
165 170 175

Arg Ala Ser Gln Ser Ile Ser Asp Phe Leu His Trp Tyr Gln Gln Lys
180 185 190

Ser His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Val Ser Gln Ser Ile
195 200 205

Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe
210 215 220

Thr Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr
225 230 235 240

Cys Gln Asn Ala His Ser Phe Pro Ser Thr Phe Gly Gly Gly Thr Lys
245 250 255

eo1f-seq1.txt

Leu Glu Ile Lys Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro
260 265 270

Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
275 280 285

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
290 295 300

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
305 310 315 320

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
325 330 335

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
340 345 350

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
355 360 365

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
370 375 380

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
385 390 395 400

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
405 410 415

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
420 425 430

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

Gln Ala Leu Pro Pro Arg
450

<210> 140

<211> 483

<212> PRT

<213> artificial sequence

<220>

<223> 2C2-V2 CAR

<400> 140

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 Val Glu Ser Gly Gly Gly Leu
Page 131

Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Thr Ser Gly Phe
 35 40 45
 Thr Phe Ser Asp Tyr Tyr Met Phe Trp Val Arg Gln Thr Pro Glu Lys
 50 55 60
 Arg Leu Glu Trp Val Ala Tyr Ile Ser Asn Gly Asp Gly Asn Thr Tyr
 65 70 75 80
 Tyr Pro Asp Thr Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
 85 90 95
 Asn Asn Thr Leu Tyr Leu Gln Met Ser Arg Leu Lys Ser Glu Asp Thr
 100 105 110
 Ala Met Tyr Tyr Cys Ala Arg Ser Ile Ser Arg Tyr Phe Asp Val Trp
 115 120 125
 Gly Ala Gly Thr Thr 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 Val Met Thr Gln Ser
 145 150 155 160
 Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser Cys
 165 170 175
 Arg Ala Ser Gln Ser Ile Ser Asp Phe Leu His Trp Tyr Gln Gln Lys
 180 185 190
 Ser His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Val Ser Gln Ser Ile
 195 200 205
 Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe
 210 215 220
 Thr Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr
 225 230 235 240
 Cys Gln Asn Ala His Ser Phe Pro Ser Thr Phe Gly Gly Gly Thr Lys
 245 250 255
 Leu Glu Ile Lys Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala
 260 265 270
 Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg
 275 280 285
 Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys

290

295

Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu
305 310 315 320

Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu
325 330 335

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
340 345 350

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
355 360 365

Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
370 375 380

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
385 390 395 400

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
405 410 415

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
420 425 430

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
435 440 445

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
450 455 460

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
465 470 475 480

Pro Pro Arg

<210> 141

<211> 669

<212> PRT

<213> artificial sequence

<220>

<223> 2C2-V3 CAR

<400> 141

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 Val Glu Ser Gly Gly Gly Leu
20 25 30

eo1f-seq1.txt

Val Gln Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Thr Ser Gly Phe
 35 40 45
 Thr Phe Ser Asp Tyr Tyr Met Phe Trp Val Arg Gln Thr Pro Glu Lys
 50 55 60
 Arg Leu Glu Trp Val Ala Tyr Ile Ser Asn Gly Asp Gly Asn Thr Tyr
 65 70 75 80
 Tyr Pro Asp Thr Leu Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
 85 90 95
 Asn Asn Thr Leu Tyr Leu Gln Met Ser Arg Leu Lys Ser Glu Asp Thr
 100 105 110
 Ala Met Tyr Tyr Cys Ala Arg Ser Ile Ser Arg Tyr Phe Asp Val Trp
 115 120 125
 Gly Ala Gly Thr Thr 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 Val Met Thr Gln Ser
 145 150 155 160
 Pro Ala Thr Leu Ser Val Thr Pro Gly Asp Arg Val Ser Leu Ser Cys
 165 170 175
 Arg Ala Ser Gln Ser Ile Ser Asp Phe Leu His Trp Tyr Gln Gln Lys
 180 185 190
 Ser His Glu Ser Pro Arg Leu Leu Ile Lys Tyr Val Ser Gln Ser Ile
 195 200 205
 Ser Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Ser Asp Phe
 210 215 220
 Thr Leu Ser Ile Asn Ser Val Glu Pro Glu Asp Val Gly Val Tyr Tyr
 225 230 235 240
 Cys Gln Asn Ala His Ser Phe Pro Ser Thr Phe Gly Gly Gly Thr Lys
 245 250 255
 Leu Glu Ile Lys Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro
 260 265 270
 Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro
 275 280 285
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr
 290 295 300

eo1f-seq1.txt

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 305 310 315 320
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 325 330 335
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 340 345 350
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 355 360 365
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 370 375 380
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 385 390 395 400
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 405 410 415
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 420 425 430
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 435 440 445
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 450 455 460
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 465 470 475 480
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr Ile Trp Ala
 485 490 495
 Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr
 500 505 510
 Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln
 515 520 525
 Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser
 530 535 540
 Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys
 545 550 555 560
 Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
 565 570 575

eo1f-seq1.txt

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
580 585 590

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg
595 600 605

Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met
610 615 620

Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly
625 630 635 640

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
645 650 655

Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
660 665

<210> 142

<211> 118

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m9E2

<400> 142

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

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

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

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

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

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

Ala Arg Ser Gly Tyr Arg Tyr Gly Phe Val Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ala

115

<210> 143

<211> 112

<212> PRT

<213> artificial sequence

<220>

<223> VL chain of m9E2

<400> 143

Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Thr Leu Gly
 1 5 10 15

Thr Ser Ala Ser Ile Ser Cys Arg Ser Ser Lys Ser Leu Leu His Ser
 20 25 30

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

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

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Asn
 85 90 95

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

<210> 144

<211> 115

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m28B9

<400> 144

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

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

Tyr Leu His Trp Met Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Ser Asp Pro Lys Phe
 Page 137

50

55

Gln Asp Lys Ala Thr Ile Asp Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

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

Ala Lys Gly Pro Trp Phe Pro Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

<210> 145

<211> 107

<212> PRT

<213> artificial sequence

<220>

<223> VL chain of m28B9

<400> 145

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

Glu Ser Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

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

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

Ser Gly Ser Gly Thr Lys Phe Ser Phe Lys Ile Ser Ser Leu Gln Ala
65 70 75 80

Glu Asp Phe Val Ser Tyr His Cys Gln Gln Leu Tyr Arg Thr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 146

<211> 120

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m26D8

<400> 146

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Met Val Arg Pro Gly Ala
Page 138

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1              5              10              15
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
      20      25      30
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
      35      40      45
Gly Lys Ile Asp Pro Ser Asp Ser Glu Thr His Tyr His Gln Lys Phe
      50      55      60
Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
      65      70      75      80
Leu Gln Phe Lys Ser Leu Thr Ser Glu Asp Ser Ala Val His Tyr Cys
      85      90      95
Ala Arg Glu Gly Ile Trp Leu Arg Tyr Ala Met Asp Tyr Trp Gly Gln
      100      105      110
Gly Thr Ser Val Thr Val Ser Ser
      115      120

<210> 147
<211> 107
<212> PRT
<213> artificial sequence

<220>
<223> VL chain of m26D8

<400> 147
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1      5      10      15
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
      20      25      30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
      35      40      45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
      50      55      60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Val Ser Ser Val Gln Ser
      65      70      75      80
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ser Tyr Pro Tyr
      85      90      95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
      100      105

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eo1f-seq1.txt

<210> 148
 <211> 121
 <212> PRT
 <213> artificial sequence
 <220>
 <223> VH chain of m15D1
 <400> 148
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Met Ser Cys Arg Ala Ser Gly Tyr Ser Ile 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 Asp Pro Asp Asn Gly Ala Thr Asn Asn Asn Gln Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser 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 Ser Glu Gly Tyr Gln Tyr Tyr Tyr Ala Met Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 149
 <211> 112
 <212> PRT
 <213> artificial sequence
 <220>
 <223> VL chain of m15D1
 <400> 149
 Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro Val Ala Leu Gly
 1 5 10 15
 Thr Ser Ala Ser Ile Ser Cys Ser Ser Ser Lys Ser Leu Leu His Ser
 20 25 30
 Asn Gly Ile Thr Tyr Leu His Trp Tyr Leu Gln Arg Pro Gly Gln Ser
 35 40 45
 Pro Gln Leu Leu Ile Tyr Arg Met Ser Asn Leu Ala Ser Gly Val Pro
 50 55 60

eo1f-seq1.txt

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Ala Gln Met
85 90 95

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

<210> 150

<211> 120

<212> PRT

<213> artificial sequence

<220>

<223> VH chain of m23F2

<400> 150

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

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

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

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

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

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

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

Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 151

<211> 111

<212> PRT

<213> artificial sequence

<220>

<223> VL chain of m23F2

eo1f-seq1.txt

<400> 151
 Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ala Leu Gly
 1 5 10 15
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Phe
 20 25 30
 Gly Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
 35 40 45
 Lys Leu Leu Ile Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile His
 65 70 75 80
 Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Leu His Ser Arg
 85 90 95
 Glu Leu Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105 110