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(54) Title: COMBINATION COMPRISING ALLOGENEIC IMMUNE CELLS DEFICIENT FOR AN ANTIGEN PRESENT ON BOTH T-CELLS AND PATHOLOGICAL CELLS AND THERAPEUTIC ANTIBODY AGAINST SAID ANTIGEN

(57) Abstract: The present invention relates to a therapeutic combination of immune cells, preferably allogeneic non-alloreactive TCR-KO immune T cells, wherein a gene coding an antigen marker X present on both T-cells and pathological cells is inactivated and a corresponding therapeutic antibody specific for said antigen marker X, method for preparing the same and use in immunotherapy.



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**COMBINATION COMPRISING ALLOGENEIC IMMUNE CELLS DEFICIENT FOR AN
ANTIGEN PRESENT ON BOTH T-CELLS AND PATHOLOGICAL CELLS AND
THERAPEUTIC ANTIBODY AGAINST SAID ANTIGEN**

5 Field of the invention

The present invention relates to a therapeutic combination of immune cells, preferably allogeneic non-alloreactive TCR-KO immune T cells, wherein a gene coding an antigen marker X present on both T-cells and pathological cells is inactivated and a corresponding therapeutic antibody specific for said antigen marker X, method for preparing the same and use in
10 immunotherapy.

The present invention also relates to rare cutting endonucleases (ex: Cas9/CRISPR, meganucleases, Zinc-finger nucleases or TAL nucleases, specific for the gene encoding said antigen marker, their use for making allogeneic therapeutic cells, alone or in combination with a therapeutic antibody specific for said marker antigen. The engineered cells can be used in
15 immune T cell depleted hosts, in the presence of said corresponding therapeutic antibody. The invention opens the way to standard and affordable adoptive immunotherapy strategies using in particular T-cells resistant to treatment with anti-T cells therapeutic antibody, for treating a cancer, an infection and an auto-immune disease.

Background of the invention

20 Adoptive immunotherapy, which involves the transfer of antigen-specific T cells generated ex vivo, is a promising strategy to treat viral infections and cancer. The T cells used for adoptive immunotherapy can be generated either by expansion of antigen-specific T cells or redirection of T cells through genetic engineering (Park, Rosenberg et al. 2011, see, e.g., Brenner et al., Current Opinion in Immunology, 22(2): 251-257 (2010). A Genetic modification
25 consists in expressing chimeric antigen receptors (CARs) in immune T cells. CAR are fusion proteins comprised of an antigen recognition moiety and T cell activation domains (see, e.g., Eshhar et al., Proc. Natl. Acad. Sci. USA, 90(2): 720-724 (1993), and Sadelain et al., Current Opinion in Immunology, 21(2): 215-223 (2009)). Often, the antigen recognition moiety of a CAR consists of an antigen-binding domain of a single-chain antibody (scFv), comprising the
30 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 successfully redirect T cell cytotoxicity, however, they failed to provide prolonged expansion and anti-tumor activity in vivo. Signaling
35 domains from co-stimulatory molecules including CD28, OX-40 (CD134), and 4-1BB (CD137)

added alone (second generation) or in combination (third generation) to the cytoplasmic domain of CARs enhance survival and increase proliferation of CAR modified T cells. Other domains seem to be determinant in the capacity of CARs to bind their target such as the hinge or stalk domain.

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 and expanded in vitro in order to amplify the number of cells 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 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 consequently 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, 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.

20 Ideally, one would like to use a standardized therapy in which allogeneic therapeutic cells could 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. The use of allogeneic cells may nevertheless encounter 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 or compatible hosts, allogeneic cells are able to engraft, but their endogenous T-cell receptors (TCR) specificities may still recognize the host tissue as foreign, resulting in graft versus host disease (GvHD), which can lead to serious tissue damage and death.

30 In order to provide less alloreactive allogeneic T-cells, preferably allogeneic non-alloreactive TCR-KO immune T cells, the inventors previously disclosed a method to genetically engineer immune cells, in which different effector genes, in particular those encoding T-cell receptors, were inactivated by using specific TAL-nucleases, better known under the trade mark TALENTM (Cellestis, 8, rue de la Croix Jarry, 75013 PARIS). This

method has proven to be highly efficient in primary cells using RNA transfection as part of a platform allowing the mass production of allogeneic T-cells (WO 2013/176915).

Effector cells and pathological cells may express a number of common antigens such as those described in WO2015121454A1.

Thus, 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 on 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).

CD38 protein is also 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).

The cell surface glycoprotein CS1 (also referred in the literature as SLAMF7, CD319 or CRACC—NCBI Reference Sequence: NP_067004.3) is highly and ubiquitously expressed on the surface of non-pathological (normal) lymphocytes and myeloma cells (Hsi ED, et al. *Clin Cancer Res* 2008 14:2775-84). CS1 is expressed at very low levels in the majority of immune effector cells, including natural killer (NK) cells, some subsets of T cells, and normal B cells, and is almost undetectable on myeloid cells (Hsi ED, et al. *Clin Cancer Res* 2008 14:2775-84). Notably, CS1 is negligibly expressed in human hematopoietic stem cells (Hsi ED, et al. *Clin Cancer Res* 2008 14:2775-84), which can be used for stem cell transplantation to treat hematologic malignancies, including MM. The functions of CS1 in MM remain incompletely understood, and it has been documented that CS1 may play a role in myeloma cell adhesion, clonogenic growth, and tumorigenicity (Benson D M Jr, et al. *J Clin Oncol* 2012 30:2013-5; Tai Y T, et al. *Blood* 2009 113:4309-18).

Similarly, cluster of Differentiation 70 (CD70, CD27LG or TNFSF7) is a member of the tumor necrosis factor (TNF) superfamily and the ligand for CD27, a TNF superfamily receptor

which is expressed on normal T cells and may be over expressed in pathological cells. The transient interaction between CD27 and CD70 provides T cell costimulation complementary to that provided by CD28. CD70 is expressed on hematological cancers such as Non-Hodgkin's Lymphoma and Hodgkin's disease as well as on solid tumors such as Glioblastoma and Renal Cell Carcinoma; with its expression on Renal Cell Carcinoma being nearly uniform (see e.g., Grewal I., et al., Expert Opinion on Therapeutic Targets, 12(3): 341-351 (2008)). CD70 antibodies are currently used to induce or produce a lymphodepletion, or to treat particular diseases (see Cancer Res 2006; 66(4): 2328-37).

Expression, even transient, of such antigens on T cells may be an obstacle during the manufacturing and production of CAR T cells due to potential target-driven T cell differentiation, exhaustion, and fratricide. To palliate this, the inventors previously disclosed genetically engineer T-cells in which, a gene encoding an antigen X expressed on both T cells and pathological cells was inactivated by using specific TAL-nucleases. In these cells, a TCR subunit may be also inactivated so that cell surface expression of endogenous TCR remains undetectable as described in WO2016142532A1 which is incorporated here in its entirety.. A CAR which gene is inserted into the genome of these cells can both, inhibit cell surface expression of the TCR and specifically redirects said cells against the antigen X while providing cells with a TCR-like mediated activity.

The inventors now disclosed a combination of said engineered cell with a therapeutic antibody specific for X. The specificity of said therapeutic antibody, for the same antigen X expressed on T cells and pathological cells, targeted by the CAR and which gene is inactivated in effector allogeneic T cells makes the combination especially efficient and fast in reducing the tumoral mass as compared to either T cells or therapeutic antibody alone.

Surprisingly, the combination of drugs of the invention allows, a better circumscription of cancer cells due to the possibility of accessing different compartments in the body where cancer cells migrated and niched, as compared to individuals treated with CART cells or the antibody specific for the corresponding antigen, alone.

Targeting the same antigen marker in cancer cells using two different pathways seem also to allow reducing significantly the amount of undesired cellular immune response (such as cytokine release syndrome).

Summary of the invention

In general, the present invention discloses a combination of immune cells comprising an inactivated antigen marker X, and a therapeutic antibody specific for X, with X being expressed on both immune cell and pathological cells. This combination is provided for the

treatment of a disease mediated by pathological cells or tissues expressing X or over expressing X as compared to normal tissue.

In particular embodiments, inactivated means that a gene encoding a protein (X) in immune cells is inactivated such as X is not expressed at the cell surface or is expressed and inactive.

5 In combination with a therapeutic antibody specific for said antigen marker X, engineered immune cells are particularly efficient in reducing the tumor mass, reducing or preventing the escape or relapse of immune cells. Examples of such antigen markers X are found in Table 4 to 14. An illustration of such antigen marker is CD38, CS1 or CD70. By antigen marker is meant the whole protein or an immune-reactive fragment thereof.

10 The present invention provides a combination comprising an immune cell comprising at least one inactivated gene coding a cell surface antigen X, with X being expressed on both immune cell and pathological cell and a therapeutic antibody specific for said cell surface antigen X.

The present invention provides a combination comprising engineered immune cells, or a population of engineered immune cells, comprising at least one inactivated gene coding a cell surface antigen X, with X being expressed on both immune cells and pathological cells, and a therapeutic antibody specific for said cell surface antigen X, preferably a homogenous population of immune cells, more preferably a homogenous population of immune cells expressing less than 90 % X at the cell surface as compared to non engineered immune cells. In preferred embodiments, the immune cells comprise an inactivated alpha and /or beta TCR gene(s) and/or inactivated beta 2 Microglobulin gene and an inactivated antigen marker X gene, and a therapeutic antibody specific for X, with X being expressed on both immune cell and pathological cells, preferably a homogenous population of immune cells, more preferably a homogenous population of immune cells expressing less than 90 % alpha beta TCR and less than 90 % X at the cell surface as compared to non engineered immune cells.

20 The combination of the invention comprises an immune cell comprising at least one inactivated gene coding a cell surface antigen, said at least one inactivated gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen and

a) a therapeutic antibody specific for said cell surface antigen.

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Preferably, the present invention provides a combination comprising :

a) an immune T cell comprising at least one inactivated gene coding a cell surface antigen, said at least one inactivated gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen and

35

b) a therapeutic antibody specific for said cell surface antigen.

The present invention provides a combination comprising :

- a) an immune T cell comprising at least one inactivated gene coding a cell surface antigen, and
- b) a therapeutic antibody specific for said cell surface antigen.

5 In other embodiments the present invention provides a combination comprising

- a) an immune T cell comprising at least one inactivated gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen and
- b) a therapeutic antibody specific for said cell surface antigen,

10 provided that said gene is not a gene coding a component of the alpha beta TCR which modification inhibits cell surface expression of the alpha beta TCR, such as a gene coding the constant part of the TCR alpha subunit (TRAC A gene), and said therapeutic antibody is not an antibody selectively binding to an antigen present at the surface of alpha beta TCR positive ($\alpha\beta$ TCR+) cells.

15 In another embodiment the present invention provides a combination comprising

- a) an immune T cell comprising at least one inactivated gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen and
- b) a therapeutic antibody specific for said cell surface antigen,

20 provided that is excluded: if said gene is a gene coding a component of the alpha beta TCR which modification inhibits cell surface expression of the alpha beta TCR, such as a gene coding the constant part of the TCR alpha subunit (TRAC A gene), said therapeutic antibody is an antibody selectively binding to an antigen present at the surface of alpha beta TCR positive ($\alpha\beta$ TCR+) cells

25 When generating "off the shelf" engineered immune cells, the invention generally concerns a combination for immunotherapy comprising :

- a) an allogeneic immune T cell(s) comprising at least one inactivated gene coding a cell surface antigen X, said at least one inactivated gene coding a cell surface antigen X comprising a genetic modification affecting cell surface expression of said cell surface antigen X and
- b) a therapeutic antibody specific for said cell surface antigen X, with X being expressed on both T immune cells and pathological cells.

30

An allogeneic immune T cell means an alpha beta TCR deficient immune T cell or cells
35 comprising more than 90 % TCR-negative T cells, preferably said allogeneic immune T cells

comprising a rare cutting endonuclease mediated TCR deficient gene, which product is not expressed at the cell surface.

In preferred embodiments, said allogeneic immune T cells comprise a TALEN-mediated TCR alpha inactivated gene, even more preferably said TALEN-mediated TCR alpha inactivated gene is inactivated by insertion of a polynucleotide into the constant part of the TCR alpha subunit (TRAC A gene), even more more preferably, said TALEN®-modified endogenous $\alpha\beta$ -TCR negative human primary T cell wherein the constant region of the genomic TCR gene (TRAC gene) comprises a genetic modification generated by a TALEN® and affecting cell surface expression of the endogenous alpha beta TCR, said genomic TRAC gene comprising from 5' to 3':

(a) a 5' region of said human genomic TRAC gene upstream,

(b) a recognition domain for a TALEN®,

(c) a gap or an insertion as compared to the wild type TRAC gene affecting the cell surface expression of the extracellular domain or transmembrane domain of the alpha beta TCR,

said insertion comprising an exogenous polynucleotide selected from a noncoding sequence such as, a stop codon, an IRES, a coding sequence such as a sequence coding for a self-cleaving peptide in frame with the TRAC open reading frame, a sequence coding a chimeric antigen receptor (CAR), a sequence coding a TCR, a sequence coding a protein conferring sensitivity to a drug, a sequence coding a protein conferring resistance to a drug, a cytokine, a termination sequence, a combination thereof,

(c') optionally a second TALEN® recognition domain,

(d) a 3' region of the genomic TRAC gene.

In more preferred embodiments said allogeneic T cell may be a human primary allogeneic T cell or a homogenous population of human primary allogeneic T cells, preferably a human primary CD8 allogeneic T cell, human primary CD4 allogeneic T cell, a combination thereof.

In even more preferred embodiments, a combination of the invention comprises a TALEN®-modified endogenous $\alpha\beta$ -TCR negative human primary allogeneic T cell comprising a recognition domain for a TALEN® comprising any one of the following sequences ttgtccacagATATC, ttgtccacagATATCCAG, CCGTGTACCAGCTGAGA, a combination thereof, and a TALEN®-inactivated antigen marker X gene, and a therapeutic antibody specific for X, with X being expressed on both immune cell and pathological cells,

In another embodiment the present invention provides a combination comprising

- a) an allogeneic immune T cell comprising at least one inactivated gene coding a cell surface antigen X comprising a genetic modification affecting cell surface expression of said cell surface antigen X and

- 5 b) a therapeutic antibody specific for said cell surface antigen X,

provided that said gene is not a gene coding a component of the alpha beta TCR which modification inhibits cell surface expression of the alpha beta TCR, such as a gene coding the constant part of the TCR alpha subunit (TRAC A gene), and said therapeutic antibody is not an antibody selectively binding to an antigen present at the surface of alpha beta TCR positive
10 ($\alpha\beta$ TCR+) cells.

Affecting cell surface expression of X means decreasing cell surface expression to undetectable level of X as compared to a positive control expressing said antigen X or inactivating the activity of said surface antigen X.

15

A genetic modification means a mutation, a deletion or an insertion, resulting in an inactivation (silencing) of a gene, and/ or inactivation of transcription, translation, and/or inactivation of protein expression, inactivation of protein expression at the cell surface, inactivation of the activity of the protein coded by said gene.

20 The following embodiments of the invention are provided:

2). The combination according to any one of item 1 and embodiments described above wherein said immune cell is an immune T cell, a hematopoietic stem cell, preferably a TCR negative immune T cell, a TCR negative hematopoietic stem cell.

In particular embodiments said immune T cell is a population of T cells, preferably homogenous
25 population of T cells.

A TCR negative immune cell means a cell comprising a TCR KO gene, or a TCR deficient gene, deficiency resulting in a disruption of cell surface expression of the TCR.

In the present invention alpha beta TCR deficient cells express less than 90%, preferably less than 95 % alpha beta TCR at the cell surface as determined by flow cytometry.

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Inhibition of cell surface expression of an antigen, means that in 80 to 90 %, preferably in more than 99%, even more preferably in more than 99.9% of total cells, cell surface expression of said antigen is undetectable by flow cytometry.

Particular embodiments encompass any one of the followings:

- 35 3) The combination according to item 1 or 2 wherein said immune cell is an immune T cell, immune NK T cell, immune CD8 T cell, immune CD4 T cells, or a T cell, preferably

an inflammatory T-cell, cytotoxic T-cell, regulatory T-cell or helper T-cell, more preferably Cytotoxic CD4 T- cell, Cytotoxic CD8 T- lymphocyte, even more preferably an immune T cell, and even more more more preferably a cytotoxic CD8 T lymphocyte.

- 4) The combination according to anyone of item 1 to 3 wherein said gene encoding an antigen marker X, is selected from any one of the genes in Table 4 to14, preferably CD38, CS1 and CD70, more preferably, CD38, or CS1.
- 5) The combination according to anyone of item 1 to 4 wherein said genetic modification comprises a mutation, an insertion or a deletion generated using a rare cutting endonuclease.
- 6) The combination according to any one of item 1 to 5 wherein said genetic modification is generated using a rare cutting endonuclease selected from a Meganuclease, a transcription activator-like (TAL)–nuclease, a zing-finger nuclease (ZFN), or a RNA/DNA guided endonucleases, preferably a transcription activator-like (TAL)—nuclease.
- 7) The combination according to any one of item 1 to 6 wherein said genetic modification is generated by a TAL-Nuclease, preferably a mRNA encoding a TAL-Nuclease.
- 8) The combination according to any one of item 1 to 7 wherein said genetic modification is an insertion and is generated by a TAL-Nuclease.
- 9) The combination according to any one of item 1 to 8 wherein said immune T cell express a recombinant TCR, a chimeric antigen receptor (CAR), a single chain CAR or a multichain CAR.
- 10) The combination according to any one of item 1 to 9 wherein said immune T cell express at least one CAR specific for a cell surface marker selected from any cluster of differentiation molecules (e.g. CD16, CD19, CD20, CD22, CD30, CD38, CD40, CD64, CD70, CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, 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), disialoganglioside GD2, o-acethyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, Flt3, WT1, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase,

ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGFI receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD123, HSP70, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, a cytokine receptor, 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, NEF, gp41); 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

11) The combination according to any one of item 1 to 10 wherein said immune T cell express a CAR which target specifically a cell surface marker selected from CD19, CD38, HSP70, CD30, FAP, HER2, CD79a, CD79b, CD123, CD22, CLL-1, MUC-1, GD2, O-acetyl-GD2, and CS1.

The combination according to any one of item 1 to 9 wherein said immune T cell express a CAR which target specifically a cell surface marker selected from CD38, HSP70, CD30, FAP, HER2, CD123, CD22, CS1, CLL-1.

The combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD38.

The combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD123.

The combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD22.

The combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CS1.

The combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CLL-1.

The combination according to any one of the above item 1 to 10 wherein said immune T cell express a recombinant TCR isolated from a tumor.

12) The combination according to any one of item 1 to 11 wherein said immune T cell express at least one CAR specific for a cell surface marker selected from any cluster of differentiation molecules (e.g. CD16, CD20, CD22, CD30, CD38, CD40, CD64,

CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, a tumor-associated surface antigen, such as ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), disialoganglioside GD2, o-acetyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGFI receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD34, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), GM-CSF, CD123, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, cytokine receptors, endoglin, a major histocompatibility complex (MHC) molecule, 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

13) The combination according to any one of item 1 to 10 wherein said immune T cell express a CAR which target specifically a cell surface marker selected from BCMA, CD33, EGFRVIII, Flt3, WT1, CD70, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10.

14) The combination according to any one of item 1 to 13 wherein said CAR comprises an extracellular binding domain, said extracellular binding domain comprising two, three or more than 3 mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab,

panitumumab, QBEND-10 and ustekinumab, preferably 2 mAb-specific epitopes specifically recognized by rituximab, or 1 mAb-specific epitopes specifically recognized by QBEND-10 and 3 mAb-specific epitopes specifically recognized by rituximab.

15) The combination according to any one of item 1 to 14 wherein said immune T cell is further engineered by genetically inactivating at least one gene encoding a components of the T-cell receptor (TCR) and/or affecting the expression of molecule of the HLA complex.

The combination according to any one of item 1 to 14 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells.

16) The combination according to any one of item 1 to 15 wherein a sequence encoding a CAR is inserted into the constant region of the TCR alpha gene or into the at least one gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen.

17) The combination according to any one of item 1 to 16 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alpha TCR, beta1 TCR, beta2 TCR.

18) The combination according to any one of item 1 to 16 wherein said immune T cell is further engineered by genetically inactivating a gene selected from beta2microglobulin, regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X-associated protein (RFXAP), and class II transactivator (CIITA), TAP-1, a combination thereof.

The combination according to any one of item 1 to 17 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and beta2microglobulin.

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor X-associated ankyrin-containing protein (RFXANK) gene.

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor 5 (RFX5) gene.

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor X-associated protein (RFXAP) gene.

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and class II transactivator (CIITA).

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and TAP-1 gene.

19) The combination according to any one of items 1 to 18 further engineered to resist hypoxia, preferably wherein said CAR comprises an intracellular domain conferring resistance to hypoxia such as at least one HIF 1 alpha domain.

20) The combination according to any one of items 1 to 19 further engineered to resist tumor-inducing inhibition of immune cells.

21) The combination according to any one of items 1 to 20 further engineered to resist tumor-inducing inhibition anti-tumor activity of immune cells mediated by any one of the following molecules, Programmed Death 1 (PD-1), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), LAG3 Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, and 2B4.

22) The combination according to anyone of 1 to item 21 comprising immune T cells with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody, preferably an anti-CD70 therapeutic antibody selected from Vorsetuzumab, Vorsetuzumab mafodotin, SGN CD70A, MDX 1203, ARGX 110, preferably an anti-CD70 therapeutic antibody ARGX-110 or MDX 1203.

23) The combination according to item 22 wherein said genetic modification is generated using a transcription activator-like (TAL)-nuclease specific for CD70, comprising at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103, comprising at least 95% homology with SEQ ID NO 104 and SEQ ID NO 105, comprising at least 95% homology with SEQ ID NO 106 and SEQ ID NO 107 specific for the sequences SEQ ID NO 73 and SEQ ID NO 74; SEQ ID NO 76 and SEQ ID NO 77; SEQ ID NO 79 and SEQ ID NO 780, respectively.

24) The combination according to anyone of item 1 to 21 comprising an immune T cell with a genetic modification of the CS-1 gene affecting cell surface expression of CS1 and an anti-CS-1 therapeutic antibody, preferably an anti-CS1 therapeutic antibody selected from Elotuzumab and ABBV838.

25) The combination according to item 24 wherein said genetic modification is generated using a transcription activator-like (TAL)-nuclease specific for CS1, comprising at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; SEQ ID NO 110 and SEQ ID NO 111; or SEQ ID NO 112 and SEQ ID NO 113 specific for the sequences: SEQ

ID NO 64 and SEQ ID NO 65; SEQ ID NO 67 and SEQ ID NO 68; SEQ ID NO 70 and SEQ ID NO 71, respectively.

26) The combination according to anyone of item 1 to 21 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody, preferably an anti-CD38 therapeutic antibody selected from Daratumumab HuMax-CD38;– Isatuximab HuMax-CD38, MOR202, MOR-03087, EDC8, and GBR 1342.

27) The combination according to item 26 wherein said genetic modification is generated using a transcription activator-like (TAL)-nuclease specific for CD38, comprising at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; SEQ ID NO 116 and SEQ ID NO 117; or SEQ ID NO 118 and SEQ ID NO 119 specific for a sequence having at least 95% homology with SEQ ID NO 1, SEQ ID NO 4; SEQ ID NO 7, respectively.

28) A pharmaceutical composition comprising a combination according to any one of item 1 or 27 and a pharmaceutically acceptable vehicle.

29) The combination according to any of the item 1 to 27 or the pharmaceutical composition according to item 28 for use in the treatment of a cancer, an infection or an autoimmune disease, preferably a cancer expressing or over expressing said cell surface antigen targeted by said therapeutic antibody, or relapse or refractory forms of said expressing or over expressing cancers.

30) The combination or the pharmaceutical composition for use according to item 29 as a treatment in a lymphodepleted and/or irradiated patient.

31) The combination or the pharmaceutical composition for use according to item 29 or 30 in the treatment or prophylaxis of cancer, wherein said cancer is selected from a hematopoietic or solid cancer, their relapse, refractory or metastatic forms.

32) The combination or the pharmaceutical composition for use according to any one of item 29 to 31 for the treatment of Renal cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia (AML), Multiple Myeloma (MM), diffuse large- cell lymphoma, follicular lymphoma and Non-Small Cell Lung Cancer.

33) The combination or the pharmaceutical composition for use according to any one of item 29 to 31 in the treatment or prophylaxis of Multiple myeloma (MM), Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Acute lymphoblastic leukemia (ALL), Hodgkin lymphoma (HL) (relapsed, refractory), Non-Hodgkin lymphoma (NHL) (relapsed, refractory), Neuroblastoma, Ewing sarcoma, Myelodysplastic syndromes, BPDCN.

34) The combination or the pharmaceutical composition for use according to any one of item 29 to 31 in the treatment or prophylaxis of Gliomas, pancreatic cancer, lung cancer, bladder cancer, colon cancer, breast cancer.

35) The combination or the pharmaceutical composition for use according to any one of item 29 to 31 for the treatment of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphoma, lymphoplasmacytic NHL, or marginal zone NHL); Hodgkin's disease (e.g., Reed-Sternberg cells); a cancer of the B-cell lineage, including, e.g., diffuse large B-cell lymphoma, follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, Cutaneous T cell lymphoma, B-cell lymphocytic leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphoma; renal cell carcinoma (e.g., clear cell and papillary); nasopharyngeal carcinoma; thymic carcinoma; glioma; glioblastoma; neuroblastoma; astrocytoma; meningioma; Waldenstrom macroglobulinemia; multiple myeloma; colon cancer, stomach cancer, and rectal carcinoma.

36) A combination according to any one of item 1 to 23 comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody ARGX 110, for use in the treatment of Cutaneous T cell lymphoma.

37) The combination to any one of item 1 to 23 comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody MDX 1203 for use in the treatment of Renal Cell Carcinoma or Non-hodgkin's Lymphoma.

CD70-expressing cancers that can be treated or prevented using the combination of the invention, for example, different subtypes of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphomas, lymphoplasmacytic NHLs, or marginal zone NHLs); Hodgkin's disease (e.g., Reed-Sternberg cells); cancers of the B-cell lineage, including, e.g., diffuse large B-cell lymphomas, follicular lymphomas, Burkitt's lymphoma, mantle cell lymphomas, B-cell lymphocytic leukemias (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphomas; renal cell carcinomas (e.g., clear cell and papillary); nasopharyngeal carcinomas; thymic carcinomas; gliomas; glioblastomas; neuroblastomas; astrocytomas; meningiomas; Waldenstrom macroglobulinemia; multiple myelomas; and colon, stomach, and rectal carcinomas. The cancer can be, for example, newly diagnosed, pre-treated or refractory or relapsed. In preferred embodiments, a CD38-expressing cancer has at least about 15,000, at least about 10,000 or at least about 5,000 CD38 molecules/cell.

In preferred embodiments, a CS1-expressing cancer has at least about 15,000, at least about 10,000 or at least about 5,000 CS1 molecules/cell.

In preferred embodiments, a CD70-expressing cancer has at least about 15,000, at least about 10,000 or at least about 5,000 CD70 molecules/cell.

5

38) The combination according to any one of item 1 to 21, 24 and 25 for the treatment of carcinoma, blastoma, and sarcoma, preferably MM, leukemia, melanoma, relapse or refractory CS-1 expressing MM or a complication related to CS-1 expressing MM.

10

39) The combination according to any one of item 1 to 21, 24 and 25 for the treatment of any one of the following CS1 expressing cancers: Multiple Myeloma, Acute Myeloid Leukemia, Myelodysplastic Syndrome, Smoldering Multiple Myeloma, monoclonal gammopathy of unknown significance (MGUS), plasma cell leukemia, Non-Hodgkin's Lymphoma.

15

40) The combination according to any one of item 1 to 21, 24 and 25 for the treatment of any one of the following CS1 expressing cancers: Multiple Myeloma, Non-Hodgkin Lymphoma, Diffuse Large B Cell Lymphoma, Mantle-Cell Lymphoma, Follicular Lymphoma, Indolent B Cell Lymphoma, Primary Mediastinal Lymphoma, Lymphoplasmacytic Lymphoma.

20

41) The combination according to any one of item 1 to 21, 24 and 25 for the treatment of any one of the following CS1 expressing cancers : NK cell lymphoma, NK or T cell lymphoma, angioimmunoblastic T-cell lymphoma (AITL), or peripheral T cell lymphoma not otherwise specified (PTCL-NOS).

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Document WO2010051391 A1 is incorporated herein in its entirety as prior art allowing the combination of CS1 deficient engineered TCR negative T cells and a therapeutic antibody to be prepared and used for the treatment of NK cell lymphoma, NK or T cell lymphoma, angioimmunoblastic T-cell lymphoma (AITL), or peripheral T cell lymphoma not otherwise specified (PTCL-NOS).

30

42) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Isatuximab for the treatment of Prostate Cancer, Non-small Cell Lung Cancer, Plasma Cell Myeloma, MM, T-cell Type Acute Leukemia, Precursor T-lymphoblastic Lymphoma or Leukaemia, prostate cancer, Non-small Cell Lung Cancer.

35

43) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Daratumumab for the

treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer, Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma, Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.

44) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of T-cell Type Acute Leukemia-Precursor T, lymphoblastic Lymphoma, Leukaemia.

45) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of a CD38-expressing or CD 38 over expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).

46) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of hematologic cancer selected from the group of leukemia, lymphoma and multiple myeloma (MM).

47) The combination according to any one of item 1 to 21, 26 and 27 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of hematologic cancer selected from the group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic myelogenous leukemia (CML-BP).

48) The combination according to any one of item 1 to 21, 26 and 27 for the treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer,

Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma, Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.

49) The combination according to any one of item 1 to 21, 26 and 27 for the treatment of T-cell Type Acute Leukemia-Precursor T, lymphoblastic Lymphoma, Leukaemia.

50) The combination according to any one of item 1 to 21, 26 and 27 for the treatment of a CD38-expressing or CD 38 over expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).

51) The combination according to any one of item 1 to 21, 26 and 27 for the treatment of hematologic cancer selected from the group of leukemia, lymphoma and multiple myeloma (MM).

52) The combination according to any one of item 1 to 21, 26 and 27 for the treatment of hematologic cancer selected from the group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic myelogenous leukemia (CML-BP), leukemia, lymphoma and multiple myeloma.

Non-limiting examples of conditions associated with CD38 expression include but are not limited to, multiple myeloma (Jackson et al. (1988), Clin. Exp. Immunol. 72: 351-356), B-cell chronic lymphocytic leukemia (B-CLL) Durig et al. (2002), Leukemia 16: 30-5; Morabito et al. (2001), Leukemia Research 25: 927-32; Marinov et al. (1993), Neoplasma 40(6): 355-8; and Jelinek et al. (2001), Br. J. Haematol. 115: 854-61), acute lymphoblastic leukemia (Keyhani et al. (1999), Leukemia Research 24: 153-9; and Marinov et al. (1993), Neoplasma 40(6): 355-8), chronic myeloid leukemia (Marinov et al. (1993), Neoplasma 40(6): 355-8), acute myeloid leukemia (Keyhani et al. (1999),

Leukemia Research 24: 153- 9), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) in
5 blastic and all subtypes of these leukemias which are defined by morphological, histochemical and immunological techniques that are well known by those of skill in the art.

53. A method for treating a patient comprising:

10 -optionally diagnosing said patient for the presence of pathological cells expressing an antigen marker on the cell surface;

-Preparing a combination of a therapeutic antibody specific for an antigen marker expressed at the cell surface of pathological cells and a population of genetically modified immune cells wherein the gene(s) encoding said antigen marker is inactivated according to any of the item
15 1 to 27 or a pharmaceutical composition of item 28,

-Administering said genetically modified cells to said patient, before, after and/or concomitantly to said therapeutic antibody.

CS 1 (also known as SLAMF7, CRACC, 19A, APEX-1, FOAP 12, and 19A; GENBANK® Accession No. NM 021181.3, Ref. Boles et al, Immunogenetics, 52:302-
20 307 (2001); Bouchon et al, J. Immunol, 167:5517-5521 (2001); Murphy et al, Biochem. J., 361 :431-436 (2002)) is a member of the CD2 subset of the immunoglobulin superfamily. Molecules of the CD2 family are involved in a broad range of immunomodulatory functions, such as co-activation, proliferation differentiation, and adhesion of lymphocytes, as well as immunoglobulin secretion, cytokine production,
25 and NK cell cytotoxicity. Several members of the CD2 family, such as CD2, CD58, and CD 150, play a role or have been proposed to play a role in a number of autoimmune and inflammatory diseases, such as psoriasis, rheumatoid arthritis, and multiple sclerosis. It has been reported that CS 1 plays a role in NK cell-mediated cytotoxicity and lymphocyte adhesion (Bouchon, A. et al, J. Immunol, 5517-5521 (2001); Murphy,
30 J. et al, Biochem. J., 361 :431-436 (2002)).

Elotuzumab is a humanized monoclonal IgG1 antibody directed against CS-1, a cell surface glycoprotein, which is highly and uniformly expressed in multiple myeloma. Elotuzumab induces significant antibody-dependent cellular cytotoxicity (ADCC)

against primary multiple myeloma cells in the presence of peripheral lymphocytes (Tai et al, Blood, 112: 1329-1337 (2008)). Results of three studies that evaluated the safety and efficacy of this drug administered alone (Zonder et al, Blood, 120(3):552-559 (2012)), in combination with bortezomib (Jakubowiak et al, J. Clin. Oncol., 30(16): 1960-1965 (Jun. 1, 2012)), or lenalidomide and low-dose dexamethasone (Lonial et al, J. Clin. Oncol., 30: 1953-1959 (2012); and Richardson et al, Blood (ASH Annual Meeting Abstracts), 116:986 (2010) for the treatment of patients with relapsed or refractory multiple myeloma, have been reported. All three combinations showed a manageable safety profile and encouraging activity. For example, a Phase VII study evaluating the safety and efficacy of Elotuzumab in combination lenalidomide and low-dose dexamethasone for the treatment of relapsed or refractory multiple myeloma demonstrated a 33 month PFS as well as a 92% response rate for patients receiving the 10 mg/kg dose (Lonial et al., J. Clin. Oncol., 31 (2013) (Suppl., Abstr. 8542)). Phase III clinical trials of lenalidomide/dexamethasone with or without Elotuzumab in previously untreated multiple myeloma patients is ongoing, while another phase III trial designed to evaluate this same combination in the first line setting is also ongoing.

54. A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; with SEQ ID NO 118 and SEQ ID NO 119; with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; with SEQ ID NO 106 and SEQ ID NO 107; with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178.

In particular embodiments a TAL-protein as any one of the TAL protein of item 54, is provided for use in the manufacturing of CART cells.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; or with SEQ ID NO 118 and SEQ ID NO 119, is provided for modifying the CD38 gene.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; or with SEQ ID NO 106 and SEQ ID NO 107, is provided for modifying the CD70 gene.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ

ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178, is provided for modifying the CS1 gene.

A polynucleotide or a vector encoding any one of the TAL protein above is provided.

A TAL- protein comprising a sequence of SEQ ID NO 114 and SEQ ID NO 115.

5 A TAL- protein comprising a sequence of SEQ ID NO 116 and SEQ ID NO 117.

A TAL- protein comprising a sequence of SEQ ID NO 118 and SEQ ID NO 119.

A TAL- protein comprising a sequence of SEQ ID NO 102 and SEQ ID NO 103.

A TAL- protein comprising a sequence of SEQ ID NO 104 and SEQ ID NO 105.

A TAL- protein comprising a sequence of SEQ ID NO 106 and SEQ ID NO 107.

10 A TAL- protein comprising a sequence of SEQ ID NO 108 and SEQ ID NO 109.

A TAL- protein comprising a sequence of SEQ ID NO 110 and SEQ ID NO 111.

A TAL- protein comprising a sequence of SEQ ID NO 112 and SEQ ID NO 113.

A TAL- protein comprising a sequence of SEQ ID NO 171 and SEQ ID NO 172.

A TAL- protein comprising a sequence of SEQ ID NO 173 and SEQ ID NO 174.

15 A TAL- protein comprising a sequence of SEQ ID NO 175 and SEQ ID NO 176.

A TAL- protein comprising a sequence of SEQ ID NO 177 and SEQ ID NO 178.

A TAL- protein according to item 54 comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; comprising a sequence having at least 95% homology with SEQ ID NO 116 and SEQ ID NO 117; comprising a sequence having at least 95% homology with SEQ ID NO 118 and SEQ ID NO 119; comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; comprising a sequence having at least 95% homology with SEQ ID NO 104 and SEQ ID NO 105; comprising a sequence having at least 95% homology with SEQ ID NO 106 and SEQ ID NO 107; comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; comprising a sequence having at least 95% homology with SEQ ID NO 110 and SEQ ID NO 111; comprising a sequence having at least 95% homology with SEQ ID NO 112 and SEQ ID NO 113; comprising a sequence having at least 95% homology with SEQ ID NO 171 and SEQ ID NO 172; comprising a sequence having at least 95% homology with SEQ ID NO 173 and SEQ ID NO 174; comprising a sequence having at least 95% homology with SEQ ID

NO 175 and SEQ ID NO 176; or comprising a sequence having at least 95% homology with SEQ ID NO 177 and SEQ ID NO 178 for use in the manufacturing of CART cells.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; with SEQ ID NO 118 and
5 SEQ ID NO 119; is provided for modifying the CD38 gene.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; with SEQ ID NO 106 and SEQ ID NO 107, is provided for modifying the CD70 gene.

A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 108
10 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113 with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178 is provided for modifying the CS1 gene.

A polynucleotide or a vector encoding a protein comprising any one of the TAL protein above
15 is provided.

A polynucleotide or a vector encoding any one of the TAL protein above is provided.

An engineered immune T cell comprising a CD38 modified gene obtained using a TAL- protein or a polynucleotide encoding a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; or with SEQ ID NO
20 118 and SEQ ID NO 119 is provided.

An engineered immune T cell comprising a CD70 modified gene obtained using a TAL- protein or a polynucleotide encoding a sequence having with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105 ; or with SEQ ID NO 106 and SEQ ID NO 107, is provided.

An engineered immune T cell comprising a CS1 modified gene obtained using a TAL- protein or a polynucleotide encoding a sequence having with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178 is provided.

55. A polynucleotide encoding a protein comprising any one of the TAL-protein of item 54.

56. A vector coding a protein comprising any one of the TAL protein of SEQ ID NO 102 and SEQ ID NO 103, SEQ ID NO 104 and SEQ ID NO 105, SEQ ID NO 106 and SEQ ID NO 107, SEQ ID NO 108 and SEQ ID NO 109, SEQ ID NO 110 and SEQ ID NO 111, SEQ ID NO 112 and SEQ ID NO 113, SEQ ID NO 114 and SEQ ID NO 115, SEQ ID NO 116 and
5 SEQ ID NO 117, SEQ ID NO 118 and SEQ ID NO 119, SEQ ID NO 171 and SEQ ID NO 172, SEQ ID NO 173 and SEQ ID NO 174, SEQ ID NO 175 and SEQ ID NO 176, or SEQ ID NO 177 and SEQ ID NO 178 for use in the manufacturing of CART cells.

57. A vector coding any one of the TAL protein of SEQ ID NO 102 and SEQ ID NO 103, SEQ ID NO 104 and SEQ ID NO 105, SEQ ID NO 106 and SEQ ID NO 107, SEQ ID NO 108 and
10 SEQ ID NO 109, SEQ ID NO 110 and SEQ ID NO 111, SEQ ID NO 112 and SEQ ID NO 113, SEQ ID NO 114 and SEQ ID NO 115, SEQ ID NO 116 and SEQ ID NO 117, SEQ ID NO 118 and SEQ ID NO 119, SEQ ID NO 171 and SEQ ID NO 172, SEQ ID NO 173 and SEQ ID NO 174, SEQ ID NO 175 and SEQ ID NO 176, or SEQ ID NO 177 and SEQ ID NO 178 for use in the manufacturing of CART cells.

15 58) A method of preparing a combination of

- 1) engineered T-cells, said engineered T cells comprising an inactivated gene coding an antigen marker, with
- 2) a therapeutic antibody specific for said antigen marker and expressed at the cell surface of a pathological cells comprising the step of:

20 (a) Genetically engineering a gene in a T-cell, which is involved in the expression or presentation of an antigen marker, said antigen marker being present both on the surface of said T-cell and the pathological cell; to inactivate said antigen marker and /or alter its binding to its receptor

25 (b) Expressing into said T-cells a transgene encoding a chimeric antigen receptor directed against said antigen marker present at the surface of said pathological cell.

(c) Combining said T-cells with a therapeutic antibody specific for said antigen marker

59) The method according to item 58, wherein said antigen marker is selected from one listed in Table 4 to 14.

30 60) The method according to item 58, wherein said antigen marker is CD38 or an immuno-reactive fragment thereof.

- 61) The method according to item 58, wherein said antigen marker is CD70 or an immuno-reactive fragment thereof.
- 62) The method according to item 58, wherein said antigen marker is CS1 or an immuno-reactive fragment thereof.
- 5 63) The method according to any one of items 58 to 62, wherein said method includes a further step of activating and expanding the T-cells.
- 64) A method according to any one of items 58 to 63, wherein said method includes a further step of purifying the resulting T-cells by excluding the cells presenting said marker antigen at their surface.
- 10 65) A method according to any one of items 58 to 64, wherein said method includes a previous step of procuring the T-cells from a donor.
- 66) A method according to any one of items 58 to 65, wherein said method includes a previous step of procuring the T-cells from a patient who is affected by the development of said pathological cells.
- 15 67) A method according to any one of items 58 to 66, wherein said T-cell is derived from a primary stem cell, iPS or hES cell.
- 68) A method according to item 67, wherein said T-cell is derived from iPS cell derived from said patient affected by the development of said pathological cells.
- 69) A method according to any one of items 58 to 68, wherein step a) is performed using a
20 rare-cutting endonuclease.
- 70) A method according to item 69, wherein step a) is performed using a TAL-nuclease.
- 71) A method according to item 70, wherein step a) is performed using a RNA-guided endonuclease
- 72) A method according to item 71, wherein the RNA-guided endonuclease is Cas9.
- 25 73) A method according to item 71, wherein RNA-guided endonuclease is split into at least 2 polypeptides, one comprising RuvC and another comprising HNH.
- 74) A method according to item 70, wherein said endonuclease is expressed from transfected mRNA.

75) A method according to any one of items 1 to 74, wherein said method includes a further step of inactivating a gene encoding a component of the T-cell receptor (TCR).

76) A method according to item 75, wherein said component of the T-cell receptor is TCR α .

5 77) A method according to any one of items 58 to 15, wherein said method includes a further step of inactivating a gene encoding a component of HLA.

78) A method according to any one of items 58 to 77, wherein said method includes a further step of inactivating a gene encoding β 2m.

10 79) A method according to any one of items 58 to 78, 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, TGFRBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB,
15 HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2 and GUCY1B3.

80) A method according to item 79, wherein said gene locus is involved into the expression of PD1 or CTLA-4 genes.

20 81) A method according to any one of items 58 to 80, wherein said method includes a further step of inactivating a gene conferring sensitivity of the T-cells to chemotherapy or immunosuppressive drugs.

82) The method according to item 81, wherein said further gene encodes CD52.

83) The method according to item 81, wherein said further gene is hypoxanthine-guanine phosphoribosyltransferase (HPRT).

25 84) The method according to item 81, wherein said further gene encodes a glucocorticoid receptor (GR).

85) The method according to item 81, wherein said further gene is involved in the DCK regulatory pathway, in particular DCK expression.

86) A method according to any one of items 1 to 85, wherein said T-cells in step a) are inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

87) The method according to item 86, wherein said T-cells are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.

88) A method according to any one of items 58 to 87, wherein said transformed T-cells are expanded in-vitro.

89) A method according to any one of items 58 to 88, wherein said transformed T-cells are expanded in-vivo.

90) A method according to any one of items 58 to 89, wherein said pathological cells are selected from malignant cells or infected cells.

91) A method according to any one of items 58 to 90, wherein said pathological cells are B-cells.

92) A method according to any one of items 58 to 91, wherein said pathological cells are solid tumor cells.

93) A method according to any one of items 58 to 92, for preparing T-cells to be used as a medicament.

94) A method according to item 93 for preparing T-cells for treating a cancer, an immune disease or an infection in a patient in need thereof.

95) A method according to item 94 for treating lymphoma.

96) A method according to item 94 for treating leukemia.

97) A method according to item 94 for treating chronic lymphocytic leukemia (CLL).

98) An engineered T-cell obtainable according to the method of any one of items 58 to 92.

99) A method for treating a patient comprising:

(a) Diagnosing said patient for the presence of pathological cells expressing at the cell surface an antigen marker in common with T-cells;

(b) preparing a population of engineered T-cells wherein cell surface expression of said antigen marker is undetectable,

(c) administering said engineered T-cells to said patient diagnosed for said pathological cells, concomitantly to the therapeutic antibody, before or after said therapeutic antibody.

According to the invention, the immune cells are engineered in order to inactivate a gene encoding an antigen marker or several antigens markers, or to inactivate the expression of the gene which product is involved in the presentation of such antigen marker on the cell surface.

Inactivation is preferably performed by a genome modification, more particularly through the expression, preferably transient, in the cell of a specific rare-cutting endonuclease able to target a genetic locus directly or indirectly involved in the production or presentation of said antigen marker at the surface of the cell. Different types of rare-cutting endonucleases can be used, such as Meganucleases, TAL-nucleases, zinc-finger nucleases (ZFN), or RNA guided endonucleases like Cas9/CRISPR. The specific rare-cutting endonucleases used here, Meganucleases, TAL-nucleases, zinc-finger nucleases (ZFN), and RNA guided endonucleases like Cas9/CRISPR are part of the present invention, their use for engineering cells for immunotherapy.

According to a preferred embodiment, the immune cells defective in at least one antigen marker X are endowed with at least one chimeric antigen receptors (CAR) allowing a specific binding of target cells bearing said targeted antigen marker X and the concomitant use of a therapeutic antibody targeting X.

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

The present invention encompasses the isolated cell, or cell lines, in particular isolated T-cells, or T 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 proteins, polynucleotides, vectors and engineered-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.

Brief description of the figures and tables

Figure 1: CD70 TALE-nucleases molecular in primary T-cells. Lines 1 and 3 correspond to exon1 and exon 2, respectively, from untreated cells. Line 2 corresponds to TALE-nuclease targeting CD70 exon1 treated cells (i.e. T02: SEQ ID NO. 104-105). Line 4 corresponds to TALE-nuclease targeting CD70 exon 2 treated T-cells (T03 SEQ ID NO. 106-107). Arrows are showing the PCR cleaved products.

Figure 2: Quantification of the percentage of CD70 positive cells (Figure 2A) and of the median fluorescence intensity (MFI) of CD70 positive cells (Figure 2B) at different time points post electroporation of untreated or TALE-nucleases treated T-cells.

Tables 4 to 14: Examples of antigen markers, which can be targeted with the engineered-cells of the invention for treating different types of cancer.

Table 5 to 13: Surface antigen markers expressed in T-cells, while being over-expressed in tumor cells from various types of cancer.

Table 5: colon tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 5 and a therapeutic antibody specific for X, for the treatment of colon cancer.

Table 6: breast tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 6 and a therapeutic antibody specific for X, for the treatment of breast cancer.

Table 7: digestive track tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 7 and a therapeutic antibody specific for X, for the treatment of digestive tract cancer.

Table 8: kidney tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers in table 8 and a therapeutic antibody specific for X, for the treatment of kidney cancer.

Table 9: liver tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 9 and a therapeutic antibody specific for X, for the treatment of liver cancer.

Table 10: lung tumor cells;

A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 10 and a therapeutic antibody specific for X, for the treatment of lung cancer.

Table 11: ovary tumor cells;

- 5 A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers in table11 and a therapeutic antibody specific for X, for the treatment of ovary cancer.

Table 12: pancreas tumor cells;

- 10 A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 12 and a therapeutic antibody specific for X, for the treatment of pancreas cancer.

Table 13: prostate tumor cells;

- 15 A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table 13 and a therapeutic antibody specific for X, for the treatment of prostate cancer.

Table 14: Main surface antigen markers expressed in T-cells, while being over-expressed in liquid tumor cells from various types of cancer (ALL, AML, CML, MDS, CLL, CTRL).

- 20 A combination comprising immune cells, comprising an inactivated antigen marker corresponding to any one of the antigen markers X in table14 and a therapeutic antibody specific for X, for the treatment of ALL, AML, CML, MDS, CLL, CTRL.

Detailed description of the invention

- 25 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.

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

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology,

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

In a general aspect, the present invention relates to new combinations of engineered immune cells with undetectable level of cell surface expression of an antigen marker X, with X usually expressed on normal non pathological immune cells, with a therapeutic antibody specific for X, for new adoptive immunotherapy strategies in treating diseases linked with the development of pathological cells expressing an antigen marker X, such as cancer cells, infected cells and cells involved in auto-immune diseases. An immune cell is a cytotoxic T cell which activation by an antigen results in lysis of the target cells.

The main objective of the invention is to target pathological cells that bear specific antigen markers in common with T-cells using two immune reagents, a therapeutic antibody and an engineered immune cell, with the particularity that both reagents target the same antigen which is normally expressed on normal immune cells and on pathological cells.

Thus, in individuals treated with the combination of the invention, an immunodepletion may be obtained but the use of immune cells may not be compromised as engineered immune cells of the present invention are resistant to the therapeutic antibody with which they are combined to and can therefore be used in an individual treated with said therapeutic antibody.

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.

Affecting cell surface expression means decreasing cell surface expression to undetectable level or inactivating the activity of said surface antigen.

5

In particular embodiments the invention provides a combination according to item 1 wherein said immune cell is an immune T cell, a hematopoietic stem cell, preferably a TCR negative immune T cell, a TCR negative hematopoietic stem cell.

10 In preferred embodiments said immune T cell is a population of T cells, preferably homogenous.

A TCR negative immune cell means a cell comprising a TCR KO gene, or a TCR deficient gene, deficiency resulting in a disruption of cell surface expression of the TCR. Preferably said TCR is an alpha beta TCR.

15 Inhibition of cell surface expression means that in 80 to 90 %, preferably in more than 99%, even more preferably in more than 99.9% of total cells, cell surface expression of said antigen is undetectable by flow cytometry.

20 In particular embodiments the invention provides a combination comprising an engineered immune CD8 T cell, immune CD4 T cells, inflammatory T-cell cytotoxic T-cell, regulatory T-cell or helper T-cell. Cytotoxic CD4 T- cell, Cytotoxic CD8 T- lymphocyte.

25 In particular embodiments the invention provides a combination according to anyone of item 1 to 3 wherein said gene is selected from any one of the genes in Table 4 to 14. A genetic modification means a mutation, an insertion or a deletion generated using a rare cutting endonuclease. Insertion comprises a polynucleotide encoding a gene, such as a CAR, a gene conferring resistance to a drug, sensitivity to a drug, encoding a cytokine.

In particular embodiments the invention provides a combination wherein said genetic modification is generated by a TAL-Nuclease, preferably a mRNA encoding a TAL-Nuclease.

30 In particular embodiments the invention provides a combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD38.

In particular embodiments the invention provides a combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD123.

35 In particular embodiments the invention provides a combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CD22.

In particular embodiments the invention provides a combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CS1.

In particular embodiments the invention provides a combination according to any one of item 11 wherein said immune T cell express a CAR targeting specifically CLL-1.

- 5 In particular embodiments the invention provides a combination according to any one of the above wherein said immune T cell express a recombinant TCR isolated from a tumor. In particular embodiments the invention provides a combination wherein said immune T cell express a CAR which target specifically a cell surface marker selected from BCMA, CD33, EGFRVIII, Flt3, WT1, CD70, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-
10 1, CHRNA2 and ADAM10.

- In particular embodiments the invention provides a combination wherein said CAR comprises an extracellular binding domain, said extracellular binding domain comprising two, or three mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab,
15 alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10 and ustekinumab, preferably 2 mAb-specific epitopes specifically recognized by rituximab, or 1 mAb-specific epitopes specifically recognized by
20 QBEND-10 and 3 mAb-specific epitopes specifically recognized by rituximab.

In most preferred embodiments the invention provides a combination according to any one of those disclosed herein wherein said immune T cell is or was further engineered by genetically inactivating at least one gene encoding a component of the T-cell receptor (TCR) and/or affecting the expression of molecule of the HLA complex.

- 25 Thus, the invention provides a combination wherein said immune T cell is engineered to be less alloreactive as compared to non-engineered cells and therefore more specific for the pathological cells intended to be treated or destroyed.

- The combination according to any one of those above wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically
30 inactivating the alphaTCR and beta2microglobulin genes,

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor X-associated ankyrin-containing protein (RFXANK) gene,

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor 5 (RFX5) gene,

5 The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and regulatory factor X-associated protein (RFXAP) gene,

The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and class II transactivator (CIITA),

10 The combination according to any one of item 1 to 15 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alphaTCR and TAP-1 gene.

In particular embodiments the invention provides a combination according to any one of the
15 above further engineered to resist hypoxia, preferably wherein said CAR or said cells comprises a domain conferring resistance to hypoxia such as at least one HIF 1 alpha domain. The combination according to the present invention further engineered to resist tumor-inducing inhibition anti-tumor activity of immune cells mediated by any one of the following molecules, Programmed Death 1 (PD-1), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), LAG3 Tim3, BTLA,
20 BY55, TIGIT, B7H5, LAIR1, SIGLEC10, and 2B4.

CD70-expressing cancers that can be treated or prevented using the combination of the invention , for example, different subtypes of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphomas, lymphoplasmacytic NHLs, or marginal zone
25 NHLs); Hodgkin's disease (e.g., Reed-Sternberg cells); cancers of the B-cell lineage, including, e.g., diffuse large B-cell lymphomas, follicular lymphomas, Burkitt's lymphoma, mantle cell lymphomas, B-cell lymphocytic leukemias (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphomas; renal cell carcinomas (e.g., clear cell and papillary); nasopharyngeal carcinomas; thymic carcinomas; gliomas;
30 glioblastomas; neuroblastomas; astrocytomas; meningiomas; Waldenstrom macroglobulinemia; multiple myelomas; and colon, stomach, and rectal carcinomas. The cancer can be, for example, newly diagnosed, pre-treated or refractory or relapsed. In some embodiments, a CD70-expressing cancer has at least about 15,000, at least about 10,000 or at least about 5,000 CD70 molecules/cell.

Document WO2010051391 A1 is incorporated herein in its entirety as prior art allowing the combination of CS1 deficient engineered TCR negative T cells and a therapeutic antibody to be prepared and used for the treatment of NK cell lymphoma, NK or T cell lymphoma, angioimmunoblastic T-cell lymphoma (AITL), or peripheral T cell lymphoma not otherwise specified (PTCL-NOS).

Non-limiting examples of conditions associated with CD38 expression include but are not limited to, multiple myeloma (Jackson et al. (1988), Clin. Exp. Immunol. 72: 351-356), B-cell chronic lymphocytic leukemia (B-CLL) Durig et al. (2002), Leukemia 16: 30-5; Morabito et al. (2001), Leukemia Research 25: 927-32; Marinov et al. (1993), Neoplasma 40(6): 355-8; and Jelinek et al. (2001), Br. J. Haematol. 115: 854-61), acute lymphoblastic leukemia (Keyhani et al. (1999), Leukemia Research 24: 153-9; and Marinov et al. (1993), Neoplasma 40(6): 355-8), chronic myeloid leukemia (Marinov et al. (1993), Neoplasma 40(6): 355-8), acute myeloid leukemia (Keyhani et al. (1999), Leukemia Research 24: 153-9), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) in blastic and all subtypes of these leukemias which are defined by morphological, histochemical and immunological techniques that are well known by those of skill in the art.

"Neoplasm" or "neoplastic condition" or "cancer" refers to a condition associated with proliferation of cells characterized by a loss of normal controls that results in one or more symptoms including, unregulated growth, lack of differentiation, local tissue invasion, and metastasis. In some embodiments of the invention, the hematologic cancer expressing CD38 is a cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL). Furthermore, it is known in the art that CD38 expression is a prognostic indicator for patients with conditions such as, for example, B-cell chronic lymphocytic leukemia (Durig et al. (2002), Leukemia 16: 30-5; and Morabito et al. (2001), Leukemia Research 25: 927-32) and acute myelogenous leukemia (Keyhani et al. (1999), Leukemia Research 24: 153-9). The combination of the invention comprising CD38 negative engineered cells and anti-CD38 therapeutic antibody is intended to be administered to patients with these particular conditions.

CS 1 (also known as SLAMF7, CRACC, 19A, APEX-1, FOAP 12, and 19A; GENBANK® Accession No. NM 021181.3, Ref. Boles et al, Immunogenetics, 52:302-307 (2001); Bouchon et al, J. Immunol, 167:5517-5521 (2001); Murphy et al, Biochem. J., 361 :431-436 (2002)) is a member of the CD2 subset of the immunoglobulin

superfamily. Molecules of the CD2 family are involved in a broad range of immunomodulatory functions, such as co-activation, proliferation differentiation, and adhesion of lymphocytes, as well as immunoglobulin secretion, cytokine production, and NK cell cytotoxicity. Several members of the CD2 family, such as CD2, CD58, and CD 150, play a role or have been proposed to play a role in a number of autoimmune and inflammatory diseases, such as psoriasis, rheumatoid arthritis, and multiple sclerosis. It has been reported that CS 1 plays a role in NK cell-mediated cytotoxicity and lymphocyte adhesion (Bouchon, A. et al, J. Immunol, 5517-5521 (2001); Murphy, J. et al, Biochem. J., 361 :431-436 (2002)).

The present invention provides a TAL- protein comprising a sequence having at least 95% homology with

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
SNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVV
AIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPALE
SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEK
KSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPD
GAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWK
VYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLT
LEEVRRKFNGEINFAAD (SEQ ID N0 114) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA

HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPAL
5 ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
TLEEVRRKFNNGEINFAAD (SEQ ID NO 115);

10 MGDPPKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
15 HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
20 ASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPAL
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
25 KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
TLEEVRRKFNNGEINFAAD (SEQ ID NO 116) and

MGDPKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALET
30 VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETV
QRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIAS
HDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
35 GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALET
VQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAI

SNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPALES
VAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEEKKS
ELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAI
5 YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYP
SSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEE
VRRKFNNGEINFAAD (SEQ ID NO 117);

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
10 LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
SNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
15 ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVV
AIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLC
QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPA
20 LESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
PDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEW
WKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
TLEEVRKFNNGEINFAAD (SEQ ID NO 118) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
30 AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
QAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVV
AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
35 QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPA

LESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL

5 TLEEVRRKFNNGEINFAAD (SEQ ID NO 119) ;

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI

10 ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQV
AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQA

15 LETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQV
VAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLC
QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRP
ALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELE
EKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
20 PDGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEW
WKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAG
TLTLEEVRRKFNNGEINFAAD (SEQ ID NO 102) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
25 LQLDTGQLLKIAGRGGTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI

ASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALET
VQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
30 SNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETV
QALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIAS
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPALESIV
35 AQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEKKSE
LRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY

TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPS
SVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEV
RRKFNNGEINFAAD (SEQ ID NO 103) ;

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL

5 SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALE
10 TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLC
15 QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPA
LESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKP
DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
20 TLEEVRRKFNNGEINFAAD (SEQ ID NO 104) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
25 ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
30 ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLC
QAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPAL
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKP
35 DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL

TLEEVRRKFNNGEINFAAD (SEQ ID NO 105) ;

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
5 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVV
AIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLC
10 QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQA
LETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLC
QAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE
15 KKSELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
DGAITYVGSPIIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
TLEEVRRKFNNGEINFAAD (SEQ ID NO 106) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
20 SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE
25 TVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
30 AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQAL
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE
KKSELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
DGAITYVGSPIIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
35 TLEEVRRKFNNGEINFAAD (SEQ ID NO 107) ;
MGDPKKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL

SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIA
SHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
5 HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALET
VQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
10 SNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALET
VAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEKKS
ELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAI
YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYP
15 SSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEE
VRRKFNNGEINFAAD (SEQ ID NO 108) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
20 TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
SNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
25 HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
SNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEK
30 KSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPD
GAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWK
VYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLT
LEEVRKFNNGEINFAAD (SEQ ID NO 109) ;

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
35 SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE

TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALET
VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
5 SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETV
QALLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIAS
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAH
GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALET
10 VAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEKKS
ELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAI
YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYP
SSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEE
VRRKFNNGEINFAAD (SEQ ID NO 110) and
15 MGDPPKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTPQVVAIASNIGGKQALET
VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
20 HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALET
VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIA
SNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
25 SNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
SIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEK
KSELRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPD
GAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWK
30 VYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLT
LEEVRKFNNGEINFAAD (SEQ ID NO 111);
MGDPPKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTPQQVVAIASNNGGKQALE
35 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA

HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
5 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEE
KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRK
10 DGAITYVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
TLEEVRRKFNNGEINFAAD (SEQ ID NO 112) and
MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
15 LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
20 ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALET
VQALLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIAS
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHG
LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQ
25 AQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQLVKSELEEKKSE
LRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIY
TVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPS
SVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEV
RRKFNNGEINFAAD (SEQ ID NO 113);
30 MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNIGGKQALET
VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQA
35 HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI

ASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQ
AHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASNIGGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLC
5 QAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPA
LESIVAQLSRPDPSGSGSGDPIRSQVLKSELEEKSELRHKLKYPHEYIELIEIAR
NSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSG
GYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNY
KAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ

10 ID NO 171) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPEQVVAI
15 ASNIGGGKQALETVQALLPVLCQAHGLTPEQVVAIASNIGGGKQALETVQALLPVLCQA
HGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGGKQALET
VQALLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
SNIGGGKQALETVQALLPVLCQAHGLTPEQVVAIASNIGGGKQALETVQALLPVLCQAH
GLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALET
20 VQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIA
SNNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPALE
SIVAQLSRPDPSGSGSGDPIRSQVLKSELEEKSELRHKLKYPHEYIELIEIARN
TQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGG
25 YNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYK
AQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ
ID NO 172);

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
30 LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNGGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVV
35 AIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGGKQALETVQALLPVLC
QAHGLTPEQVVAIASNIGGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQAL

ETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPAL
ESIVAQLSRPDPSGSGSGGDPISRSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARN
5 STQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSG
GYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNY
KAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ
ID NO 173) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
10 SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIA
SNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALE
15 TVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASNIGGKQALETV
QALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIAS
NNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHG
20 LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPALESIV
AQLSRPDPSGSGSGGDPISRSQLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQ
DRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNL
PIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQL
TRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ ID NO
25 174);

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHDGGKQALET
VQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIA
30 SHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA
HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASNIGGKQALETV
QALLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIAS
NIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALET
35 VQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIA
SNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQA

HGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGGRPALESI
VAQLSRPDPSGSGSGGDPIRSQVLKSELEEKKSELRHKLYVPHEYIELIEIARNST
QDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGY
NLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLVSGHFKGNYKA
5 QLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ ID
NO 175) and

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
SQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
10 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
15 AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE
TVQALLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIA
SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPALESI
VAQLSRPDPSGSGSGGDPIRSQVLKSELEEKKSELRHKLYVPHEYIELIEIARNST
20 QDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYSGGY
NLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLVSGHFKGNYKA
QLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFAAD (SEQ ID
NO 176);

MGDPKKKRKVIDIADLRTLGYSSQQQKEKIKPKVRSTVAQHHEALVGHGFTHAHIVAL
25 SQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
LQLDTGQLLKIARKGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQAL
30 ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVV
AIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLC
QAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPQQVVAI
ASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQ
35 AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGGRPAL
ESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEE

KKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKP
 DGAIYTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWW
 KVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL
 TLEEVRKFNNGEINFAAD (SEQ ID NO 177) and

5 MGDPPKKRKVIDIADLRTLGYSSQQQKEIKPKVRSTVAQHHEALVGHGFTHAHIVAL
 SQHPAALGTAVVKYQDMIAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPP
 LQLDTGQLLKIAGRGGVTAVEAVHAWRNALTGAPLNLTTPQQVVAIASNNGGKQALE
 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
 ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQA
 10 HGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALE
 TVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAI
 ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
 AHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALE
 TVQALLPVLCQAHGLTPEQVVAIASNIGGKQALETVQALLPVLCQAHGLTPEQVVAIA
 15 SNIGGKQALETVQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAH
 GLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKPALESI
 VAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLGDPISRSQVLKSELEEKKS
 ELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAI
 YTVGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYP
 20 SSVTEFKFLFVSGHFKGNYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTLTLEE
 VRRKFNNGEINFAAD (SEQ ID NO 178).

The invention provides a TAL- protein comprising a sequence having at least 95% homology
 with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; with
 SEQ ID NO 118 and SEQ ID NO 119; with SEQ ID NO 102 and SEQ ID NO 103; with SEQ
 25 ID NO 104 and SEQ ID NO 105; with SEQ ID NO 106 and SEQ ID NO 107; with SEQ ID NO
 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112
 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and
 SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and
 SEQ ID NO 178 for use in the manufacturing of CART cells.

30 The invention provides a TAL- protein comprising a sequence having at least 95% homology
 with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; or with
 SEQ ID NO 118 and SEQ ID NO 119; is provided for modifying the CD38 gene,

The invention provides a TAL- protein comprising a sequence having at least 95% homology
 with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; or with
 35 SEQ ID NO 106 and SEQ ID NO 107, is provided for modifying the CD70 gene.

The invention provides a TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178 is provided for modifying the CS1 gene.

The invention provides a polynucleotide or a vector encoding any one of the TAL protein above.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 114 and SEQ ID NO 115.

10 The invention provides a TAL- protein comprising a sequence of SEQ ID NO 116 and SEQ ID NO 117.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 118 and SEQ ID NO 119.

15 The invention provides a TAL- protein comprising a sequence of SEQ ID NO 102 and SEQ ID NO 103.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 104 and SEQ ID NO 105.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 106 and SEQ ID NO 107.

20 The invention provides a TAL- protein comprising a sequence of SEQ ID NO 108 and SEQ ID NO 109.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 110 and SEQ ID NO 111.

25 The invention provides a TAL- protein comprising a sequence of SEQ ID NO 112 and SEQ ID NO 113.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 171 and SEQ ID NO 172.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 173 and SEQ ID NO 174.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 175 and SEQ ID NO 176.

The invention provides a TAL- protein comprising a sequence of SEQ ID NO 177 and SEQ ID NO 178.

- 5 The invention provides a TAL- protein according to item 54 comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; comprising a sequence having at least 95% homology with SEQ ID NO 116 and SEQ ID NO 117; comprising a sequence having at least 95% homology with SEQ ID NO 118 and SEQ ID NO 119; comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; comprising a sequence having at least 95% homology with SEQ ID NO 104 and SEQ ID NO 105; comprising a sequence having at least 95% homology with SEQ ID NO 106 and SEQ ID NO 107; comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; comprising a sequence having at least 95% homology with SEQ ID NO 110 and SEQ ID NO 111; comprising a sequence having at least 95% homology with SEQ ID NO 112 and SEQ ID NO 113; comprising a sequence having at least 95% homology with SEQ ID NO 171 and SEQ ID NO 172; comprising a sequence having at least 95% homology with SEQ ID NO 173 and SEQ ID NO 174; comprising a sequence having at least 95% homology with SEQ ID NO 175 and SEQ ID NO 176; or comprising a sequence having at least 95% homology with SEQ ID NO 177 and SEQ ID NO 178 for use in the manufacturing of CART cells.

The invention provides a TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; or with SEQ ID NO 118 and SEQ ID NO 119; is provided for modifying the CD38 gene.

- 25 The invention provides a TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; or with SEQ ID NO 106 and SEQ ID NO 107, is provided for modifying the CD70 gene.

- A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178 is provided for modifying the CS1 gene.

The invention provides a polynucleotide or a vector encoding a protein comprising any one of the TAL protein above is provided.

A polynucleotide or a vector encoding any one of the TAL protein above is provided.

An engineered immune T cell comprising a CD38 modified gene obtained using a TAL-protein or a polynucleotide encoding a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; with SEQ ID NO 116 and SEQ ID NO 117; or with SEQ ID NO 118 and SEQ ID NO 119 is provided.

The invention provides an engineered immune T cell comprising a CD70 modified gene obtained using a TAL- protein or a polynucleotide encoding a sequence having with SEQ ID NO 102 and SEQ ID NO 103; with SEQ ID NO 104 and SEQ ID NO 105; or with SEQ ID NO 106 and SEQ ID NO 107, is provided.

An engineered immune T cell comprising a CS1 modified gene obtained using a TAL- protein or a polynucleotide encoding a sequence having with SEQ ID NO 108 and SEQ ID NO 109; with SEQ ID NO 110 and SEQ ID NO 111; with SEQ ID NO 112 and SEQ ID NO 113; with SEQ ID NO 171 and SEQ ID NO 172; with SEQ ID NO 173 and SEQ ID NO 174; with SEQ ID NO 175 and SEQ ID NO 176; or with SEQ ID NO 177 and SEQ ID NO 178 is provided.

The T-cells according to the invention may be endowed with a chimeric antigen receptor directed the antigen marker that is commonly expressed by the pathological cells and T-cells. The expression "known to be present" means that the antigen marker is reported to be found on the surface of the T-cells 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 antigen marker on the surface of the engineered T-cell, thereby preventing the chimeric antigen receptor from reacting with the engineered T-cell surface. In this respect, the method for preparing the T cells of the invention may include a further step of purifying the resulting T-cells by excluding the cells presenting said marker antigen on their surface.

Therapeutic antibodies

Therapeutic antibodies used in the present study may be any therapeutic antibody specific for an antigen expressed on immune cells used for immunotherapy such as T cells of precursors, and on pathological cells preferably any one of the antigens described in tables 4 to 14 of the present invention.

As non-limiting examples of anti-CD70 therapeutic antibodies that may be used with and/or combined to engineered cells with a CD70 KO or inactivated gene, the following therapeutic antibody may be combined with cells in which the CD70 gene was inactivated:

Anti CD70 therapeutic mAbs

Vorsetuzumab (or SGN-70 or h1F6) as disclosed in WO200473656, preferably (h1F6 clone) as disclosed in WO2006113909 which is a humanized h1F6.

ARGX 110 (or Anti-CD70 SIMPLE Antibody) (clone 41D12 or ARGX110), as
5 disclosed in WO2012123586 which is incorporated here in its entirety.

SGN CD70A, SGN-CD70A is an antibody-drug conjugate that combines an anti-CD70 monoclonal antibody with a synthetic DNA cross-linking molecule, pyrrolobenzodiazepine (PBD) dimer.

Vorsetuzumab mafodotin (SGN-75),

10 MDX 1203 (BMS-936561) as disclosed in WO2008074004 or in WO2007038637 or in disclosed in WO2009126934.

In a preferred embodiment, the combination of the invention comprises CD70 KO immune cells and ARGX 110 and is used for the treatment of Renal Cell Carcinoma or for Non-hodgkin's Lymphoma.

15 As examples of anti-CD38 therapeutic antibodies that may be used and combined to CD38 KO engineered cells, the following may be combined with cells in which the CD38 gene was inactivated:

Anti CD38 therapeutic mAbs

20 Daratumumab (DARZALEX; Humanised anti-CD38 monoclonal antibody; HuMax-CD38; HuMax®-CD38 - Genmab; JNJ-54767414). The combination of the invention may be especially efficient for the treatment of MM.

Isatuximab (Anti-CD38 monoclonal antibody - Sanofi; hu38SB19; SAR-650984) as described in U.S. Patent No. 8,153,765, incorporated here in its entirety.

25 MOR202 (or MOR-03087) from MorphoSys as described in W01999/62526 (Mayo Foundation); W0200206347 (Crucell Holland); US2002164788 (Jonathan Ellis) which is incorporated by reference in its entirety; W02005/103083 (MorphoSys AG), US serial no. 10/588,568, which is incorporated by reference in its entirety, W02006/125640 (MorphoSys AG), US serial no. 11/920,830, which is incorporated by reference in its entirety, and (MorphoSys AG), US serial no. 12/089,806, which is incorporated by reference in its entirety;
30 W02006099875 (Genmab), US serial no. 11/886,932, which is incorporated by reference in its

entirety; and W008/047242 (Sanofi-Aventis), US serial no. 12/441,466, which is incorporated by reference in its entirety.

The combination of the invention of CD38 KO immune cells and therapeutic anti-CD38 MOR202 antibody may be used for the treatment of MM.

- 5 Other Ab may be used such as EDC8 (from Wisconsin Alumni Research Foundation) or GBR 1342 from Glenmark Pharmaceuticals S.A. (bispec for CD3 and CD38), in particular for the treatment of MM.

As example of anti-CS1 antibody that may be used and combined to engineered cells in which the CS1 gene was inactivated the following are preferred:

10 Anti CS1 therapeutic mAbs

Elotuzumab (BMS-901608; Empliciti; HuLuc63; PDL063) from PDL BioPharma (developed by AbbVie; Bristol-Myers Squibb), for use in the treatment of MM

ABBV838 for use in the treatment of MM.

- 15 Elotuzumab is a humanized monoclonal IgG1 antibody directed against CS-1, a cell surface glycoprotein, which is highly and uniformly expressed in multiple myeloma.

- Elotuzumab induces significant antibody-dependent cellular cytotoxicity (ADCC) against primary multiple myeloma cells in the presence of peripheral lymphocytes (Tai et al, Blood, 112: 1329-1337 (2008)). Results of three studies that evaluated the safety and efficacy of this drug administered alone (Zonder et al, Blood, 120(3):552-559 (2012)), in combination with bortezomib (Jakubowiak et al, J. Clin. Oncol., 30(16): 1960-1965 (Jun. 1, 2012)), or lenalidomide and low-dose dexamethasone (Lonial et al, J. Clin. Oncol., 30: 1953-1959 (2012); and Richardson et al, Blood (ASH Annual Meeting Abstracts), 116:986 (2010) for the treatment of patients with relapsed or refractory multiple myeloma, have been reported. All three combinations showed a manageable safety profile and encouraging activity. For example, a Phase VII study evaluating the safety and efficacy of Elotuzumab in combination lenalidomide and low-dose dexamethasone for the treatment of relapsed or refractory multiple myeloma demonstrated a 33 month PFS as well as a 92% response rate for patients receiving the 10 mg/kg dose (Lonial et al., J. Clin. Oncol., 31 (2013) (Suppl., Abstr. 8542)). Phase III clinical trials of lenalidomide/dexamethasone with or without Elotuzumab in previously untreated multiple myeloma patients is ongoing, while another phase III trial designed to evaluate this same combination in the first line setting is also ongoing. Accordingly, the invention provides a combination of CS-1 deficient engineered cells
- 20
- 25
- 30

and a therapeutic antibody specific for CS1 namely in combination with bortezomib or lenalidomide and low-dose dexamethasone for the treatment of patients with relapsed or refractory multiple myeloma.

Combination – step of combining

- 5 Combining said engineered T cells with a therapeutic antibody specific for said antigen marker, may mean that cells and antibody are incubated together so that said therapeutic antibody binds to cells in which the antigen is still expressed. The therapeutic antibody is therefore bound to engineered cells that express the antigen marker. Incubation with appropriate reagent allows an antibody mediated purification in vitro or in vivo of engineered cells that do not
- 10 express the marker antigen. An appropriate reagent may be beads, preferably magnetic beads coated with a protein that binds to antibodies, such as G or A proteins or protein of the complement. Purification may be performed in vivo or in vitro. A therapeutic antibody is an antibody with therapeutic activity, preferably approved by health authorities. Therapeutic activity means an activity that improves health condition in a patient suffering a disease.
- 15 All these examples of therapeutically effective antibody are administered in individual in need thereof. A skilled person will administer it in combination with engineered cells accordingly to the present invention, each at the efficient doses to improve the health condition.

In certain embodiments of the present invention, engineered cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) the therapeutic antibody

20 targeting the cell surface antigen marker which expression is inactivated in said cells.

The active dose(s) of antibody and of engineered immune cells and route of injection are known of the skilled person. The present invention provides a combination adapted to the patients so that the first injection of the combination allows 1) immunodepleting the patient in T cells, 2) grafting said patient with engineered cells 3) destroying pathological cells and /or

25 avoiding relapse refractory forms of said pathological cells.

As shown in Table 4, this invention is applicable to an important number of antigen marker candidates reported to be expressed by tumor cells, but also by T-cells. Some of them, like CD38, have been used as specific markers in diagnostic methods for a while, especially with respect to Leukemia pathological cells, but not in therapy. Indeed, although these markers

30 were identified in the art as quite specific markers, they could not be used as targets for immunotherapy because antibodies directed against these markers would have destroyed or interfered with patients' T-cells.

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.

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 recombination or non-homologous end joining (NHEJ). However, NHEJ is an imperfect repair process that often results in changes to the DNA sequence at the site of the cleavage. Mechanisms involve rejoining of what remains of the two DNA ends through direct re-ligation (Critchlow and Jackson 1998) or via the so-called microhomology-mediated end joining (Betts, Brenchley et al. 2003; Ma, Kim et al. 2003). Repair via non-homologous end joining (NHEJ) often results in small insertions or deletions and can be used for the creation of specific gene knockouts. Said modification may be a substitution, deletion, or addition of at least one nucleotide. Cells in which a cleavage-induced mutagenesis event, i.e. a mutagenesis event consecutive to an NHEJ event, has occurred can be identified and/or selected by well-known method in the art.

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 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).

Cas 9

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.

5 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
10 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). 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
15 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
20 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
25 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
30 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. Rearranging 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

5 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.

10 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

15 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

20 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

25 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

30 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,

35 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.

Preferably, the Cas9 variants according to the invention have an amino acid sequence sharing at least 70 %, preferably at least 80 %, more preferably at least 90%, and even more preferably 95 % identity with Cas9 of *S. Pyogenes* (COG3513).

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-Crel 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.

The present invention provides a rare-cutting endonuclease for inactivating a gene which product is expressed or overexpressed on pathological cells and normal T cell and which product is expressed on the surface of normal T-cells selected from any one of those disclosed in table 4 to table 14.

In preferred embodiment the rare-cutting endonuclease of the invention is specific for the CD38 gene or for the CD70 gene or for the CS1 gene.

The rare-cutting endonuclease as above is provided and selected from a Meganuclease, a transcription activator-like (TAL)-nuclease, a zing-finger nuclease (ZFN), or a RNA/DNA guided endonucleases.

The rare-cutting endonuclease as above is provided and selected from a Meganuclease, a transcription activator-like (TAL)-nuclease, a zing-finger nuclease (ZFN), or a RNA/DNA guided endonucleases.

The rare-cutting endonuclease provided here is a transcription activator-like effector (TALE)-nuclease specific for CD38, CD70 or CS-1 gene.

The rare-cutting endonuclease of the invention may be for example specific for one of the following SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7, SEQ ID NO.63, SEQ ID NO.66, SEQ ID NO.69, SEQ ID NO.72, SEQ ID NO.75, SEQ ID NO.78, SEQ ID NO. 167, SEQ ID NO. 168, SEQ ID NO. 169 or SEQ ID NO. 170.

The present invention encompasses TAL-nucleases having at least 95% homology with any one of the TAL-nucleases comprising **SEQ ID NO 114** and **SEQ ID NO 115**; **SEQ ID NO 116** and **SEQ ID NO 117**; **SEQ ID NO 118** and **SEQ ID NO 119**; **SEQ ID NO 102** and **SEQ ID NO 103**; **SEQ ID NO 104** and **SEQ ID NO 105**; **SEQ ID NO 106** and **SEQ ID NO 107**; **SEQ ID NO 108** and **SEQ ID NO 109**; **SEQ ID NO 110** and **SEQ ID NO 111**; **SEQ ID NO 112** and **SEQ ID**

NO 113; SEQ ID NO 171 and SEQ ID NO 172; SEQ ID NO 173 and SEQ ID NO 174; SEQ ID NO 175 and SEQ ID NO 176; or SEQ ID NO 177 and SEQ ID NO 178.

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.

TAL-nucleases

"TALE-nuclease" or "MBBBD-nuclease" refers to engineered proteins resulting from the fusion of a DNA binding domain typically derived from Transcription Activator Like Effector proteins (TALE) or Modular Base-per-Base Binding domain (MBBBD), with a catalytic domain having endonuclease activity. Such catalytic domain usually comes from enzymes, such as for instance I-TevI, ColE7, 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 TALEN™ (Collectis, 8 rue de la Croix Jarry, 75013 Paris, France).

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.

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 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 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 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-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.

In a preferred embodiment, a TAL nuclease of the invention comprises a sequence selected from any one the following pair of sequences: SEQ ID NO 102 and SEQ ID NO 103, SEQ ID NO 104 and SEQ ID NO 105, SEQ ID NO 106 and SEQ ID NO 107, SEQ ID NO 108 and SEQ ID NO 109, SEQ ID NO 110 and SEQ ID NO 111, SEQ ID NO 112 and SEQ ID NO 113, SEQ ID NO 114 and SEQ ID NO 115, SEQ ID NO 116 and SEQ ID NO 117, SEQ ID NO 118 and SEQ ID NO 119, SEQ ID NO 171 and SEQ ID NO 172, SEQ ID NO 173 and SEQ ID NO 174, SEQ ID NO 175 and SEQ ID NO 176, SEQ ID NO 177 and SEQ ID NO 178; or a sequence having least 95% homology with any one of said pair of sequences.

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,...) 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 1. 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 1 : 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

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. In a preferred embodiment the use of adeno associated viral vectors and more preferably of AAV6, or AAV9 vectors is particularly suited for expressing a gene including chimeric antigen
5 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.

Non alloreactive T cells:

10 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 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. The engineered
15 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 procuring the T-cells from a donor and to inactivate genes thereof involved in MHC recognition
20 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-cell receptor complex present on the cell surface. Each
25 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
30 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
35 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 allogeneic 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), preferably by inserting a sequence into the endogenous TCR gene (genomic TCR gene), by using a viral vector preferably an adeno associated virus.

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 noted in Table 2 (+ for lower ratio of TCR negative cells, ++ for intermediate ratio, +++ for higher ratio).

Table 2: appropriate target sequences for the guide RNA using Cas9 in T-cells

Exon TCR	Position	Strand	Target genomic sequence	SEQ ID	efficiency
Ex1	78	-1	GAGAATCAAAATCGGTGAATAGG	120	+++
Ex3	26	1	TTCAAAACCTGTCAGTGATTGGG	121	+++
Ex1	153	1	TGTGCTAGACATGAGGTCTATGG	122	+++
Ex3	74	-1	CGTCATGAGCAGATTAAACCCGG	123	+++
Ex1	4	-1	TCAGGGTTCTGGATATCTGTGGG	124	+++
Ex1	5	-1	GTCAGGGTTCTGGATATCTGTGG	125	+++
Ex3	33	-1	TTCGGAACCCAATCACTGACAGG	126	+++
Ex3	60	-1	TAAACCCGGCCACTTTCAGGAGG	127	+++
Ex1	200	-1	AAAGTCAGATTTGTTGCTCCAGG	128	++
Ex1	102	1	AACAAATGTGTCACAAAGTAAGG	129	++
Ex1	39	-1	TGGATTTAGAGTCTCTCAGCTGG	130	++
Ex1	59	-1	TAGGCAGACAGACTTGTCAGTGG	131	++
Ex1	22	-1	AGCTGGTACACGGCAGGGTCAGG	132	++
Ex1	21	-1	GCTGGTACACGGCAGGGTCAGGG	133	++
Ex1	28	-1	TCTCTCAGCTGGTACACGGCAGG	134	++
Ex3	25	1	TTTCAAAACCTGTCAGTGATTGG	135	++
Ex3	63	-1	GATTAAACCCGGCCACTTTCAGG	136	++
Ex2	17	-1	CTCGACCAGCTTGACATCACAGG	137	++
Ex1	32	-1	AGAGTCTCTCAGCTGGTACACGG	138	++
Ex1	27	-1	CTCTCAGCTGGTACACGGCAGGG	139	++
Ex2	12	1	AAGTTCCTGTGATGTCAAGCTGG	140	++
Ex3	55	1	ATCCTCCTCCTGAAAGTGGCCGG	141	++
Ex3	86	1	TGCTCATGACGCTGCGGCTGTGG	142	++
Ex1	146	1	ACAAAAGTGTGCTAGACATGAGG	143	+
Ex1	86	-1	ATTTGTTTGAGAATCAAAATCGG	144	+
Ex2	3	-1	CATCACAGGAAGCTTTCTAAAAGG	145	+
Ex2	34	1	GTCGAGAAAAGCTTTGAAACAGG	146	+

Ex3	51	-1	CCACTTTCAGGAGGAGGATTTCGG	147	+
Ex3	18	-1	CTGACAGGTTTTGAAAGTTTAGG	148	+
Ex2	43	1	AGCTTTGAAACAGGTAAGACAGG	149	+
Ex1	236	-1	TGGAATAATGCTGTTGTTGAAGG	150	+
Ex1	182	1	AGAGCAACAGTGCTGTGGCCTGG	151	+
Ex3	103	1	CTGTGGTCCAGCTGAGGTGAGGG	152	+
Ex3	97	1	CTGCGGCTGTGGTCCAGCTGAGG	153	+
Ex3	104	1	TGTGGTCCAGCTGAGGTGAGGGG	154	+
Ex1	267	1	CTTCTTCCCCAGCCCAGGTAAGG	155	+
Ex1	15	-1	ACACGGCAGGGTCAGGGTTCTGG	156	+
Ex1	177	1	CTTCAAGAGCAACAGTGCTGTGG	157	+
Ex1	256	-1	CTGGGGAAGAAGGTGTCTTCTGG	158	+
Ex3	56	1	TCCTCCTCCTGAAAGTGGCCGGG	149	+
Ex3	80	1	TTAATCTGCTCATGACGCTGCGG	160	+
Ex3	57	-1	ACCCGGCCACTTTTCAGGAGGAGG	161	+
Ex1	268	1	TTCTTCCCCAGCCCAGGTAAGGG	162	+
Ex1	266	-1	CTTACCTGGGCTGGGGAAGAAGG	163	+
Ex1	262	1	GACACCTTCTTCCCCAGCCCAGG	164	+
Ex3	102	1	GCTGTGGTCCAGCTGAGGTGAGG	165	+
Ex3	51	1	CCGAATCCTCCTCCTGAAAGTGG	166	+

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 proteasome 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.

The present invention provides an engineered T cell comprising any one of the genetic modification to a sequence as shown in SEQ ID NO.1, SEQ ID NO.4, or SEQ ID NO.7 of the CD38 gene, of SEQ ID NO.63, SEQ ID NO.66, SEQ ID NO. 69, SEQ ID NO.167, SEQ ID NO.168, SEQ ID NO.169 or SEQ ID NO.170 of the CS1 gene or of SEQ ID NO.72, SEQ ID NO.75 or SEQ ID NO.78 of the CD70 gene.

The present invention provides a combination comprising an engineered T cell comprising any one of the genetic modification to a sequence as shown in SEQ ID NO.1, SEQ ID NO.4, SEQ ID NO.7 of the CD38 gene and a therapeutic antibody specific for the antigen marker encoded by the CD38 gene.

The present invention provides a combination comprising an engineered T cell comprising any one of the genetic modification to a sequence as shown in SEQ ID NO.63, SEQ ID NO.66, SEQ ID NO. 69, SEQ ID NO.167, SEQ ID NO.168, SEQ ID NO.169 or SEQ ID NO.170 of the CS1 gene and a therapeutic antibody specific for the antigen marker encoded by the CS1 gene.

The present invention provides a combination comprising an engineered T cell comprising any one of the genetic modification to a sequence as shown in SEQ ID NO.72, SEQ ID NO.75 or SEQ ID NO.78 of the CD70 gene and a therapeutic antibody specific for the antigen marker encoded by the CD70 gene.

The engineered T cell according to the above comprises a genetic modification to a sequence of SEQ ID NO.1 by a TAL protein comprising a sequence SEQ ID NO 114 and SEQ ID NO 115, a genetic modification to a sequence of SEQ ID NO.4 by a TALEN comprising a SEQ ID N° 116 and SEQ ID NO 117, or a genetic modification to a sequence of SEQ ID NO.7 by a TAL protein comprising a sequence SEQ ID N° 118 and SEQ ID NO 119; and/or a genetic

modification to a sequence of SEQ ID NO.63 by a TAL protein of sequence SEQ ID NO 108 and SEQ ID NO 109, a genetic modification to a sequence of SEQ ID NO.66 by a TAL protein of sequence SEQ ID NO 110 and SEQ ID NO 111, a genetic modification to a sequence of SEQ ID NO. 69 by a TAL protein of sequence SEQ ID NO 112 and SEQ ID NO 113, a genetic
5 modification to a sequence of SEQ ID NO.167 by a TAL protein of sequence SEQ ID NO 171 and SEQ ID NO 172, a genetic modification to a sequence of SEQ ID NO.168 by a TAL protein of sequence SEQ ID NO 173 and SEQ ID NO 174, or a genetic modification to a sequence of SEQ ID NO.169 by a TAL protein of sequence SEQ ID NO 171 and SEQ ID NO 172; and/or a genetic modification to a sequence of SEQ ID NO.72 by a TAL protein of sequence SEQ ID
10 NO 102 and SEQ ID NO 103, a genetic modification to a sequence of SEQ ID NO.75 by a TAL protein of sequence SEQ ID NO 104 and SEQ ID NO 105, a genetic modification to a sequence of SEQ ID NO.78 by a TAL protein of sequence SEQ ID NO 106 and SEQ ID NO 107.

The engineered T cell according to the above comprising an insertion of a polynucleotide
15 sequence encoding a gene, preferably a CAR, preferably at the locus described above, into the TRAC gene and at least one a genetic inactivation to a sequence of SEQ ID NO.1 by a TAL protein comprising a sequence SEQ ID NO 114 and SEQ ID NO 115; a genetic modification to a sequence of SEQ ID NO.4 by a TALEN comprising a SEQ ID NO 116 and SEQ ID NO 117, a genetic modification to a sequence of SEQ ID NO.7 by a TAL protein
20 comprising a sequence SEQ ID NO 118 and SEQ ID NO 119, a genetic modification to a sequence of SEQ ID NO.63 by a TAL protein of sequence SEQ ID NO 108 and SEQ ID NO 109, a genetic modification to a sequence of SEQ ID NO.66 by a TAL protein of sequence SEQ ID NO 110 and SEQ ID NO 111, a genetic modification to a sequence of SEQ ID NO. 69 by a TAL protein of sequence SEQ ID NO 112 and SEQ ID NO 113, of the CS1 gene, a genetic
25 modification to a sequence of SEQ ID NO.72 by a TAL protein of sequence SEQ ID NO 102 and SEQ ID NO 103, a genetic modification to a sequence of SEQ ID NO.75 by a TAL protein of sequence SEQ ID NO 104 and SEQ ID NO 105, a genetic modification to a sequence of SEQ ID NO.78 of the CD70 gene by a TAL protein of sequence SEQ ID NO 106 and SEQ ID NO 107.

30 The TAL-nuclease of the invention as any one of the above is provided for use as a medicament, alone or in combination with an therapeutic antibody specific for the same gene.

The combination of the invention as any one of the above and below is provided for the
35 treatment of cancer, infections or auto-immune disease.

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, most patients were treated with chemotherapy and immune depleting agents as a standard of care, prior to receiving T-cell immunotherapy. One 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 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; or an engineered T cell
- (b) Selecting at least one drug;
- (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 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 is the inactivation of the CD3 normally expressed at the surface of the T-cell can confer resistance to anti-CD3 antibodies such as teplizumab.

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 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 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 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, 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. Glucocorticosteroids 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. 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 glycosylphosphatidylinositol- (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 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 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 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 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 an antigen marker X expressed on both T cell and pathological cells and of at least two genes selected from the group consisting of CD52, dCK, GR, TCR alpha, TCR beta, a combination thereof. The inventor succeeded in engineering at least 5 genes in a primary cell as disclosed in PA 201670503.

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.

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 dCK and GR, dCK and TCR alpha, dCK 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.

Engineering highly active T cells for immunotherapy

According to the present invention, the cells can be selected from the group consisting of T-cells, preferably inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In another embodiment, said cell can be from the group

consisting of CD4⁺ T-lymphocytes and CD8⁺ T-lymphocytes. They can be extracted from blood or derived from stem cells. The cells may also be stem cells preferably cells can be adult stem cells, embryonic stem cells. In another embodiments cells may be non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34⁺ cells, or CD8⁺ 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 blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available and known to those skilled in the art, may be used. In another embodiment, said cell can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In another embodiment, said cell is part of a mixed population of cells which present different phenotypic characteristics. In the scope of the present invention is also encompassed a cell line obtained from a transformed T- cell according to the method previously described.

The cells used here may have been already engineered, that is to say inactivated by deleting a TCR subunit, preferably a TCR alpha subunit, made resistant to PNA by inactivating the dCK gene, made resistant to alentuzumab by deleting the D52 gene as described in WO2015075195A1, or in Valton J, Guyot V, Marechal A, et al. A Multidrug-resistant Engineered CAR T Cell for Allogeneic Combination Immunotherapy. Molecular Therapy. 2015;23(9):1507-1518. doi:10.1038/mt.2015.104.

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-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-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, {Meyaard, 1997 #122}), SIGLEC10 (GeneBank accession number: AY358337.1), 2B4 (also known as CD244, accession number: NM_001166664.1), PPP2CA, PPP2CB, PTPN6, PTPN22, CD96, CRTAM, SIGLEC7 {Nicoll, 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 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 checkpoint, in particular PD1 and/or CTLA-4 or any immune-checkpoint proteins referred to in Table 3.

Table 3: 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
Death receptors	CD244(2b4)	CD244
	TRAIL	TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7
	FAS	FADD, FAS
Cytokine signalling	TGF-beta signaling	TGFBR2, TGFBR1, 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

Engineered cells encompasses an engineered immune cell comprising at least one inactivated gene X and expressing a chimeric antigen receptor (CAR) or a component of a recombinant T-cell receptor; wherein said cell has been genetically modified to reduce or inactivate the expression of at least one additional endogenous polynucleotide sequence selected from:

a) polynucleotide sequence(s), which expression is(are) involved into reduction of glycolysis and calcium signaling in response to a low glucose condition, such as SERCA3 to increase calcium signaling, miR101 and mir26A to increase glycolysis, BCAT to mobilize glycolytic reserves; and/or

b) polynucleotide sequence(s), which expression up regulate(s) immune checkpoint proteins (e.g. TIM3, CEACAM, LAG3, TIGIT), such as IL27RA, STAT1, STAT3; and/or

c) polynucleotide sequence(s), which expression mediate(s) interaction with HLA-G, such as ILT2 or ILT4; and/or

d) polynucleotide sequence(s), which expression is(are) involved into the down regulation of T-cell proliferation such as SEMA7A, SHARPIN to reduce Treg proliferation, STAT1 to lower apoptosis, PEA15 to increase IL-2 secretion and RICTOR to favor CD8 memory differentiation; and/or

e) polynucleotide sequence(s), which expression is(are) involved into the down regulation of T-cell activation, such as mir21; and/or

f) polynucleotide sequence(s), which expression is(are) involved in signaling pathways responding to cytokines, such as JAK2 and AURKA

g) polynucleotide sequence(s), which expression is(are) involved in T-cell exhaustion, such as DNMT3, miRNA31, MT1A, MT2A, PTGER2.

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).

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.

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, 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, prostase, prostase 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.

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
5 derived from FcεRI 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.

10 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:

- 15 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 transmembrane domain of FcεRI gamma chain, whereby different polypeptides
20 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 can be in juxtamembrane positions (i.e. adjacent to the cell membrane
25 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 between the different
30 subunits into the multi-chain CAR. This type of architecture has been recently described by the applicant in PCT/US2013/058005.

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 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 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 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 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 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 invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the multi-chain CAR is

expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines.

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 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.

The combination of the invention comprises an engineered T cell according to the above endowed with at least one chimeric antigen receptor (CAR) or exogenous TCR.

The combination of the invention comprises an engineered allogeneic T cell according to the above, engineered to be less alloreactive, by engineering or deleting genes encoding components of the HLA complex, either to inactivated cell surface expression , by deleting

genes encoding components of the HLA complex, or modifying the sequence to match MHC molecules to those of the patient.

The combination of the invention comprises an engineered allogeneic T cell according to the above, engineered to be less alloreactive, by engineering or deleting genes encoding components of the HLA complex, selected from beta2microglobulin, regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X-associated protein (RFXAP), and class II transactivator (CIITA), TAP-1, a combination thereof.

The engineered T cell of the combination of the invention according to the above wherein said CAR is specific for: a cluster of differentiation molecule, a tumor-associated surface antigen, a lineage-specific or tissue specific antigen or a virus-specific surface antigen is provided.

The engineered T cell according to the above may comprise a CAR wherein said CAR is targeting (and specific for) any of the following antigen surface marker: CD16, CD64, CD78, CD96, CLL1, CD116, CD117, CD71, CD45, CD71, CD123 and CD138; or 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, 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); or CD3, CD4, CD8, CD24, CD25, CD33, CD34, CD38, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), GM-CSF, cytokine receptors, endoglin, a major histocompatibility complex (MHC) molecule, BCMA (CD269, TNFRSF17); or 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 the therapeutic antibody combined.

The CAR endowed in the engineered cells in the combination of the invention may be specific for (e.g. CD16, CD19, CD20, CD22, CD30, CD38, CD40, CD64, CD70, CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, 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), disialoganglioside GD2, o-acethyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, Flt3, WT1, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGF1 receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD123, HSP70, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, a cytokine receptor, 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, NEF, gp41); 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

The combination of the invention may comprise an immune T cell expressing a CAR which targets specifically a cell surface marker selected from CD19, CD38, HSP70, CD30, FAP, HER2, CD79a, CD79b, CD123, CD22, CLL-1, MUC-1, GD2, O-acetyl-GD2, GD3, ROR 1, and CS1.

The combination of the invention may comprise an immune T cell expressing a CAR which targets specifically a cell surface marker selected from any cluster of differentiation molecules (e.g. CD16, CD20, CD22, CD30, CD38, CD40, CD64, CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, a tumor-associated surface antigen, such as ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), disialoganglioside GD2, o-acethyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, M-CSF, prostase, prostase specific

antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGFI receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD34, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), GM-CSF, CD123, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, cytokine receptors, endoglin, a major histocompatibility complex (MHC) molecule, 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

The combination of the invention may comprise an immune T cell expressing a CAR which targets specifically a cell surface marker selected from BCMA, CD33, EGFRVIII, Flt3, WT1, CD70, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10.

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- γ , 1L-4, 1L-7, GM-CSF, -10, -2, 1L-15, TGF β , 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, α -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.

Therapeutic applications

The combination of the invention as described above are intended to be used as a medicament for treating, among others, cancer, infections or immune diseases in a patient in need thereof.

Said treatment can be ameliorating, curative or prophylactic. It may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment. By autologous, it is meant that cells, cell line or population of cells used for treating patients are originating from said patient or from a Human Leucocyte Antigen (HLA) compatible donor. By allogeneic is meant that the cells or population of cells used for treating patients are not originating from said patient but from a donor.

The combination of the above comprises T cells according to one of the previous methods may be pooled, frozen, and administrated to one or several patients. 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 thereof in combination with a therapeutic antibody directed against the antigen deleted in cells with which Ab are combined of the invention.

Said treatments are primarily intended to patients diagnosed with cancer, viral infection, autoimmune disorders or Graft versus Host Disease (GvHD). Cancers are preferably leukemias and lymphomas, which have liquid tumors, but may also concern solid tumors. Types of cancers to be treated with the CARs of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

The present invention provides in Tables 4 to 14 with examples of antigen markers, which can be targeted with the combination of the invention for treating different types of cancer as illustrated in table 5 to 14.

Preferred antigen markers used for the immunotherapy of the present invention are more particularly those of table 5 to 14.

Pathological conditions

The following pathological conditions may be treated efficiently with the combination of the invention : Multiple myeloma (MM), Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Acute lymphoblastic leukemia (ALL), Hodgkin lymphoma (HL) (relapsed, refractory), Non-Hodgkin lymphoma (NHL) (relapsed, refractory), Neuroblastoma, Ewing sarcoma, Myelodysplastic syndromes, BPDCN.

The combination or the pharmaceutical composition of the invention is also especially efficient in the treatment or prophylaxis of Gliomas, pancreatic cancer, lung cancer, bladder cancer, colon cancer, breast cancer.

The combination or the pharmaceutical composition of the invention is adapted for the treatment of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphoma, lymphoplasmacytic NHL, or marginal zone NHL); Hodgkin's disease (e.g., Reed-Sternberg cells); a cancer of the B-cell lineage, including, e.g., diffuse large B-cell lymphoma, follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, Cutaneous T cell lymphoma, B-cell lymphocytic leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphoma; renal cell carcinoma (e.g., clear cell and papillary); nasopharyngeal carcinoma; thymic carcinoma; glioma; glioblastoma; neuroblastoma; astrocytoma; meningioma; Waldenstrom macroglobulinemia; multiple myeloma; colon cancer, stomach cancer, and rectal carcinoma.

A combination comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody ARGX 110, is used in the treatment of Cutaneous T cell lymphoma.

A combination comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody MDX 1203 is intended for its use in the treatment of Renal Cell Carcinoma or Non-hodgkin's Lymphoma.

The combination directed to CS1 is used for the treatment of carcinoma, blastoma, and sarcoma, preferably MM, leukemia, melanoma, relapse or refractory CS-1 expressing MM or a complication related to CS-1 expressing MM.

The combination directed to CS1 is used for the treatment of any one of the following CS1 expressing cancers: Multiple Myeloma, Acute Myeloid Leukemia, Myelodysplastic Syndrome, Smoldering Multiple Myeloma, monoclonal gammopathy of unknown significance (MGUS), plasma cell leukemia, Non-Hodgkin's Lymphoma.

The combination directed to CS1 expressing cancers is used for the treatment of Multiple Myeloma, Non-Hodgkin Lymphoma, Diffuse Large B Cell Lymphoma, Mantle-Cell Lymphoma, Follicular Lymphoma, Indolent B Cell Lymphoma, Primary Mediastinal Lymphoma, Lymphoplasmacytic Lymphoma.

The combination directed to CS1 may be used for the treatment of any one of the following CS1 expressing cancers : NK cell lymphoma, NK or T cell lymphoma, angioimmunoblastic T-cell lymphoma (AITL), or peripheral T cell lymphoma not otherwise specified (PTCL-NOS).

The combination directed to CD38 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Isatuximab is for example for the treatment of Prostate Cancer, Non-small Cell Lung Cancer, Plasma Cell Myeloma, MM, T-cell Type Acute Leukemia, Precursor T-lymphoblastic Lymphoma or Leukaemia, prostate cancer, Non-small Cell Lung Cancer.

The combination comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Daratumumab may be used for the treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer, Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma, Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.

The combination comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of T-cell Type Acute Leukemia-Precursor T, lymphoblastic Lymphoma, Leukaemia is provided.

The combination comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of a CD38-expressing or CD 38 over expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).

The combination comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of hematologic cancer selected from the group of leukemia, lymphoma and multiple myeloma (MM).

The combination comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, (directed to CD38) for the treatment of hematologic cancer selected from the group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic myelogenous leukemia (CML-BP).

The combination directed to CD38 for the treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer, Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma,

Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.

The combination as above directed to CD38 for the treatment of T-cell Type Acute Leukemia-
5 Precursor T, lymphoblastic Lymphoma, Leukaemia.

The combination directed to CD38 for the treatment of a CD38-expressing or CD 38 over
expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia
(CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and
10 Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).

The combination directed to CD38 for the treatment of hematologic cancer selected from the
group of leukemia, lymphoma and multiple myeloma (MM).

15 The combination directed to CD38 for the treatment of hematologic cancer selected from the
group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic
myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or
chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia
(AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia
20 syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic
myelogenous leukemia (CML-BP), leukemia, lymphoma and multiple myeloma.

The present combination of the invention, when armed with specific CARs directed against
patient's own immune cells, especially T-cells, allow the inhibition or regulation of said cells,
25 which is a key step for treating auto-immune disease, such as rheumatoid polyarthritis,
systemic lupus erythematosus, Sjogren's syndrome, scleroderma, fibromyalgia, myositis,
ankylosing spondylitis, insulin dependent diabetes of type I, Hashimoto's thyroiditis, Addison's
disease, Crohn's disease, Celiac's disease, amyotrophic lateral sclerosis (ALS) and multiple
sclerosis (MS). Accordingly the present invention encompass a method for treating an immune
30 disease by directing engineered T-cells and therapeutic antibody as previously described
against patient's own T-cells.

The above combination can take place in combination with one or more therapies selected
from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy,
gene therapy, hormone therapy, laser light therapy and radiation therapy.

35 The combination of the invention may comprise allogeneic cells 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, comprising a dCK inactivated gene. As with the therapeutic antibody of the combination disclosed here as an invention, 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) a therapeutic antibody and 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, 1993). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery. 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.

According to one embodiment, said combination 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 of the combination of the invention 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.

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

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 administered in one or more doses. In another embodiment, said effective amount of cells are administered as a single dose. In another embodiment, said effective amount of cells are administered as more than one dose over a period time. Timing of administration of the combination of the invention is within the judgment of managing physician and depends on the clinical condition of the patient. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type and of a given antibody of the combination, 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 with administered. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

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

EXAMPLES

The cells or population of cells may be obtained from any source, such as a blood bank or a donor.

Identification of surface antigen marker expressed on the surface of T-cells, while being overexpressed in solid tumors involved into different types of cancer (Tables 5 to 13)

We used BioGPS microarray data from a panel of normal tissues (Human U133A/GNF1H Gene Atlas) cancer microarray data that also can be downloaded from BioGPS (Human Primary Tumors (U95)) uniprot data that contains the subcellular localization.

We drew the distribution of values coming from normal tissues and determined a threshold value of 5 for the relative expression.

We browsed all the genes assayed with microarrays (44.000 probes representing about 13 000 genes) and checked their localization in the membrane (protein not referred to as being a membrane protein were discarded). Expression in CD8+ T-cells was checked from the BioGPS database. The genes were listed according to the type of cancer where the corresponding expression was the highest (Tables 5 to 13).

Identification of surface antigen marker expressed on the surface of T-cells, while being overexpressed in different liquid blood tumors (Table 14)

For that study, no RNA-seq data were available and thus we used microarray data that were obtained from a large study from the MILE consortium (Microarray Innovations in Leukemia), involving 11 laboratories (http://www.ngrl.org.uk/wessex/downloads/tm08/TM08-S4-1_KenMills.pdf - Haferlach et al. 2010, <http://www.ncbi.nlm.nih.gov/pubmed/20406941>). This raw data include results for ALL (acute lymphoblastic leukemia), AML (acute myelogenous leukemia), CLL (chronic lymphoblastic leukemia) and CML (chronic myelogenous leukemia) and MDS (myelodysplastic syndrome). We also used uniprot data for subcellular localization as usual.

We first drew the overall distribution of values from all genes on all studied tissues. Then, to have an idea of the level necessary for expression, we took a list of genes which are expressed in some liquid tumors and for which therapeutic antibodies are available (CD52, CD 20, CD33, CD19, CD25, CD44, CD47, CD96, CD116, CD117, CD135, TIM-3). For each gene, we looked at the value obtained in the tumor in which it is expressed. Then, we computed the average for each tumor and gene pair for which the gene seems to give a cell membrane protein (cell membrane localization + description of at least one transmembrane domain in the protein). We discarded genes for which the expression in all the tissues was below this threshold of 0.15. We listed and ranked in Table 14, those genes which relative expression in T-cells was above 0.2. Thus, Table 4 provides putative antigen marker candidates for targeting liquid tumor cells as per the invention, in particular for treating ALL, AML, CLL, CML and MDS.

Therapeutic antibody specific for an the antigen identified, when available, and already under clinic phase below, were used.

Several administration protocols were tested first in an animal model of CD38 expressing pathological tissues.

5 Under these experimental conditions, the dose of Ab and cells necessary to obtained optimized reduction of pathological cells was estimated.

In human subjects, the amount of Ab and cells was determined based on clinical data (Drent E, Groen RWJ, Noort WA, et al. Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma. Haematologica. 10 2016;101(5):616-625. doi:10.3324/haematol.2015.137620).

Additionally, cells were engineered to be less alloreactive and/or be used in lymphodepleted individuals (TCR-negative and MHC-negative).

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

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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 20 (Dynabeads®). The 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 viral 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 fused to a 25 sequence encoding the variable chain of an anti-CD38 antibody. For security improvement of the transformed T-cell, a suicide gene sensitive to rituximab and QBEN 10 was be introduced as described in WO 2013/153391.

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

Inactivation of the TCR alpha in said cells was performed as previously described to eliminate 30 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. Thus, cells were also engineered to inactivate the TCR alpha subunit and alter the alpha beta TCR expression at the cell surface.

It is also possible to inactivate 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 dCK and immunosuppressive agent is a PNA or a combination of fludarabine and cytarabine as described in WO 2013/176915.

Gene Inactivation is performed by electroporation of 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 targeted gene (e.g. CD38) 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.

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 additional immunosuppressive treatment such as CAMPATH1-H, a humanized monoclonal anti-CD52 antibody, in combination with the therapeutic Ab of the combination disclosed here (eg anti-CD70 Ab).

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.

Functional analysis of the engineered T-cells electroporated with a monocistronic mRNA encoding for an anti-CD38 single chain chimeric antigen receptor (CAR CD38):

To verify that genome engineering did not affect the ability of the engineered T-cells to present anti-tumor activity, especially when provided with a chimeric antigen receptor (CAR CD38), The engineered anti-CD38 CAR T-cells were incubated for 4 hours with Daudi cells expressing CD38 on their surface. The cell surface upregulation of CD107a, a marker of cytotoxic granule release by T lymphocytes (called degranulation) was measured by flow cytometry analysis (Betts, Brenchley et al. 2003).

24 hours post electroporation, cells were stained with a fixable viability dye eFluor-780 and a PE-conjugated goat anti mouse IgG F(ab')₂ fragment specific to assess the cell surface expression of the CAR on the live cells. The vast majority of the live T-cells genetically disrupted for CD38, express the anti-CD38 CAR on their surface. T cells were co-cultured with Daudi (CD38⁺) cells for 6 hours and analyzed by flow cytometry to detect the expression of the degranulation marker CD107a at their surface (Betts, Brenchley et al. 2003).

The results showed that CD38 disrupted T-cells kept the same ability to degranulate in response to PMA /ionomycin (positive control) or CD38+ Daudi cells. CD107 upregulation is dependent on the presence of a CD38+. These data suggest that the genome engineering of the present T-cells had no negative impact on the ability of T cells to mount a controlled anti-tumor response.

Combination with therapeutic anti-CD38 Ab.

The following anti-CD38 Ab were combined with CD38-negative, anti-CD38 CAR allogeneic (TCR KO) T engineered cells: Daratumumab (DARZALEX), Isatuximab or MOR-03087).

The combination of the invention of CD38 KO immune cells and therapeutic anti-CD38 MOR202 or DARZALEX (daratumumab) antibody were used for the treatment of MM.

The resulting data show that daratumumab at the dose of 8 mg/kg of body weight, administered as an intravenous infusion for 8 weeks weekly or bi weekly in combination with anti-CD38 CAR T cells administered the last week, and a second time, significantly reduce tumor mass volume in all patients, with no relapse observed at 24 weeks as compared to individual treated with Ab alone or engineered cells alone.

Examples of TAL proteins inactivating the CD70 gene

A mRNA encoding a TALE-nuclease cleaving the following CD70 genomic sequence were synthesized from plasmid carrying the coding sequences (SEQ ID NO. 104-105, or SEQ ID NO. 106-107) downstream from a T7 promoter, using mMMESSAGE mMACHINE™ T7 ULTRA Transcription Kit according to manufacturer's protocol (Ambion™). T lymphocytes isolated from peripheral blood were activated using anti-CD3/CD28 activator beads (Life technologies) and 5 million cells were then transfected by electroporation with 10 µg of each of 2 mRNAs encoding both half TALE-nuclease (or non coding RNA as controls) using a CytoLVT-P instrument. A sample of engineered T-cells were harvested on the day of electroporation and at one, two, five and eight days post electroporation, the remaining T-cells were grown in culture media (REF). Fourteen days post electroporation, all T-cells were harvested. For each time point, the harvested T cells were labeled with fluorochrome-conjugated anti-CD70 monoclonal antibody (ref. 130-104-357, Miltenyi Biotec and according to manufacturer's protocol) and analyzed by flow cytometry for the presence of CD70 at their cell surface. At day 5 post electroporation, genomic DNA was also extracted on harvested T-cells and Polymerase Chain Reactions (PCR) were performed to amplify the targeted CD70 loci (Exon 1 for SEQ ID NO. 104-105 or Exon2 for SEQ ID NO. 106-107), these PCR were then submitted to the T7 Endonuclease 1 assay [New England Biolabs, see Vouillot et al. (2015) Comparison of T7E1 and Surveyor Mismatch Cleavage Assays to Detect Mutations

Triggered by Engineered Nucleases. G3. 5(3): 407–415.] allowing the quantification of nucleases activity.

The results show that all TALE-nucleases specific for the CD70 gene showed cleavage activity using T7 Endonuclease 1 assay at day 5 post electroporation (Figure 1). The TALE-nuclease activities of T02 (SEQ ID NO. 104-105) and T03 (SEQ ID NO. 106-107) were quantified at 54% and 20%, respectively as compared to control.

In addition, TALE-nucleases T02 and T03 were also able to decrease either the percentage of CD70 positive cells and/or the intensity of the labelling measured by the Median Fluorescence Intensity as compared to mock engineered cells (controls) (Figure 2). This is demonstrating that these TALE-nucleases could efficiently inactivate the CD70 gene and the expression of CD70 at the surface of primary T-cells.

In one example (T01), CD70 at cell surface was inactivated (inactive) although partly still expressed at the cell surface. This is presenting another advantage as compared to CD70 deficient cells, probably by disrupting the signaling properties of CD70.

Cells obtained then, were combined with an anti-CD70 therapeutic antibody (CD70 Ab).

The anti-CD70 Ab was the following: Vorsetuzumab (or SGN-70 or h1F6) as disclosed in WO200473656, preferably (h1F6 clone) as disclosed in WO2006113909 which is a humanized h1F6, ARGX 110, SGN CD70A, SGN-CD70A, Vorsetuzumab mafodotin (SGN-75), and MDX 1203 (BMS-936561). The dose chosen was half of the dose giving 50 activity.

In another combination for the study above, the Ab was ARGX 2, at a dose of 5, and 10 mg/kg 3 weeks every 3 weeks with 5×10^5 and 5×10^6 cells at the same time, or at the end of the 3 weeks (Clin Cancer Res October 4 2017 DOI: 10.1158/1078-0432.CCR-17-0613).

Anti-CD70 Ab was used to selectively destroy anti-CD70 expressing cells in individuals suffering a cancer. The cells expressed a CAR allowing the cancer to be targeted and could resist the treatment with anti-CD70 Ab. The amount of engineered cells was also divided by 2 as compared to the effective dose 50 or used at the effective dose 50.

The results surprisingly showed that the combination of the invention was 7 to 10 times more efficient in reducing the tumor mass than either one of the component (engineered cells alone or anti-CD70 Ab alone) of the combination (engineered cells alone or antibody alone). Moreover, individuals receiving both components of the combination had significantly slower relapse and less refractory events than individuals treated with one component of the combination of this invention. The lymphodepletion induced by the CD70 Ab in these

individuals and the in vivo selection of more active CD70 negative immune cells used for therapy (by in vivo depletion of any CD70 positive cells) account for these results.

In one arm, cells were engineered to alter cell surface expression of alpha beta TCR as previously described and combined with a CD70 cell surface depletion.

Table 4: Cluster of differentiation (CD) antigen markers of various cancers found to be expressed on the surface of T-cells

Antigen	Other Names	Structure	main Distribution	Function
CD1a	T6	IgSF, MHC-like	cortical thymocytes, Langerhans cells, DC	antigen presentation, with beta2m
CD1b	T6	IgSF, MHC-like	cortical thymocytes, Langerhans cells, DC	antigen presentation, with beta2m
CD1c	T6	IgSF, MHC-like	cortical thymocytes, Langerhans cells, DC, B subset	antigen presentation, with beta2m
CD1d		IgSF, MHC-like	intestinal epith, B subset, monolow, DC	antigen presentation, with beta2m
CD3 gamma, CD3 delta	T3	IgSF	T, thymocyte subset	with TCR, TCR surface expression / signal transduction
CD3 epsilon	T3	IgSF	T, thymocyte subset	with TCR, TCR surface expression / signal transduction
CD4	T4	IgSF	thymocyte subset, T subset, mono, mac	MHC class II coreceptor, HIV receptor, T cell differentiation / activation
CD5	T1, Tp67	Scavenger R SF	thymocytes, T, B subset, B-CLL	CD72 receptor, TCR or BCR signaling, T-B interaction
CD7		IgSF	hematopoietic progenitors, thymocytes, T, NK	T costimulation
CD8a	T8, Leu-2	IgSF	thymocyte subset, T subset, NK	MHC class I coreceptor, receptor for some mutated HIV-1, T cell differentiation / activation
CD8b		IgSF	thymocyte subset, T subset	
CD9	p24, MRP-1	TM4SF	pre-B, eosinophils, basophils, platelets, Tact	cellular adhesion and migration
CD10	CALLA, NEP, gp100	type II TM	B precursors, T precursors, neutrophils	zinc-binding metalloproteinase, B cell development
CD11a	LFA-1, integrin alphaL	Integrin family	lymph, gran, mono, mac	CD11a / CD18 receptor for ICAM-1, -2, -3, intercellular adhesion, T costimulation
CD11b	Mac-1, integrin alphaM	Integrin family	myeloid cells, NK	binds CD54, ECM, iC3b
CD11c	p150, 95, CR4, integrin alphaX	Integrin family	DC, myeloid cells, NK, B, T subset	binds CD54, fibrinogen and iC3b
CD13	Aminopeptidase N, APN	type II TM	myeloid cells	zinc-binding metalloproteinase, antigen processing, receptor for corona virus strains
CD14	LPS-R	GPI-linked	mono, mac, Langerhans cells, granlow	receptor for LPS/LBP, LPS recognition

CD15	Lewis-x, Lex	CHO	neutrophils, eosinophils, mono	adhesion
CD16a	FcgammaRIIIA	IgSF	neutrophils, mac, NK	component of low affinity Fc receptor, phagocytosis and ADCC
CD16b	FcgammaRIIIB	IgSF	neutrophils	component of low affinity Fc receptor, phagocytosis and ADCC
CD20	B1, Bp35	TM4SF	B, T subset	B cell activation
CD21	C3DR, CR2, EBV-R	CCRSF	B, FDC, T subset	complement C3d and EBV receptor, complex with CD19 and CD81, BCR coreceptor
CD22	BL-CAM, Siglec-2	IgSF, sialoadhesins	B	adhesion, B-mono, B-T interactions
CD23	FcepsilonRII	C-type lectin	B, activated mac, eosinophils, FDC, platelets	CD19-CD21-CD81 receptor, IgE low affinity receptor, signal transduction
CD24	BA-1	GPI-linked	thymocytes, erythrocytes, peripheral lymph, myeloid	binds P-selectin
CD25	Tac, p55	type I TM	Tact, Bact, lymph progenitors	IL-2Ralpha, with IL-2Rbeta and gamma to form high affinity complex
CD31	PECAM-1	IgSF	mono, platelets, gran, endoth, lymph subset	CD38 receptor, adhesion
CD33	p67, Siglec-3	IgSF, sialoadhesins	myeloid progenitors, mono, gran, DC, mast cells, Tact	adhesion
CD37		TM4SF	B, Tlow, granlow	signal transduction
CD38	T10		variable levels on majority of hematopoietic cells, high expression on plasma cells, B and Tact	ecto-ADP-ribosyl cyclase, cell activation
CD40		TNFRSF	B, mono, mac, FDC, endoth, T subset	CD154 receptor, B differentiation / costimulation, isotype-switching, rescues B cells from apoptosis
CD43	Leukosialin, sialophorin	Sialomucin, type I TM	leukocytes, except resting B, plateletslow	inhibition of T cell interaction, CD54R, adhesion
CD44	H-CAM, Pgp-1	hyaladherin family	hematopoietic and non-hematopoietic cells, except platelets, hepatocytes, testis	binds hyaluronic acid, adhesion
CD45	LCA, T200, B220		hematopoietic cells, multiple isoforms from alternative splicing	tyrosine phosphatase, enhanced TCR & BCR signals
CD45RA			B, T subset(naive), mono	exon A isoforms of CD45
CD45RB			T subset, B, mono, mac, gran	exon B isoforms of CD45

CD45RO					Tact, memory T, B subset, mono, mac, gran	isoform of CD45 lacking A, B, C exons
CD46	MCP	CCRSF			nucleated cells	membrane cofactor protein, binds C3b & C4b allowing degradation by Factor I, measles virus receptor
CD47	IAP	IgSF			hematopoietic cells, epith, endoth, fibroblasts, other tissues	leukocyte adhesion, migration, activation
CD48	Blast-1	IgSF			broad, all leukocytes	cell adhesion
CD52	CAMPATH-1				thymocytes, T, B (not plasma cells), mono, mac	
CD53		TM4SF			leukocytes, DC, osteoblasts, osteoclasts	signal transduction
CD55	DAF	GPI-linked			hematopoietic, endoth	binds C3b, complement regulation
CD56	NCAM	IgSF			NK, T subset, neurons, some large granular lymphocyte leukemias, myeloid leukemias	adhesion
CD57	HNK-1, Leu-7				NK subset, T subset	
CD58	LFA-3	IgSF			hematopoietic, non-hematopoietic cells	CD2 receptor, adhesion
CD59	Protectin, MAC-inhibitor	GPI-linked			hematopoietic, non-hematopoietic cells	binds complement C8 and C9, blocks assembly of membrane attack complex
CD60a	GD3	CHO			T subset, platelets, thymic epith, astrocytes	costimulation
CD63	LIMP, LAMP-3	TM4SF			activated platelets, mono, mac	lysosomal membrane protein, moves to cell surface after activation
CD68	Macrosialin, gp110	Sialomucin			intracellularly in mono, mac, neutrophils, basophils, large lymph, mast cells, DC, myeloid progenitors, liver	
CD69	AIM	C-type lectin			Tact, B, NK and gran, thymocytes, platelets, Langerhans cells	signal transduction
CD70	Ki-24	TNFSF			Bact and Tact	CD27 ligand, T and B cell costimulation
CD74	Ii, invariant chain	B, mac, mono, Langerhans cells, DC, Tact				MHC class II traffic and function
CD79a	Iga	IgSF		B		component of BCR, BCR surface expression and signal transduction
CD79b	Igb	IgSF		B		component of BCR, BCR surface expression and signal transduction
CD81	TAPA-1	TM4SF			T, B, NK, thymocytes, DC, endoth, fibroblast, neuroblastomas, melanomas	complex with CD19 & CD21, signaling, T costimulation
CD82	R2	TM4SF			leukocytes	signal transduction

CD83		HB15	IgSF	Bact and Tact, DC, Langerhans cells	
CDw84				mono, platelets, B, T subset, mac subset	
CD86		B70, B7-2	IgSF	mono, DC, Bact and Tact	binds to CD28, CD152, T costimulation
CD87		UPA-R	GPI-linked	gran, mono, NK, Tact, endoth, fibroblasts	urokinase plasminogen activator receptor, inflammatory cell invasion, metastasis
CD90		Thy-1	IgSF, GPI-linked	CD34+ hematopoietic subset, neurons	hematopoietic stem cell and neuron differentiation
CD94		KP43	C-type lectin	NK, T subset	complex with NKG2, inhibits NK function
CD95		Apo-1, Fas	TNFRSF	lymph (high upon activation), mono, neutrophils	FasL (CD178) receptor, apoptosis
CD96		TACTILE	IgSF	NK, Tact	adhesion of activated T and NK
CD97			TM7SF	Bact and Tact, mono, gran	
CD98		4F2		T, B, NK, gran, all human cell lines	cellular activation
CD99		MIC2, E2		leukocytes	T cell activation, adhesion
CD100				hematopoietic cells except immature bone marrow cells, RBC and platelets	cell adhesion, cellular activation
CD103		HML-1, alpha6, integrin alphaE	Integrin family	intraepithelial lymph, lymph subset, activated lymph	with integrin beta7, binds E-cadherin, lymph homing/retention
CD107a		LAMP-1		activated platelets, T, endoth, metastatic tumors	a lysosomal membrane protein
CD107b		LAMP-2		activated platelets, T, endoth, metastatic tumors	a lysosomal membrane protein
CD109				Tact and platelets, CD34+ subset, endoth	
CD123		IL-3R	CRSF	lymph subset, basophils, hematopoietic progenitors, mac, DC, megakaryocytes	IL-3Ralpha, with CDw131
CD146		MUC18, S-endo	IgSF	endoth, melanomas, FDC, Tact	adhesion
CD154		CD40L, gp39, TRAP	TNFSF	Tact	CD40 ligand, B and DC costimulation
CD158a		p58.1	IgSF, KIR family	NK subset, T subset	inhibition of NK cell cytolytic activity, MHC class-I specific NK receptor
CD158b		p58.2	IgSF, KIR family	NK subset, T subset	inhibition of NK cell cytolytic activity, MHC class-I specific NK receptor
CD163		130kD	Scavenger receptor SF	mono, mac	
CD164		MGC-24	epith, mono, lymphflow, bone	hematopoietic progenitor cell-stromal cell interaction	

CD168	RHAMM	marrow stromal cells, CD34+ erythroid progenitors	mono, T subset, thymocyte subset, intracellularly in breast cancer cells	adhesion, tumor migration, metastasis
CD171	L1	IgSF	CNS, PNS, glial cells, mono, T subset, B, DC, several human tumor cells	kidney morphogenesis, lymph node architecture, T costimulation, neurohistogenesis, homotypic interaction, binds CD9, CD24, CD56, CD142, CD166, integrins
CD177	NB1		neutrophil subset	
CD178	FasL, CD95L	TNFSF	Tact, testis	CD95 ligand, apoptosis, immune privilege, soluble form in serum
CD180	RP-105	LRRF, TLR family	B subset, mono, DC	B cell activation, LPS signaling, with MD-1
CD182	CXCR2, IL-8RB	GPCR1 family	neutrophils, basophils, NK, T subset, mono	binding of IL-8 induces chemotaxis of neutrophils
CD185	CXCR5, BLR1	GPCR1 family	mature B and Burkitt Lymphoma cells	with chemokine BLC, possible regulatory function in Burkitt Lymphomagenesis and/or B differentiation, activation of mature B
CD191	CCR1, MIP-1alphaR, RANTES-R	GPCR1 family	T, mono, stem cell subset	binds C-C type chemokines and transduces signal by increasing intracellular calcium ion levels
CD193	CCR3, CKR3	GPCR1 family	eosinophils, lower expression in neutrophils and mono, T subset	binds eotaxin, eotaxin-3, MCP-3, MCP-4, RANTES & MIP-1delta, alternative coreceptor with CD4 for HIV-1 infectiongg
CD196	CCR6, LARC receptor, DRY6	GPCR1 family	T subset, B, DC subset	binds MIP-3alpha/LARC
CD197	CCR7		T subset, DC Subset	6CKine and MIP-2beta receptor
CD200	OX-2		thymocytes, endoth, B, Tact	inhibition of immune response
CD209	DC-SIGN		DC subset	ICAM-3 receptor, HIV-1 binding protein
CD227	MUC1, EMA	Mucin family, type I TM	epith, stem cell subset, FDC, mono, B subset, some myelomas	adhesion, signaling, binds CD169, CD54, & selectins

CD231	TALLA-1, A15	TM4SF	T leukemias, neuroblastomas, brain neurons	marker for T cell acute lymphoblastic leukemia
CD246	ALK, Ki-1		anaplastic T cell leukemias, small intestine, testis, brain, not on normal lymph	brain development, implicated in ALK lymphomas
CD254	TRANCE, RANKL, OPGL	TNFSF	lymph node & BM stroma Tact	binds OPG and RANK, osteoclast differentiation, enhances DC to stimulate naive-T proliferation
CD263	TRAIL-R3, DcR1, LIT		peripheral blood lymphocytes	receptor for TRAIL but lacks death domain
CD272	BTLA	IgSF	Tact, B, remains on Th1	HVEM receptor, inhibitory response
CD273	B7DC, PD-L2, PDGD1L2	IgSF	DC subset, mono, mac	PD-1 receptor, costimulation or suppression of T proliferation
CD276	B7-H3	B7 Family, ASV	in vitro cultured DC and mono, Tact, mammary tissue	costimulation, T activation
CD277	BT3.1, butyrophilin SF3 A1, BTF5	B7/BT family, ASV	T, B, NK, mono, DC, endoth, CD34+ cells, tumor cell lines	T activation
CD279	PD1, SLEB2		Tact and Bact	B7-H1 & B7-DC receptor, autoimmune disease and peripheral tolerance
CD298	Na+/K+-ATPase beta3 subunit		broad	transport sodium & potassium ions across membrane
CD300a	CMRF35H, IRC1, IRp60	IgSF, ASV	NK, mono, neutrophils, T and B subset and lymphocytic cell lines, AML	unknown
CD300c	CMRF35A, LIR	IgSF	mono, neutrophils, monocytic cell lines, B & T subsets	unknown
CD304	BDC4, neuropilin 1	semaphorin family	neurons, CD4+/CD25+ Treg, DC, endothelial and tumor cells	interacts with VEGF165 & semaphorins, co-receptor with plexin, axonal guidance, angiogenesis, cell survival, migration
CD305	LAIR1	IgSF, ASV	NK, B, T, mono	inhibitory receptor on NK and T cells
CD314	NKG2D, KLR	Type II lectin-like receptor	NK, CD8+ activated, NK1.1+ T, some myeloid cells	binds MHC class I, MICA, MICB, Rae1 & ULBP4, activates cytolysis and cytokine production, costimulation
CD317	BST2, HM1.24	Type II	B, T, NK, mono, DC, fibroblast cell line, myeloma	pre-B cell growth, overexpressed in multiple myeloma

CD319	CS1, CRACC, SLAMF7	SLAM receptor family	B Cells, Dendritic Cells, NK, NKT	multiple myeloma
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Table 5: antigen markers expressed on the surface of both colon tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
EPCAM	Epithelial cell adhesion molecule	2,97	13,99
IFITM1	Interferon-induced transmembrane protein 1	10,55	13,06
CLDN4	Claudin-4	2,87	11,62
CDH17	Cadherin-17	1,85	11,52
CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1	3,33	10,84
SLC26A3	Chloride anion exchanger	2,57	10,59
ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	9,28	10,51
SI	Isomaltase	2,86	10,46
ABCB1	Multidrug resistance protein 1	6,09	10,24
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	3,36	9,99
FCGRT	IgG receptor FcRn large subunit p51	4,8	9,98
EPHB3	Ephrin type-B receptor 3	5,23	9,74
DSG2	Desmoglein-2	3,04	8,5
EPHB4	Ephrin type-B receptor 4	6,5	8,44
GUCY2C	Heat-stable enterotoxin receptor	2,23	8,05
EPHA2	Ephrin type-A receptor 2	2,8	7,95
LY6G6D	Lymphocyte antigen 6 complex locus protein G6f	2,02	7,91
CD97	CD97 antigen subunit beta	7,7	7,87
SIGMAR1	Sigma non-opioid intracellular receptor 1	4,58	7,85
EREG	Epiregulin	2,93	6,9
FAIM2	Protein lifeguard 2	2,94	6,82
PIGR	Secretory component	4,2	6,8
SLC7A6	Y+L amino acid transporter 2	8,06	6,55
SCNN1D	Amiloride-sensitive sodium channel subunit delta	1,77	5,74
GPR35	G-protein coupled receptor 35	1,98	5,5
ABCG2	ATP-binding cassette sub-family G member 2	1,79	5,35
LPAR4	Lysophosphatidic acid receptor 4	2,93	5,05
GPR161	G-protein coupled receptor 161	2,71	4,96
CD1C	T-cell surface glycoprotein CD1c	2,73	4,89
SGCA	Alpha-sarcoglycan	2,32	4,84
CD22	B-cell receptor CD22	4,12	4,75
CD22	B-cell receptor CD22	3,58	4,75
CD22	B-cell receptor CD22	2,73	4,75
CD22	B-cell receptor CD22	2,14	4,75
SLC22A18	Solute carrier family 22 member 18	2,32	4,62
HTR7	5-hydroxytryptamine receptor 7	3,02	4,46
LCT	Phlorizin hydrolase	2,32	4,24
CD33	Myeloid cell surface antigen CD33	3,42	4,14
PVR	Poliovirus receptor	5,07	4,07
PLXDC1	Plexin domain-containing protein 1	5,85	3,99
P2RY2	P2Y purinoceptor 2	2,15	3,97
CHRNA2	Neuronal acetylcholine receptor subunit beta-2	6,31	3,88

PTGDR	Prostaglandin D2 receptor	4,08	3,65
NCR1	Natural cytotoxicity triggering receptor 1	2,63	3,33
GYPA	Glycophorin-A	3,18	3,31
TNFRSF8	Tumor necrosis factor receptor superfamily member 8	2	2,75
KEL	Kell blood group glycoprotein	1,93	2,48
EDA	Ectodysplasin-A, secreted form	2,7	2,42
ACE	Angiotensin-converting enzyme, soluble form	2,39	2,19
DRD2	D(2) dopamine receptor	2,49	1,97
CXCR3	C-X-C chemokine receptor type 3	4,19	1,66
MC2R	Adrenocorticotrophic hormone receptor	1,94	1,43

Table 6: antigen markers expressed on the surface of both breast tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ABCA8	ATP-binding cassette sub-family A member 8	3,15	7,73
ABCC10	Multidrug resistance-associated protein 7	6,48	5,29
ABCC6	Multidrug resistance-associated protein 6	2,67	2,17
ACCN2	Acid-sensing ion channel 1	3,62	2,49
ADAM12	Disintegrin and metalloproteinase domain-containing protein 12	4,96	7,72
ADCYAP1R1	Pituitary adenylate cyclase-activating polypeptide type I receptor	2,17	2,88
ADRA1A	Alpha-1A adrenergic receptor	3,31	4,85
ADRA1B	Alpha-1B adrenergic receptor	1,49	1,6
ADRA1D	Alpha-1D adrenergic receptor	2,39	3,38
ADRA2A	Alpha-2A adrenergic receptor	2,64	1,79
ADRB3	Beta-3 adrenergic receptor	2,36	2,16
AGER	Advanced glycosylation end product-specific receptor	2,85	2,38
AGTR2	Type-2 angiotensin II receptor	3,08	3,7
ALK	ALK tyrosine kinase receptor	4,97	4,27
ANO3	Anoctamin-3	2,39	3,69
ANPEP	Aminopeptidase N	3,26	10,78
APLNR	Apelin receptor	2,47	2,06
AQP2	Aquaporin-2	2,12	1,43
ATP10A	Probable phospholipid-transporting ATPase VA	3,96	6,02
ATP2B2	Plasma membrane calcium-transporting ATPase 4	2,75	4,81
ATP2B3	Plasma membrane calcium-transporting ATPase 3	3,7	4,14
ATP4A	Potassium-transporting ATPase alpha chain 1	1,56	11,49
ATP4B	Potassium-transporting ATPase subunit beta	2,49	13,56
ATP6V0A2	V-type proton ATPase 116 kDa subunit a isoform 2	2,51	2,57
ATRN	Attractin	4,09	9,44
AVPR1A	Vasopressin V1a receptor	2,52	4,03
AVPR1B	Vasopressin V1b receptor	2,97	3,32
AVPR2	Vasopressin V2 receptor	2,68	2,93
BAI1	Brain-specific angiogenesis inhibitor 1	2,73	0,33

BAI2	Brain-specific angiogenesis inhibitor 2	2,34	4,14
BAI3	Brain-specific angiogenesis inhibitor 3	2,73	4,76
BDKRB1	B1 bradykinin receptor	2,07	3,28
BRS3	Bombesin receptor subtype-3	2,74	4,12
BTF3	Butyrophilin subfamily 3 member A2	11,29	13,02
C18orf1	Low-density lipoprotein receptor class A domain-containing protein 4	3,18	8,45
C3AR1	C3a anaphylatoxin chemotactic receptor	3,04	5,15
C6orf105	Androgen-dependent TFPI-regulating protein	2,34	3,84
CASR	Extracellular calcium-sensing receptor	2,52	5
CCBP2	Atypical chemokine receptor 2	1,72	3,29
CCKAR	Cholecystokinin receptor type A	2,46	3
CCKBR	Gastrin/cholecystokinin type B receptor	2,25	5,66
CCR2	C-C chemokine receptor type 2	5,94	3,56
CCR3	C-C chemokine receptor type 3	1,89	4,17
CCR6	C-C chemokine receptor-like 2	3,33	5,23
CCR8	C-C chemokine receptor type 8	2,28	3,93
CCR9	C-C chemokine receptor type 9	1,68	1,98
CD1A	T-cell surface glycoprotein CD1a	1,98	4,88
CD1B	T-cell surface glycoprotein CD1b	2,35	4,94
CD1D	Antigen-presenting glycoprotein CD1d	2,82	4,96
CD300C	CMRF35-like molecule 6	2,04	5,04
CD4	T-cell surface glycoprotein CD4	2,84	6,17
CD40LG	CD40 ligand, soluble form	2,1	3,49
CD5	T-cell surface glycoprotein CD5	3,14	1,01
CD63	CD63 antigen	8,6	13,18
CD84	SLAM family member 5	4,7	3,17
CDH15	Cadherin-15	2,07	3,55
CDH19	Protocadherin-16	2,82	8,4
CDH22	Cadherin-22	3	4,9
CDH8	Cadherin-8	3,63	5,87
CDON	Cell adhesion molecule-related/down-regulated by oncogenes	2,35	3,61
CHRNA4	Neuronal acetylcholine receptor subunit alpha-4	2,14	3,33
CHRNA5	Neuronal acetylcholine receptor subunit alpha-5	2,2	4,88
CHRNA6	Neuronal acetylcholine receptor subunit alpha-6	2,26	4,93
CHRNB3	Neuronal acetylcholine receptor subunit beta-3	1,85	3,91
CHRNE	Acetylcholine receptor subunit epsilon	2,56	2,83
CLDN3	Claudin-3	2,91	13,56
CLDN7	Claudin-7	1,89	12,87
CLDN8	Claudin-8	2,46	10,67
CLDN9	Claudin-9	1,74	1,69
CLEC4M	C-type lectin domain family 4 member M	2,7	3,32
CMKLR1	Chemokine-like receptor 1	2,62	5
CNNM2	Metal transporter CNNM2	2,47	5,32
CNR2	Cannabinoid receptor 2	2,38	3,66
CRHR1	Corticotropin-releasing factor receptor 1	2,15	10,71
CRHR2	Corticotropin-releasing factor receptor 2	2,32	6,44
CSF1	Processed macrophage colony-stimulating factor 1	5,63	7,61
CSF1R	Macrophage colony-stimulating factor 1 receptor	2,2	4,02
CSF3R	Granulocyte colony-stimulating factor receptor	1,85	2,8
CX3CL1	Processed fractalkine	2,35	9,31

CXCR5	C-X-C chemokine receptor type 5	2,07	6,06
DAGLA	Sn1-specific diacylglycerol lipase alpha	2,6	2,11
DRD1	D(1A) dopamine receptor	2,67	5,71
DRD3	D(3) dopamine receptor	2,72	4,99
DRD4	D(4) dopamine receptor	1,49	0,89
DRD5	D(1B) dopamine receptor	2,26	4,91
DSC2	Desmocollin-2	2,26	11,12
DSCAM	Down syndrome cell adhesion molecule	2,54	3,76
DSG1	Desmoglein-1	2,62	7,71
EMR2	EGF-like module-containing mucin-like hormone receptor-like 2	2,25	3,38
EPHA5	Ephrin type-A receptor 5	2,42	7,48
EPHA7	Ephrin type-A receptor 7	2,61	4,87
ERBB3	Receptor tyrosine-protein kinase erbB-3	2,39	12,76
F2RL2	Proteinase-activated receptor 3	3,2	5,16
FAM168B	Myelin-associated neurite-outgrowth inhibitor	8,34	11,16
FAP	Seprase	1,87	10,15
FAS	Tumor necrosis factor receptor superfamily member 6	5,68	7,24
FASLG	FasL intracellular domain	2,23	2,66
FCAR	Immunoglobulin alpha Fc receptor	2,8	3,85
FCER1A	High affinity immunoglobulin epsilon receptor subunit alpha	2,54	4,59
FCGR2A	Low affinity immunoglobulin gamma Fc region receptor II-a	2,77	8,81
FCGR2B	Low affinity immunoglobulin gamma Fc region receptor II-b	2,46	5,35
FGFR2	Fibroblast growth factor receptor 2	4,01	9,83
FGFR4	Fibroblast growth factor receptor 4	2,56	7,42
FLT3LG	Fms-related tyrosine kinase 3 ligand	7,86	4,37
FPR1	fMet-Leu-Phe receptor	3,38	5,92
FPR3	N-formyl peptide receptor 3	1,91	2,61
FSHR	Follicle-stimulating hormone receptor	1,89	3,78
FZD5	Frizzled-5	2,82	5,2
FZD5	Frizzled-5	1,81	5,2
FZD9	Frizzled-9	2,66	3,16
GABRA1	Gamma-aminobutyric acid receptor subunit alpha-1	2,2	6,26
GABRA5	Gamma-aminobutyric acid receptor subunit alpha-5	2,49	3,24
GABRA6	Gamma-aminobutyric acid receptor subunit alpha-6	2,54	2,98
GABRB1	Gamma-aminobutyric acid receptor subunit beta-1	1,89	2,37
GABRB2	Gamma-aminobutyric acid receptor subunit beta-2	2,26	3,89
GABRG3	Gamma-aminobutyric acid receptor subunit gamma-3	2,23	2,85
GABRP	Gamma-aminobutyric acid receptor subunit pi	2,93	12,34
GABRR1	Gamma-aminobutyric acid receptor subunit rho-1	2,35	3,47
GABRR2	Gamma-aminobutyric acid receptor subunit rho-2	4,16	5,43
GALR2	Galanin receptor type 2	1,85	0,46
GALR3	Galanin receptor type 3	0,68	0,48
GCGR	Glucagon receptor	1,38	3,4
GHRHR	Growth hormone-releasing hormone receptor	1,61	3,49
GJA5	Gap junction alpha-5 protein	1,72	2,05
GJA8	Gap junction alpha-8 protein	2,39	6,51
GJC1	Gap junction delta-3 protein	1,94	3,89
GLP1R	Glucagon-like peptide 1 receptor	5,72	3,41
GLRA1	Glycine receptor subunit alpha-1	2,15	3,87
GLRA3	Glycine receptor subunit alpha-3	3,19	3,1
GNRHR	Gonadotropin-releasing hormone receptor	2,72	4,1

GPNMB	Transmembrane glycoprotein NMB	2,14	13,94
GPR1	G-protein coupled receptor 1	3,83	4,1
GPR135	Probable G-protein coupled receptor 135	4,15	1,91
GPR143	G-protein coupled receptor 143	1,93	3,65
GPR15	G-protein coupled receptor 15	1,81	4,41
GPR17	Uracil nucleotide/cysteinyl leukotriene receptor	1,93	1,74
GPR171	Probable G-protein coupled receptor 171	7,73	6,32
GPR18	N-arachidonyl glycine receptor	7,05	3,52
GPR182	G-protein coupled receptor 182	1,66	1,29
GPR19	Probable G-protein coupled receptor 19	1,89	5,26
GPR20	G-protein coupled receptor 20	2,02	2,53
GPR3	G-protein coupled receptor 3	3,01	5,36
GPR31	12-(S)-hydroxy-5,8,10,14-eicosatetraenoic acid receptor	1,63	1,64
GPR37L1	Prosaposin receptor GPR37L1	2,23	4
GPR39	G-protein coupled receptor 39	1,81	1,36
GPR44	Prostaglandin D2 receptor 2	2	2,32
GPR45	Probable G-protein coupled receptor 45	2,78	5,31
GPR6	G-protein coupled receptor 6	2,56	3,38
GPR65	Psychosine receptor	6,59	4,5
GPR68	Ovarian cancer G-protein coupled receptor 1	2,12	1,09
GPR98	G-protein coupled receptor 98	1,89	4,7
GRIA1	Glutamate receptor 1	4,17	4,77
GRIA3	Glutamate receptor 3	2,51	6,83
GRIK2	Glutamate receptor ionotropic, kainate 5	2,56	4,94
GRIK3	Glutamate receptor ionotropic, kainate 3	2,05	3,58
GRIN1	Glutamate receptor ionotropic, NMDA 1	4,52	1,49
GRIN2B	Glutamate receptor ionotropic, NMDA 2B	2,22	3,56
GRIN2C	Glutamate receptor ionotropic, NMDA 2C	2,56	3,37
GRM1	Metabotropic glutamate receptor 1	3,21	3,69
GRM2	Metabotropic glutamate receptor 2	2,04	0,44
GRM3	Metabotropic glutamate receptor 3	2,39	3,41
GRM4	Metabotropic glutamate receptor 4	5,2	3,78
GRM5	Metabotropic glutamate receptor 5	2,26	5,28
GRM7	Metabotropic glutamate receptor 7	2,86	3,07
GYPB	Glycophorin-B	2,43	4,02
HBP1	Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1	7,32	9,27
HCRT2	Orexin receptor type 2	2,32	2,42
HTR1B	5-hydroxytryptamine receptor 1B	2,82	3,51
HTR1D	5-hydroxytryptamine receptor 1D	2,29	2,33
HTR1E	5-hydroxytryptamine receptor 1E	1,72	2,4
HTR2A	5-hydroxytryptamine receptor 2A	2,1	3,67
HTR2C	5-hydroxytryptamine receptor 2C	2,49	5,18
HTR4	5-hydroxytryptamine receptor 4	3,86	4,25
ICAM4	Intercellular adhesion molecule 4	2,51	2,16
ICOS	Inducible T-cell costimulator	3,91	3,86
IL6R	Interleukin-6 receptor subunit alpha	4,24	3,08
IL6R	Interleukin-6 receptor subunit alpha	2,64	3,08
IL6ST	Interleukin-6 receptor subunit beta	9,43	12,67
IL9R	Interleukin-9 receptor	2,71	2,86
ITGB3	Integrin beta-3	4,16	3,69
KCNA3	Potassium voltage-gated channel subfamily A member 3	2,09	4,9

KCND2	Potassium voltage-gated channel subfamily D member 2	2,67	4,25
KCNH1	Potassium voltage-gated channel subfamily H member 1	2,31	4,48
KCNJ4	Inward rectifier potassium channel 4	2,43	3,49
KCNMA1	Calcium-activated potassium channel subunit alpha-1	2,35	7,17
KCNS1	Potassium voltage-gated channel subfamily S member 1	5,66	6,49
KCNV2	Potassium voltage-gated channel subfamily V member 2	2,38	4,06
KIR2DL4	Killer cell immunoglobulin-like receptor 2DL4	1,68	3,31
KIR3DL1	Killer cell immunoglobulin-like receptor 3DL1	2,56	2,73
KIR3DL3	Killer cell immunoglobulin-like receptor 3DL3	1,7	3,06
KLRG1	Killer cell lectin-like receptor subfamily G member 1	8,3	5,76
LAMP1	Lysosome-associated membrane glycoprotein 1	10,9	13,6
LHCGR	Lutropin-choriogonadotropic hormone receptor	2,23	4,92
LNPEP	Leucyl-cystinyl aminopeptidase, pregnancy serum form	2,68	5,05
LPAR2	Lysophosphatidic acid receptor 2	5,5	4,23
LRIG2	Leucine-rich repeats and immunoglobulin-like domains protein 2	3,35	5,48
LRRTM2	Leucine-rich repeat transmembrane neuronal protein 2	2,42	4,24
LTB4R	Leukotriene B4 receptor 1	4,96	2,26
MAS1	Proto-oncogene Mas	1,91	3,11
MC1R	Melanocyte-stimulating hormone receptor	2,94	0,96
MC5R	Melanocortin receptor 5	2,28	1,63
MEP1B	Meprin A subunit beta	2,61	3,87
MFSD5	Molybdate-anion transporter	1,98	4,72
MOG	Myelin-oligodendrocyte glycoprotein	3,08	4,74
MTNR1B	Melatonin receptor type 1B	1,61	1,67
MUC1	Mucin-1 subunit beta	2,73	13,68
MUSK	Muscle, skeletal receptor tyrosine-protein kinase	2,39	4,75
NCAM2	Neural cell adhesion molecule 2	2,12	4,49
NCR2	Natural cytotoxicity triggering receptor 2	4,79	7,09
NCR3	Natural cytotoxicity triggering receptor 3	4,55	2,74
NIPA2	Magnesium transporter NIPA2	6,77	3,9
NLGN1	Neurologin-1	2,62	7,71
NLGN4Y	Neurologin-4, Y-linked	2,52	5,26
NMBR	Neuromedin-B receptor	1,68	2,47
NPHS1	Nephrin	2,74	4,33
NPY2R	Neuropeptide Y receptor type 2	2,68	4,43
NPY5R	Neuropeptide Y receptor type 5	2,38	5,05
NTSR2	Neurotensin receptor type 2	1,72	3
OPRD1	Delta-type opioid receptor	2,26	2,14
OPRL1	Nociceptin receptor	2,31	1,51
OPRM1	Mu-type opioid receptor	3,18	4,01
OR10H3	Olfactory receptor 10H3	1,63	4,02
OR1E1	Olfactory receptor 1E1	3,04	4,77
OR2F1	Olfactory receptor 2F1	2,64	5,73
OR2F2	Olfactory receptor 2F2	2,19	2,3
OR2H1	Olfactory receptor 2H1	3,39	3,82
OR2H2	Olfactory receptor 2H2	3,79	6,37
OR2J2	Olfactory receptor 2J2	2,41	2,16
OR2J2	Olfactory receptor 2J2	1,93	2,16
OR5I1	Olfactory receptor 5I1	1,85	2,8
OR7E24	Olfactory receptor 7E24	2,5	3,47
P2RX7	P2X purinoceptor 7	2,36	2,15

PANX1	Pannexin-1	2,14	4,38
PCDHA9	Protocadherin alpha-9	2,82	3,56
PCDHB11	Protocadherin beta-11	1,91	5,23
PCDHGA8	Protocadherin gamma-A8	3,13	4,48
PLA2R1	Soluble secretory phospholipase A2 receptor	2,91	5,16
PLXNA3	Plexin-A3	2,42	3,25
POP1	Blood vessel epicardial substance	1,74	2,59
PPYR1	Neuropeptide Y receptor type 4	2,2	2,75
PTGER1	Prostaglandin E2 receptor EP1 subtype	1,96	0,94
PTGFR	Prostaglandin F2-alpha receptor	2,75	4,89
PTGIR	Prostacyclin receptor	2,78	2,12
PTPRJ	Receptor-type tyrosine-protein phosphatase eta	2,63	4,6
PTPRR	Receptor-type tyrosine-protein phosphatase R	2,47	9,99
PVRL1	Poliovirus receptor-related protein 1	2,52	4,51
PVRL2	Poliovirus receptor-related protein 2	3,84	10,05
ROS1	Proto-oncogene tyrosine-protein kinase ROS	2,93	3,38
S1PR2	Sphingosine 1-phosphate receptor 2	1,74	1,17
S1PR4	Sphingosine 1-phosphate receptor 4	4	0,21
SCNN1B	Amiloride-sensitive sodium channel subunit beta	1,89	3,16
SCNN1G	Amiloride-sensitive sodium channel subunit gamma	2,23	2,61
SEMA4D	Semaphorin-4D	10,66	1,56
SEMA6A	Semaphorin-6A	4,55	7,81
SEMA6C	Semaphorin-6C	5,02	3,73
SGCB	Beta-sarcoglycan	2,69	3,45
SGCB	Beta-sarcoglycan	2,04	3,45
SLC12A3	Solute carrier family 12 member 3	2,26	3,36
SLC14A1	Urea transporter 1	2,97	6,21
SLC14A2	Urea transporter 2	2,85	4,4
SLC16A1	Monocarboxylate transporter 1	3,46	8,84
SLC16A2	Monocarboxylate transporter 8	1,77	5,17
SLC16A6	Monocarboxylate transporter 7	2,41	11,66
SLC22A1	Solute carrier family 22 member 1	2,95	11,61
SLC22A6	Solute carrier family 22 member 6	2,26	2,53
SLC5A12	Sodium-coupled monocarboxylate transporter 2	2,98	4,45
SLC6A1	Sodium- and chloride-dependent GABA transporter 1	2,45	4,3
SLC6A4	Sodium-dependent serotonin transporter	2,17	2,66
SLC6A6	Sodium- and chloride-dependent taurine transporter	2,54	4,13
SLC7A7	Y+L amino acid transporter 1	2,22	9,78
SLC8A1	Sodium/calcium exchanger 1	2,07	2,36
SLC9A1	Sodium/hydrogen exchanger 1	3,15	5,54
SLC9A3	Sodium/hydrogen exchanger 3	2,12	3,15
SLCO1A2	Solute carrier organic anion transporter family member 1A2	3,87	4,98
SLCO2B1	Solute carrier organic anion transporter family member 2B1	4,43	8,92
SORT1	Sortilin	2,93	4,6
SSTR2	Somatostatin receptor type 2	3,08	4,47
SSTR3	Somatostatin receptor type 3	2,23	1,5
SSTR4	Somatostatin receptor type 4	1,83	1,53
SSTR5	Somatostatin receptor type 5	2,57	1,47
TACR1	Substance-P receptor	2,66	3,2
TACR3	Neuromedin-K receptor	2,32	5,7
TLR6	Toll-like receptor 6	2,2	4,58
TMPRSS6	Transmembrane protease serine 6	4,02	3,69

TNFSF11	Tumor necrosis factor ligand superfamily member 11,	2,57	5,18
TNFSF14	Tumor necrosis factor ligand superfamily member 14, soluble form	3,34	2,83
TPO	Thyroid peroxidase	1,96	1,89
TRAT1	T-cell receptor-associated transmembrane adapter 1	7,51	5,29
TRHR	Thyrotropin-releasing hormone receptor	2	4,18
TRPM1	Transient receptor potential cation channel subfamily M member 1	2,43	5,22
TSHR	Thyrotropin receptor	2,9	4,87
TSHR	Thyrotropin receptor	2,12	4,87
UNC93A	Protein unc-93 homolog A	2,64	4,94
VIPR2	Vasoactive intestinal polypeptide receptor 2	2,58	3,37
ZP2	Processed zona pellucida sperm-binding protein 2	1,94	3,55

Table 7: antigen markers expressed on the surface of both digestive tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ACVR1B	Activin receptor type-1B	5,16	10,48
AMIGO2	Amphoterin-induced protein 2	6,73	8,2
ATP1B1	Sodium/potassium-transporting ATPase subunit beta-1	2,64	12,31
ATP8B1	Probable phospholipid-transporting ATPase IC	8,22	2,17
CCR7	C-C chemokine receptor type 7	10,25	11,52
CD164	Sialomucin core protein 24	10,27	12,12
CD180	CD180 antigen	2,5	6,47
CD40	Tumor necrosis factor receptor superfamily member 5	5,02	6
CD53	Leukocyte surface antigen CD53	10,79	11,3
CD79A	B-cell antigen receptor complex-associated protein alpha chain	3,74	9,17
CD79B	B-cell antigen receptor complex-associated protein beta chain	3,6	6,66
CD8B	T-cell surface glycoprotein CD8 beta chain	8,43	2,62
CELSR1	Cadherin EGF LAG seven-pass G-type receptor 1	2,72	8,68
CLCN5	H(+)/Cl(-) exchange transporter 5	2,71	4,97
CLDN18	Claudin-18	3,05	14,51
CLIC1	Chloride intracellular channel protein 1	9,94	13,83
COL13A1	Collagen alpha-1(XIII) chain	2,96	6,24
DIO3	Type III iodothyronine deiodinase	2,04	2,9
EDNRA	Endothelin-1 receptor	2,9	8,96
EMR1	EGF-like module-containing mucin-like hormone receptor-like 1	1,83	7,29
ENPP1	Nucleotide pyrophosphatase	2,57	9,66
EPHB1	Ephrin type-B receptor 1	2,02	6,33
EPHB1	Ephrin type-B receptor 1	1,81	6,33
F2R	Proteinase-activated receptor 1	3,04	9,78
F2RL1	Proteinase-activated receptor 2, alternate cleaved 2	3,31	9,47
FCER2	Low affinity immunoglobulin epsilon Fc receptor soluble form	2,49	8,77
GABBR1	Gamma-aminobutyric acid type B receptor subunit 1	5,1	8,52

GABRA3	Gamma-aminobutyric acid receptor subunit alpha-3	2,12	3,84
GPR183	G-protein coupled receptor 183	4,79	10,22
GPR37	Prosaposin receptor GPR37	3,1	8,23
GPRC5A	Retinoic acid-induced protein 3	1,87	13,69
GRPR	Gastrin-releasing peptide receptor	2,04	3,35
GYPC	Glycophorin-C	9,22	7,58
IL1R2	Interleukin-1 receptor type 2, soluble form	2,82	12,83
KIAA0319	Dyslexia-associated protein KIAA0319	2,43	5,61
LAMP2	Lysosome-associated membrane glycoprotein 2	4,05	11,29
LRP8	Low-density lipoprotein receptor-related protein 8	4,24	8,84
LSR	Lipolysis-stimulated lipoprotein receptor	4,99	11,48
MICB	MHC class I polypeptide-related sequence B	5,27	9,89
MMP16	Matrix metalloproteinase-16	3,19	6,18
MS4A1	B-lymphocyte antigen CD20	2,15	8,02
MYOF	Myoferlin	2,41	11,56
NAT1	Sodium-coupled neutral amino acid transporter 3	3,49	12,09
NFASC	Neurofascin	3,78	8,28
NPY1R	Neuropeptide Y receptor type 1	2,32	6,93
OR2B6	Olfactory receptor 2B6	2,78	4,24
P2RY10	Putative P2Y purinoceptor 10	3,39	6,62
PCDH1	Protocadherin-1	4,45	10,07
PROM1	Prominin-1	2,52	11,77
PSEN1	Presenilin-1 CTF12	2,94	8,83
PTGER2	Prostaglandin E2 receptor EP2 subtype	6,33	6,74
PTGER4	Prostaglandin E2 receptor EP4 subtype	8,62	5,12
PTPRK	Receptor-type tyrosine-protein phosphatase kappa	2,14	10,9
RET	Extracellular cell-membrane anchored RET cadherin 120 kDa fragment	2,38	12,3
SERINC3	Serine incorporator 3	7,93	12,01
SIT1	Sodium- and chloride-dependent transporter XTRP3	5,92	4,82
SLAMF1	Signaling lymphocytic activation molecule	4,4	9,03
SLC29A1	Equilibrative nucleoside transporter 1	2,07	6,12
SLC39A6	Zinc transporter ZIP6	6,69	15,23
SLC7A5	Large neutral amino acids transporter small subunit 1	3,79	10,98
STX4	Syntaxin-4	5,68	7,67
TGFBR3	Transforming growth factor beta receptor type 3	7,55	7,29
TGOLN2	Trans-Golgi network integral membrane protein 2	9,59	11,3
TLR1	Toll-like receptor 1	2,34	4,57
TMED10	Transmembrane emp24 domain-containing protein 10	9,34	12,24
TMEM97	Transmembrane protein 97	2,75	9,02
TNF	Tumor necrosis factor, soluble form	1,63	3,18
TNFRSF17	Tumor necrosis factor receptor superfamily member 17	1,89	10,47
TNFRSF1B	Tumor necrosis factor-binding protein 2	5,51	9,4
VDAC1	Voltage-dependent anion-selective channel protein 1	6,52	11,5

Table 8: antigen markers expressed on the surface of both kidney tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ADORA3	Adenosine receptor A3	1,89	4,56
ATP11A	Probable phospholipid-transporting ATPase 1H	3,62	8,8
BSG	Basigin	4,77	11,34
BTN3A2	Butyrophilin subfamily 3 member A2	10,86	8,19
C10orf72	V-set and transmembrane domain-containing protein 4	2,04	6,85
CADM3	Cell adhesion molecule 3	3,57	6,39
CD8A	T-cell surface glycoprotein CD8 alpha chain	10,35	6,6
CDH16	Cadherin-16	2,17	7,09
CDH4	Cadherin-4	2,15	3,6
CDH5	Cadherin-5	2,5	9,55
CHL1	Processed neural cell adhesion molecule L1-like protein	2,69	10,43
CHRNA1	Acetylcholine receptor subunit beta	2,12	3,6
CLIC4	Chloride intracellular channel protein 4	3,34	13,12
CNR1	Cannabinoid receptor 1	2,26	5,64
CRIM1	Processed cysteine-rich motor neuron 1 protein	3,57	12,39
CSPG4	Chondroitin sulfate proteoglycan 4	3,33	6,59
CYBB	Cytochrome b-245 heavy chain	2,86	8,07
EDNRB	Endothelin B receptor	3,04	8,97
FLT1	Vascular endothelial growth factor receptor 1	2,75	8,5
FZD1	Frizzled-1	2,72	7,59
GJC2	Gap junction gamma-2 protein	2,09	2,94
GLRB	Glycine receptor subunit beta	2,51	7,15
GPER	G-protein coupled estrogen receptor 1	2,34	8,64
GPM6A	Neuronal membrane glycoprotein M6-a	2,95	6,88
GPR162	Probable G-protein coupled receptor 162	2,75	2,81
GPR4	G-protein coupled receptor 4	2,93	8,09
GRM8	Metabotropic glutamate receptor 8	3,43	8,25
HLA-DPB1	HLA class II histocompatibility antigen, DP beta 1 chain	9,93	13,99
HTR6	5-hydroxytryptamine receptor 6	4,83	10,07
INSR	Insulin receptor subunit beta	3,44	8,95
ITM2B	Bri23 peptide	11,16	12,19
KCNJ1	ATP-sensitive inward rectifier potassium channel 1	2,5	4,17
KDR	Vascular endothelial growth factor receptor 2	2,99	9,95
KL	Klotho peptide	2,83	7,59
LAIR1	Leukocyte-associated immunoglobulin-like receptor 1	5,64	4,25
MFAP3	Microfibril-associated glycoprotein 3	3,7	7,3
MFAP3L	Microfibrillar-associated protein 3-like	3,44	8,7
MICA	MHC class I polypeptide-related sequence A	4,07	2,01
NCAM1	Neural cell adhesion molecule 1	2,45	7,31
NOTCH3	Notch 3 intracellular domain	3,21	12,41
NOTCH4	Notch 4 intracellular domain	5,89	8,84
OLR1	Oxidized low-density lipoprotein receptor 1, soluble form	2,84	8,41
P2RY14	P2Y purinoceptor 14	2,63	4,63
PCDH17	Protocadherin-17	1,7	7,36
PDGFRB	Platelet-derived growth factor receptor beta	2,68	10,48

PECAM1	Platelet endothelial cell adhesion molecule	7,7	10,85
PLXND1	Plexin-D1	5,02	11,68
PPAP2B	Lipid phosphate phosphohydrolase 3	4,17	12,46
PTAFR	Platelet-activating factor receptor	3,01	4,81
PTGER3	Prostaglandin E2 receptor EP3 subtype	4,76	10,26
PTH1R	Parathyroid hormone/parathyroid hormone-related peptide receptor	2,35	7,31
RAMP3	Receptor activity-modifying protein 3	1,79	8,84
ROR2	Tyrosine-protein kinase transmembrane receptor ROR2	3,2	5,98
S1PR1	Sphingosine 1-phosphate receptor 1	5,17	6,51
SCARB1	Scavenger receptor class B member 1	3,01	10,4
SLC13A3	Solute carrier family 13 member 3	3,32	7,89
SLC16A4	Monocarboxylate transporter 5	2,88	12,54
SLC17A3	Sodium-dependent phosphate transport protein 4	1,58	11,55
SLC28A1	Sodium/nucleoside cotransporter 1	4,76	6,3
SLC2A5	Solute carrier family 2, facilitated glucose transporter member 5	2,74	8,5
SLC39A14	Zinc transporter ZIP14	2,66	11,63
SLC6A13	Sodium- and chloride-dependent GABA transporter 2	2,75	7,44
SLC7A8	Large neutral amino acids transporter small subunit 2	5,03	10,46
SLCO2A1	Solute carrier organic anion transporter family member 2A1	3,46	8,06
TBXA2R	Thromboxane A2 receptor	4,01	3,64
TGFBR2	TGF-beta receptor type-2	10,41	10,94
THSD7A	Thrombospondin type-1 domain-containing protein 7A	3,05	8
TIE1	Tyrosine-protein kinase receptor Tie-1	2,04	4,41
TNFRSF1A	Tumor necrosis factor-binding protein 1	6,84	10,52
TNFSF12	Tumor necrosis factor ligand superfamily member 12, secreted form	4,35	4,1
VAMP5	Vesicle-associated membrane protein 5	3,49	6,18

Table 9: antigen markers expressed on the surface of both liver tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ABCB4	Multidrug resistance protein 3	2,02	3,7
ADAM10	Disintegrin and metalloproteinase domain-containing protein 10	9,42	9,41
ATR	Anthrax toxin receptor 1	6,98	9,9
BST2	Bone marrow stromal antigen 2	7,38	12,45
BTN3A3	Butyrophilin subfamily 3 member A3	9,72	7,48
C9	Complement component C9b	2,41	10,52
CHRND	Acetylcholine receptor subunit delta	2,43	4,05
CLDN14	Claudin-14	2,79	2,4
EPOR	Erythropoietin receptor	4,67	10,55
ERBB2	Receptor tyrosine-protein kinase erbB-2	2,36	14,12
F2RL3	Proteinase-activated receptor 4	2,17	2,61
GJB1	Gap junction beta-1 protein	2,96	9,4
GPR126	G-protein coupled receptor 126	2,23	11,32
IL1R1	Interleukin-1 receptor type 1, soluble form	2,88	12,57
ITGB1	Integrin beta-1	8,76	13,48
NAALADL1	N-acetylated-alpha-linked acidic dipeptidase-like protein	3,03	1,46
OR7A5	Olfactory receptor 7A5	1,51	3,83
SGCD	Delta-sarcoglycan	3,99	7,21
SIGLEC6	Sialic acid-binding Ig-like lectin 6	3,57	3,49
SLC38A3	Sodium-coupled neutral amino acid transporter 3	1,89	8,91
TFR2	Transferrin receptor protein 2	2,74	10,47

Table 10: antigen markers expressed on the surface of both lung tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ABCB6	ATP-binding cassette sub-family B member 6, mitochondrial	2,88	9,82
ABCC1	Multidrug resistance-associated protein 1	7,05	8,16
ACCN1	Acid-sensing ion channel 2	2,25	0,8
ADAM23	Disintegrin and metalloproteinase domain-containing protein 23	2,51	4,73
ADORA1	Adenosine receptor A1	4,49	8,22
ADORA2B	Adenosine receptor A2b	1,66	7,5
AJAP1	Adherens junction-associated protein 1	1,85	6,24
APLP1	C30	2,22	6,02
AQP3	Aquaporin-3	8,38	13,88
ATP10D	Probable phospholipid-transporting ATPase VD	2,43	7,4
ATP1A3	Sodium/potassium-transporting ATPase subunit alpha-3	3,01	3,13
ATP1B2	Sodium/potassium-transporting ATPase subunit beta-2	3,21	3,8
ATP1B3	Sodium/potassium-transporting ATPase subunit beta-3	8,6	14,26
AXL	Tyrosine-protein kinase receptor UFO	2,51	9,58
BEST1	Bestrophin-1	2,49	4,44
BTC	Betacellulin	2,86	4,59
BTN3A1	Butyrophilin subfamily 3 member A1	10,66	11,63
CALCR	Calcitonin receptor	2,95	8,62
CALCRL	Calcitonin gene-related peptide type 1 receptor	2,12	7,67
CCR1	C-C chemokine receptor type 1	2,63	9,77
CD163	Soluble CD163	2,66	8,76
CD300A	CMRF35-like molecule 8	7,96	4,23
CD300A	CMRF35-like molecule 8	2,29	4,23
CD68	Macrosialin	4,02	8,92
CD74	HLA class II histocompatibility antigen gamma chain	9,1	13,44
CD86	T-lymphocyte activation antigen CD86	2,93	5,04
CHRNA3	Neuronal acetylcholine receptor subunit alpha-3	2,54	4,62
CHRNA3	Neuronal acetylcholine receptor subunit alpha-3	2	4,62
CKAP4	Cytoskeleton-associated protein 4	6,15	11,94
CLCA2	Calcium-activated chloride channel regulator 2, 35 kDa form	2,99	9,81
CLDN5	Claudin-5	3,66	7,73
CLSTN1	CTF1-alpha	8,26	12,51
CNIH3	Protein cornichon homolog 3	2,7	6,09
COMT	Catechol O-methyltransferase	7,78	12,13
CSPG5	Chondroitin sulfate proteoglycan 5	2,84	5,69
CXCR6	C-X-C chemokine receptor type 6	3,16	3,91
CXCR7	Atypical chemokine receptor 3	2,5	8,95
DCHS1	Protocadherin-16	4,29	2,28
DSC3	Desmocollin-2	2,82	8,95
DSG3	Desmoglein-3	2,23	10,73

EGFR	Epidermal growth factor receptor	3,8	10,92
FAT2	Protocadherin Fat 2	2,25	9,29
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	3,13	8,96
FCGR1A	High affinity immunoglobulin gamma Fc receptor I	2,09	9,65
FLT4	Vascular endothelial growth factor receptor 3	3,19	3,36
FPR2	N-formyl peptide receptor 2	2,9	7,14
FURIN	Furin	6,42	7,5
FZD6	Frizzled-6	2,64	10,45
GABBR2	Gamma-aminobutyric acid type B receptor subunit 2	3,79	9,19
GABRB3	Gamma-aminobutyric acid receptor subunit beta-3	2,46	8,83
GABRD	Gamma-aminobutyric acid receptor subunit delta	1,72	1,67
GABRE	Gamma-aminobutyric acid receptor subunit epsilon	1,85	9,18
GIPR	Gastric inhibitory polypeptide receptor	3,43	5,37
GJA1	Gap junction alpha-1 protein	2,84	12,65
GJB3	Gap junction beta-3 protein	3,72	3,79
GJB5	Gap junction beta-5 protein	1,77	6,69
GLRA2	Glycine receptor subunit alpha-2	2,26	6,15
GPR109B	Hydroxycarboxylic acid receptor 3	1,77	2,91
GPR12	G-protein coupled receptor 12	2	1,76
GPR176	Probable G-protein coupled receptor 176	2,05	3,86
GPR50	Melatonin-related receptor	2,26	3,16
GRIK1	Glutamate receptor ionotropic, kainate 1	4,66	5,65
GRIN2D	Glutamate receptor ionotropic, NMDA 2D	2,17	2,32
HCRTR1	Orexin receptor type 1	2,34	3,56
HLA-DPA1	HLA class II histocompatibility antigen, DP alpha 1 chain	8,31	12,86
HLA-DQA1	HLA class II histocompatibility antigen, DQ alpha 1 chain	2,35	11,44
HLA-DQB1	HLA class II histocompatibility antigen, DQ beta 1 chain	7,4	12,71
HLA-DRA	HLA class II histocompatibility antigen, DR alpha chain	6,42	14,18
HLA-DRB4	HLA class II histocompatibility antigen, DR beta 4 chain	2,72	11,24
IGSF9B	Protein turtle homolog B	3,92	2,81
IL1RAP	Interleukin-1 receptor accessory protein	3,99	11,4
IL1RL1	Interleukin-1 receptor-like 1	2,55	5,15
IL4R	Soluble interleukin-4 receptor subunit alpha	4,15	9,56
IL7R	Interleukin-7 receptor subunit alpha	11,62	11,26
ITGA6	Integrin alpha-6 light chain	7,99	12,76
JPH3	Junctophilin-3	2,34	2,5
KCNS3	Potassium voltage-gated channel subfamily S member 3	2,45	8,91
KIT	Mast/stem cell growth factor receptor Kit	2,85	8,67
KITLG	Soluble KIT ligand	2,58	7,27
LILRB3	Leukocyte immunoglobulin-like receptor subfamily B member 3	5,65	8,03
LILRB4	Leukocyte immunoglobulin-like receptor subfamily B member 4	3,12	10,44
LPAR1	Lysophosphatidic acid receptor 1	4,12	5,47
LPHN3	Latrophilin-3	2,85	6,43
MMP24	Processed matrix metalloproteinase-24	5,19	5,73
MPZ	Myelin protein P0	2,56	3,63
MUC4	Mucin-4 beta chain	3,04	10,34
NCKAP1L	Nck-associated protein 1-like	6,69	7,51
NKG7	Protein NKG7	10,92	3,66
NOTCH2	Notch 2 intracellular domain	6,62	6,22

NRCAM	Neuronal cell adhesion molecule	2,78	8,16
NRG2	Neuregulin-2	3,55	9,22
NRXN1	Neurexin-1	2,56	5,33
NTRK2	BDNF/NT-3 growth factors receptor	2,56	10,7
NTSR1	Neurotensin receptor type 1	1,74	9,74
P2RY1	P2Y purinoceptor 1	2,34	7,62
P2RY6	P2Y purinoceptor 6	4,27	5,79
PCDH8	Protocadherin-8	2,67	9,29
PCDHA3	Protocadherin alpha-3	2,14	3,54
PIK3IP1	Phosphoinositide-3-kinase-interacting protein 1	8,68	3,47
PLXNA2	Plexin-A2	2,88	7,3
PRR4	Processed poliovirus receptor-related protein 4	3,24	8,02
PTPRE	Receptor-type tyrosine-protein phosphatase epsilon	6,03	7,92
PTPRO	Receptor-type tyrosine-protein phosphatase U	10,46	9,01
PTPRU	Receptor-type tyrosine-protein phosphatase U	3,72	6,18
RABAC1	Prenylated Rab acceptor protein 1	7,54	8,82
SCTR	Secretin receptor	2,2	2,48
SECTM1	Secreted and transmembrane protein 1	2,42	6,9
SGCE	Epsilon-sarcoglycan	2,15	9,65
SGCG	Gamma-sarcoglycan	2,56	5,74
SLC16A3	Monocarboxylate transporter 4	5,89	12,72
SLC16A7	Monocarboxylate transporter 2	5,39	6,97
SLC20A2	Sodium-dependent phosphate transporter 2	2,51	12,69
SLC26A4	Pendrin	3,57	9,39
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	5,1	5,83
SLC4A7	Sodium bicarbonate cotransporter 3	4,89	8,7
SLCO3A1	Solute carrier organic anion transporter family member 3A1	4,87	7,91
SYNE2	Nesprin-2	9,43	10,43
TACR2	Substance-K receptor	2,23	6,68
TFRC	Transferrin receptor protein 1, serum form	7,32	14,31
TMEFF1	Tomoregulin-1	3,22	5,05
TMPRSS11D	Transmembrane protease serine 11D catalytic chain	2,35	8,32

Table 11: antigen markers expressed on the surface of both ovary tumor cells and T-cells;

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ACVR2B	Activin receptor type-2B	2,1	4,26
ADAM28	Disintegrin and metalloproteinase domain-containing protein 28	2,83	9,22
ADRA2C	Alpha-2C adrenergic receptor	4,6	5,13
ATP2B1	Plasma membrane calcium-transporting ATPase 1	5,3	11,49
ATP2B4	Plasma membrane calcium-transporting ATPase 4	8,21	10,1
ATP7A	Copper-transporting ATPase 1	3,91	7,31
CD200	OX-2 membrane glycoprotein	2,83	10,51
CD47	Leukocyte surface antigen CD47	9,88	10,42
CDH12	Cadherin-12	2,31	5,91

CDH18	Cadherin-18	2,28	4,79
CDH2	Cadherin-2	3,72	11,97
CDH6	Cadherin-6	2,77	8,68
CDIPT	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	8,88	10,73
CELSR2	Cadherin EGF LAG seven-pass G-type receptor 2	2,66	8,38
CHRNA1	Acetylcholine receptor subunit alpha	2,42	5,71
CLSTN3	Calsyntenin-3	3,87	4,54
CX3CR1	CX3C chemokine receptor 1	9	11,42
DDR1	Epithelial discoidin domain-containing receptor 1	3,83	12,36
EPHA1	Ephrin type-A receptor 1	2,02	5,96
EPHA4	Ephrin type-A receptor 4	2,39	8,56
ERBB4	ERBB4 intracellular domain	2,29	9,76
FGFR1	Fibroblast growth factor receptor 1	5,42	11,4
FGFR3	Fibroblast growth factor receptor 3	2,95	11,35
FZD2	Frizzled-2	1,91	8,06
FZD7	Frizzled-7	2,55	10,24
GJA4	Gap junction alpha-4 protein	2,04	6,7
GPR125	Probable G-protein coupled receptor 125	2,35	7,88
GPR56	GPR56 C-terminal fragment	8,6	11,27
GPR64	G-protein coupled receptor 64	2,04	8,57
GPRC5B	G-protein coupled receptor family C group 5 member B	1,96	10,29
GRIA2	Glutamate receptor 2	1,96	11,78
GRIK5	Glutamate receptor ionotropic, kainate 5	5,79	3,36
GRIN2A	Glutamate receptor ionotropic, NMDA 2A	1,68	2,96
HEG1	Protein HEG homolog 1	4,8	10,1
HRH1	Histamine H1 receptor	2,31	6,26
HTR3A	5-hydroxytryptamine receptor 3A	2,1	9,35
IFITM2	Interferon-induced transmembrane protein 2	10,27	11,36
IFITM3	Interferon-induced transmembrane protein 3	8,55	13,48
KCNH2	Potassium voltage-gated channel subfamily H member 2	2,09	5,36
KCNJ12	ATP-sensitive inward rectifier potassium channel 12	2,29	2,21
L1CAM	Neural cell adhesion molecule L1	2,61	8,73
LGR5	Leucine-rich repeat-containing G-protein coupled receptor 5	2,45	12,12
LPHN1	Latrophilin-1	4,5	5,56
LPHN1	Latrophilin-1	1,63	5,56
LPHN2	Latrophilin-2	1,93	7,14
MGA	Glucoamylase	5,15	5,65
NEO1	Neogenin	1,85	10,31
NPTN	Neuroplastin	8,46	13,14
NRG1	Neuregulin-1	2,61	6,53
NTRK1	High affinity nerve growth factor receptor	2,09	2,49
PCDH7	Protocadherin-7	2,89	8,52
PCDH9	Protocadherin-9	2,99	6,15
PDGFRA	Platelet-derived growth factor receptor alpha	3,69	8,44
PDGFRA	Platelet-derived growth factor receptor alpha	2,26	8,44
PLXNB1	Plexin-B1	2,26	6,71
PLXNB2	Plexin-B2	3,1	10,68
PODXL	Podocalyxin	2,73	11,41
PRSS8	Prostasin heavy chain	2,07	10,77
PTH2R	Parathyroid hormone 2 receptor	1,85	8,67
PVRL3	Poliovirus receptor-related protein 3	2,56	10,15

SCNN1A	Amiloride-sensitive sodium channel subunit alpha	5,97	10,63
SLC29A2	Equilibrative nucleoside transporter 2	2,93	1,89
SSPN	Sarcospan	3,49	9,16
STAR	Heat-stable enterotoxin receptor	2,36	7,13
TGFA	Transforming growth factor alpha	2,64	1,71
TMED1	Transmembrane emp24 domain-containing protein 1	4,79	9,3
TMEM59	Transmembrane protein 59	8,83	12,74
TNFRSF25	Tumor necrosis factor receptor superfamily member 25	7,53	4,27
TYRO3	Tyrosine-protein kinase receptor TYRO3	4,11	10,27
UPK2	Uroplakin-2	2,29	7,49

Table 12: antigen markers expressed on the surface of both pancreas tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ADAM9	Disintegrin and metalloproteinase domain-containing protein 9	3,49	10,99
B4GALT1	Processed beta-1,4-galactosyltransferase 1	7,44	8,99
BDKRB2	B2 bradykinin receptor	2,52	4,44
CA9	Carbonic anhydrase 9	3,34	11,9
CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	2,36	4,54
CD58	Lymphocyte function-associated antigen 3	6,51	8,16
CDH11	Cadherin-11	2,85	10,38
CDH3	Cadherin-3	1,96	10,91
CFTR	Cystic fibrosis transmembrane conductance regulator	3,12	11,45
CHRNA4	Neuronal acetylcholine receptor subunit beta-4	2,38	0,66
CLDN10	Claudin-10	2,36	11,5
CXCR4	C-X-C chemokine receptor type 4	11,74	10,98
DAG1	Beta-dystroglycan	5,65	10,98
DDR2	Discoidin domain-containing receptor 2	2,34	8
DMPK	Myotonic-protein kinase	3,7	4,21
FAT1	Protocadherin Fat 1, nuclear form	3,3	12,45
HTR2B	5-hydroxytryptamine receptor 2B	2,22	7,73
LDLR	Low-density lipoprotein receptor	2,93	12,14
NCKAP1	Nck-associated protein 1	3,34	11,99
PMP22	Peripheral myelin protein 22	2,09	10,66
PNPLA2	Patatin-like phospholipase domain-containing protein 2	5,46	3,45
PNPLA2	Patatin-like phospholipase domain-containing protein 2	2,35	3,45
TEK	Angiopoietin-1 receptor	3,87	8,52
TGFBR1	TGF-beta receptor type-1	2,17	4,3

Table 13: antigen markers expressed on the surface of both prostate tumor cells and T-cells

Antigen	Protein Name	Relative expression in T-Cell	Relative Expression in colon cancer cells
ACCN3	Acid-sensing ion channel 3	2,47	2,03
ADRB1	Beta-1 adrenergic receptor	2,85	5,09
ADRB2	Beta-2 adrenergic receptor	5,74	9,43
AGTR1	Type-1 angiotensin II receptor	2,81	11,62
ALPL2	Amyloid-like protein 2	7,06	13,06
ATP1A2	Sodium/potassium-transporting ATPase subunit alpha-2	3,07	7,55
ATP8A1	Probable phospholipid-transporting ATPase 1A	7,23	9,16
CADM1	Cell adhesion molecule 1	4,42	12,28
CHRM3	Muscarinic acetylcholine receptor M3	1,85	9,23
CHRNA2	Neuronal acetylcholine receptor subunit alpha-2	2,83	5,34
CXADR	Coxsackievirus and adenovirus receptor	3,31	12,74
DPP4	Dipeptidyl peptidase 4 soluble form	6,42	11,22
ECE1	Endothelin-converting enzyme 1	7,14	4,7
ENPP4	Bis(5'-adenosyl)-triphosphatase ENPP4	6,57	7,49
EPHA3	Ephrin type-A receptor 3	2,84	7,85
ERG	Potassium voltage-gated channel subfamily H member 2	2,72	11,3
FAM38A	Piezo-type mechanosensitive ion channel component 1	8,4	9,57
FOLH1	Glutamate carboxypeptidase 2	2,96	13,18
GABRA2	Gamma-aminobutyric acid receptor subunit alpha-2	3	6,42
GHR	Growth hormone-binding protein	2,52	6,84
GPM6B	Neuronal membrane glycoprotein M6-b	3,22	6,56
GPR116	Probable G-protein coupled receptor 116	3,69	10,09
HBEGF	Heparin-binding EGF-like growth factor	2,87	8,12
JAM3	Junctional adhesion molecule C	4,29	7,26
KCND3	Potassium voltage-gated channel subfamily D member 3	3,09	9,77
LIFR	Leukemia inhibitory factor receptor	2,71	6,8
LRBA	Lipopolysaccharide-responsive and beige-like anchor protein	5,35	9,26
MME	Neprilysin	2,62	8,05
NOV	Plexin-A1	2,43	10,41
NRP1	Neuropilin-1	3,17	7,85
OPRK1	Kappa-type opioid receptor	2,07	4,92
PLXNB3	Plexin-B3	2,57	3,59
PPAP2A	Lipid phosphate phosphohydrolase 1	3,6	11,55
SCAMP5	Secretory carrier-associated membrane protein 5	3,03	8,43
SLC23A2	Solute carrier family 23 member 2	3,55	7,04
SLC2A4	Solute carrier family 2, facilitated glucose transporter member 4	2,67	5,96
SLC36A1	Proton-coupled amino acid transporter 1	3,38	9,28
SLC4A4	Electrogenic sodium bicarbonate cotransporter 1	3,14	11,29
STIM1	Stromal interaction molecule 1	3,68	6,51
TMPRSS2	Transmembrane protease serine 2 catalytic chain	2,67	9,63
TRPV6	Transient receptor potential cation channel subfamily V member 6	4,84	8,09
VIPR1	Vasoactive intestinal polypeptide receptor 1	4,41	7,73

YIPF3	Protein YIPF3, 36 kDa form III	4	4,3
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Table 14: antigen markers expressed on the surface of T-cells and overexpressed in liquid tumor cells (ALL, AML, CML, MDS, CLL, CTRL)

Antigen	Protein Name	Relative expression on T cell
CD63	CD63 antigen	0.83
CXCR4	C-X-C chemokine receptor type 4	0.82
IFITM2	Interferon-induced transmembrane protein 2	0.82
ITM2B	Bri23 peptide	0.81
BTF3	Butyrophilin subfamily 3 member A2	0.8
HLA-DRB1	HLA class II histocompatibility antigen, DRB1-12 beta chain	0.79
HLA-DRA	HLA class II histocompatibility antigen, DR alpha chain	0.78
IFITM3	Interferon-induced transmembrane protein 3	0.78
NKG7	Protein NKG7	0.78
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	0.78
IFITM1	Interferon-induced transmembrane protein 1	0.76
NPTN	Neuroplastin	0.76
GYPC	Glycophorin-C	0.76
GPR160	Probable G-protein coupled receptor 160	0.76
HLA-DPB1	HLA class II histocompatibility antigen, DP beta 1 chain	0.75
BRI3	CT-BRI3	0.75
SLC38A2	Sodium-coupled neutral amino acid transporter 2	0.74
C5AR1	C5a anaphylatoxin chemotactic receptor 1	0.74
CDIPT	CDP-diacylglycerol--inositol 3-phosphatidyltransferase	0.73
TNFSF13B	Tumor necrosis factor ligand superfamily member 13b, soluble form	0.73
CSF3R	Granulocyte colony-stimulating factor receptor	0.73
HLA-DPA1	HLA class II histocompatibility antigen, DP alpha 1 chain	0.71
CD164	Sialomucin core protein 24	0.71
CD97	CD97 antigen subunit beta	0.7
C3AR1	C3a anaphylatoxin chemotactic receptor	0.69
P2RY8	P2Y purinoceptor 8	0.68
BSG	Basigin	0.68
APLP2	Amyloid-like protein 2	0.67
TFRC	Transferrin receptor protein 1, serum form	0.67
MGAM	Glucoamylase	0.67
GYPA	Glycophorin-A	0.67
TMED10	Transmembrane emp24 domain-containing protein 10	0.66
FCGRT	IgG receptor FcRn large subunit p51	0.66
CKAP4	Cytoskeleton-associated protein 4	0.66
DYSF	Dysferlin	0.66
SPPL2A	Signal peptide peptidase-like 2A	0.65

LAMP2	Lysosome-associated membrane glycoprotein 2	0.65
SLC7A5	Large neutral amino acids transporter small subunit 1	0.65
TNFRSF1B	Tumor necrosis factor-binding protein 2	0.64
TREM1	Triggering receptor expressed on myeloid cells 1	0.64
GPR183	G-protein coupled receptor 183	0.63
SERINC3	Serine incorporator 3	0.63
CD58	Lymphocyte function-associated antigen 3	0.63
GYPB	Glycophorin-B	0.63
RABAC1	Prenylated Rab acceptor protein 1	0.62
KCNH2	Potassium voltage-gated channel subfamily H member 2	0.62
FPR1	fMet-Leu-Phe receptor	0.62
P2RY13	P2Y purinoceptor 13	0.62
CLEC5A	C-type lectin domain family 5 member A	0.62
SLC7A7	Y+L amino acid transporter 1	0.61
MICB	MHC class I polypeptide-related sequence B	0.61
CD300LF	CMRF35-like molecule 1	0.61
GJB6	Gap junction beta-6 protein	0.61
ATP1A1	Sodium/potassium-transporting ATPase subunit alpha-1	0.6
PTGER4	Prostaglandin E2 receptor EP4 subtype	0.6
CD8A	T-cell surface glycoprotein CD8 alpha chain	0.6
PTGER2	Prostaglandin E2 receptor EP2 subtype	0.6
GPR97	Probable G-protein coupled receptor 97	0.6
IMP3	Signal peptide peptidase-like 2A	0.59
LAMP1	Lysosome-associated membrane glycoprotein 1	0.59
LILRB3	Leukocyte immunoglobulin-like receptor subfamily B member 3	0.59
GPR109B	Hydroxycarboxylic acid receptor 3	0.59
SAT2	Sodium-coupled neutral amino acid transporter 2	0.58
GPR65	Psychosine receptor	0.58
AMICA1	Junctional adhesion molecule-like	0.58
PAG1	Phosphoprotein associated with glycosphingolipid-enriched microdomains 1	0.58
ENPP4	Bis(5'-adenosyl)-triphosphatase ENPP4	0.57
SLC40A1	Solute carrier family 40 member 1	0.57
OLR1	Oxidized low-density lipoprotein receptor 1, soluble form	0.57
LRRC33	Negative regulator of reactive oxygen species	0.56
IL7R	Interleukin-7 receptor subunit alpha	0.56
LAIR1	Leukocyte-associated immunoglobulin-like receptor 1	0.56
ITM2C	CT-BRI3	0.56
GPR84	G-protein coupled receptor 84	0.56
SLC12A7	Solute carrier family 12 member 7	0.55
PTAFR	Platelet-activating factor receptor	0.55
CD33	Myeloid cell surface antigen CD33	0.55
SLC22A16	Solute carrier family 22 member 16	0.55
CCR7	C-C chemokine receptor type 7	0.54
TLR1	Toll-like receptor 1	0.54

TGOLN2	Trans-Golgi network integral membrane protein 2	0.54
YIPF3	Protein YIPF3, 36 kDa form III	0.54
BST2	Bone marrow stromal antigen 2	0.54
MAGT1	Magnesium transporter protein 1	0.54
TMEM173	Stimulator of interferon genes protein	0.54
ERMAP	Erythroid membrane-associated protein	0.54
CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1	0.54
NIPA2	Magnesium transporter NIPA2	0.53
PECAM1	Platelet endothelial cell adhesion molecule	0.53
CD1D	Antigen-presenting glycoprotein CD1d	0.53
TMEM59	Transmembrane protein 59	0.53
NCKAP1L	Nck-associated protein 1-like	0.53
FAS	Tumor necrosis factor receptor superfamily member 6	0.53
IL6R	Interleukin-6 receptor subunit alpha	0.53
TNFRSF1A	Tumor necrosis factor-binding protein 1	0.53
KEL	Kell blood group glycoprotein	0.53
TMEM149	IGF-like family receptor 1	0.52
SLC3A2	4F2 cell-surface antigen heavy chain	0.52
ORAI1	Calcium release-activated calcium channel protein 1	0.52
XKR8	XK-related protein 8, processed form	0.52
C9orf46	Plasminogen receptor (KT)	0.52
TMEM127	Transmembrane protein 127	0.52
SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	0.52
FCGR1B	High affinity immunoglobulin gamma Fc receptor IB	0.52
CXCR2	C-X-C chemokine receptor type 2	0.52
IL4R	Soluble interleukin-4 receptor subunit alpha	0.51
HSD17B7	3-keto-steroid reductase	0.51
SEMA4D	Semaphorin-4D	0.51
ZDHHC5	Palmitoyltransferase ZDHHC5	0.51
ADRB2	Beta-2 adrenergic receptor	0.51
S1PR4	Sphingosine 1-phosphate receptor 4	0.51
PILRA	Paired immunoglobulin-like type 2 receptor alpha	0.51
LTB4R	Leukotriene B4 receptor 1	0.51
SORT1	Sortilin	0.51
SLCO4C1	Solute carrier organic anion transporter family member 4C1	0.51
ANO10	Anoctamin-10	0.51
CLSTN1	CTF1-alpha	0.5
RHBDF2	Inactive rhomboid protein 2	0.5
CCR1	C-C chemokine receptor type 1	0.5
EPCAM	Epithelial cell adhesion molecule	0.5
PNPLA2	Patatin-like phospholipase domain-containing protein 2	0.49
SLC12A6	Solute carrier family 12 member 6	0.49
SLC30A1	Zinc transporter 1	0.49
GPR27	Probable G-protein coupled receptor 27	0.49

EPOR	Erythropoietin receptor	0.49
CD79A	B-cell antigen receptor complex-associated protein alpha chain	0.48
HLA-DQB1	HLA class II histocompatibility antigen, DQ beta 1 chain	0.48
HBP1	Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1	0.48
ABCA7	ATP-binding cassette sub-family A member 7	0.48
RAG1AP1	Sugar transporter SWEET1	0.48
CD47	Leukocyte surface antigen CD47	0.48
CXCL16	C-X-C motif chemokine 16	0.48
SLC14A1	Urea transporter 1	0.48
TGFBR2	TGF-beta receptor type-2	0.47
LRBA	Lipopolysaccharide-responsive and beige-like anchor protein	0.47
MFSD5	Molybdate-anion transporter	0.47
RELT	Tumor necrosis factor receptor superfamily member 19L	0.47
ATP2B4	Plasma membrane calcium-transporting ATPase 4	0.47
FURIN	Furin	0.47
GAPT	Protein GAPT	0.47
NFAM1	NFAT activation molecule 1	0.47
ATP2B1	Plasma membrane calcium-transporting ATPase 1	0.46
SLC26A11	Sodium-independent sulfate anion transporter	0.46
STX4	Syntaxin-4	0.46
NAT1	Sodium-coupled neutral amino acid transporter 3	0.46
STIM1	Stromal interaction molecule 1	0.46
SLC39A4	Zinc transporter ZIP4	0.46
ESYT2	Extended synaptotagmin-2	0.46
TM7SF3	Transmembrane 7 superfamily member 3	0.46
SEMA4A	Semaphorin-4A	0.46
CYBB	Cytochrome b-245 heavy chain	0.46
FCAR	Immunoglobulin alpha Fc receptor	0.46
GABBR1	Gamma-aminobutyric acid type B receptor subunit 1	0.45
CD53	Leukocyte surface antigen CD53	0.45
SIGLEC10	Sialic acid-binding Ig-like lectin 10	0.45
S1PR1	Sphingosine 1-phosphate receptor 1	0.45
BTN3A2	Butyrophilin subfamily 3 member A2	0.45
NOTCH2	Notch 2 intracellular domain	0.45
PIK3IP1	Phosphoinositide-3-kinase-interacting protein 1	0.45
FAM168B	Myelin-associated neurite-outgrowth inhibitor	0.45
LPAR2	Lysophosphatidic acid receptor 2	0.45
ATP1B3	Sodium/potassium-transporting ATPase subunit beta-3	0.45
FLVCR1	Feline leukemia virus subgroup C receptor-related protein 1	0.45
SECTM1	Secreted and transmembrane protein 1	0.45
SLC38A5	Sodium-coupled neutral amino acid transporter 5	0.45
GPR18	N-arachidonyl glycine receptor	0.44
LMBR1L	Protein LMBR1L	0.44
ABCC1	Multidrug resistance-associated protein 1	0.44

SLC22A18	Solute carrier family 22 member 18	0.44
CSF1R	Macrophage colony-stimulating factor 1 receptor	0.44
EMR1	EGF-like module-containing mucin-like hormone receptor-like 1	0.44
FPR2	N-formyl peptide receptor 2	0.44
KIT	Mast/stem cell growth factor receptor Kit	0.44
MS4A1	B-lymphocyte antigen CD20	0.43
MICA	MHC class I polypeptide-related sequence A	0.43
GPR172A	Solute carrier family 52, riboflavin transporter, member 2	0.43
F11R	Junctional adhesion molecule A	0.43
ADAM10	Disintegrin and metalloproteinase domain-containing protein 10	0.43
FAM38A	Piezo-type mechanosensitive ion channel component 1	0.43
CD68	Macrosialin	0.43
SLC26A6	Solute carrier family 26 member 6	0.43
MCOLN1	Mucolipin-1	0.43
SLCO3A1	Solute carrier organic anion transporter family member 3A1	0.43
PPAP2B	Lipid phosphate phosphohydrolase 3	0.43
ICAM4	Intercellular adhesion molecule 4	0.43
CXCR1	C-X-C chemokine receptor type 1	0.43
CD300A	CMRF35-like molecule 8	0.43
RELL1	RELT-like protein 1	0.43
TAPBPL	Tapasin-related protein	0.42
FCGR2C	Low affinity immunoglobulin gamma Fc region receptor II-c	0.42
SLC16A6	Monocarboxylate transporter 7	0.42
TMED1	Transmembrane emp24 domain-containing protein 1	0.42
CD86	T-lymphocyte activation antigen CD86	0.42
SLC16A3	Monocarboxylate transporter 4	0.42
SLC2A5	Solute carrier family 2, facilitated glucose transporter member 5	0.42
SLC29A1	Equilibrative nucleoside transporter 1	0.42
SLC16A14	Monocarboxylate transporter 14	0.42
P2RY2	P2Y purinoceptor 2	0.42
SUCNR1	Succinate receptor 1	0.42
BTN3A1	Butyrophilin subfamily 3 member A1	0.41
LAT2	Linker for activation of T-cells family member 2	0.41
PLXND1	Plexin-D1	0.41
ECE1	Endothelin-converting enzyme 1	0.41
TGFBR1	TGF-beta receptor type-1	0.41
CCRL2	C-C chemokine receptor-like 2	0.41
TFR2	Transferrin receptor protein 2	0.41
SLC44A1	Choline transporter-like protein 1	0.41
ITGA6	Integrin alpha-6 light chain	0.41
PMP22	Peripheral myelin protein 22	0.41
LAX1	Lymphocyte transmembrane adapter 1	0.4
AMIGO2	Amphoterin-induced protein 2	0.4
SLC38A1	Sodium-coupled neutral amino acid transporter 1	0.4

SLC41A1	Solute carrier family 41 member 1	0.4
C2orf89	Metalloprotease TIK1	0.4
ABCC10	Multidrug resistance-associated protein 7	0.4
CLDN15	Claudin-15	0.4
SLC39A6	Zinc transporter ZIP6	0.4
SLC16A5	Monocarboxylate transporter 6	0.4
TTYH3	Protein tweety homolog 3	0.4
ATP7A	Copper-transporting ATPase 1	0.4
COMT	Catechol O-methyltransferase	0.4
SLC17A5	Sialin	0.4
TMIGD2	Transmembrane and immunoglobulin domain-containing protein 2	0.4
CLEC7A	C-type lectin domain family 7 member A	0.4
SLC31A1	High affinity copper uptake protein 1	0.4
LRRC4	Leucine-rich repeat-containing protein 4	0.4
P2RY10	Putative P2Y purinoceptor 10	0.39
ATP10D	Probable phospholipid-transporting ATPase VD	0.39
BTN3A3	Butyrophilin subfamily 3 member A3	0.39
LIME1	Lck-interacting transmembrane adapter 1	0.39
TNF	Tumor necrosis factor, soluble form	0.39
PAQR8	Membrane progesterin receptor beta	0.39
OXER1	Oxoeicosanoid receptor 1	0.39
TRAT1	T-cell receptor-associated transmembrane adapter 1	0.39
GPBAR1	G-protein coupled bile acid receptor 1	0.39
SLC36A1	Proton-coupled amino acid transporter 1	0.39
PTPRE	Receptor-type tyrosine-protein phosphatase epsilon	0.39
PROM1	Prominin-1	0.39
CD74	HLA class II histocompatibility antigen gamma chain	0.38
CNST	Consortin	0.38
TMEM49	Vacuole membrane protein 1	0.38
CLIC4	Chloride intracellular channel protein 4	0.38
NAALADL1	N-acetylated-alpha-linked acidic dipeptidase-like protein	0.38
ANTXR2	Anthrax toxin receptor 2	0.38
FGFR1	Fibroblast growth factor receptor 1	0.38
IL1RAP	Interleukin-1 receptor accessory protein	0.38
ATP1B2	Sodium/potassium-transporting ATPase subunit beta-2	0.38
ABCG2	ATP-binding cassette sub-family G member 2	0.38
CLEC12A	C-type lectin domain family 12 member A	0.38
HLA-DQA1	HLA class II histocompatibility antigen, DQ alpha 1 chain	0.37
B4GALT1	Processed beta-1,4-galactosyltransferase 1	0.37
CNNM3	Metal transporter CNNM3	0.37
ATP1B1	Sodium/potassium-transporting ATPase subunit beta-1	0.37
SLC39A1	Zinc transporter ZIP1	0.37
ATRN	Attractin	0.37
CYSLTR1	Cysteinyl leukotriene receptor 1	0.37

TRPV2	Transient receptor potential cation channel subfamily V member 2	0.37
SLC27A1	Long-chain fatty acid transport protein 1	0.37
GPR171	Probable G-protein coupled receptor 171	0.37
DAGLB	Sn1-specific diacylglycerol lipase beta	0.37
KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	0.37
FZD6	Frizzled-6	0.37
CSF2RA	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha	0.37
PTH2R	Parathyroid hormone 2 receptor	0.37
MARCH1	E3 ubiquitin-protein ligase MARCH1	0.36
BACE2	Beta-secretase 2	0.36
CD5	T-cell surface glycoprotein CD5	0.36
TMEM219	Insulin-like growth factor-binding protein 3 receptor	0.36
XPR1	Xenotropic and polytropic retrovirus receptor 1	0.36
CD1C	T-cell surface glycoprotein CD1c	0.36
CNNM2	Metal transporter CNNM2	0.36
TMEM88	Transmembrane protein 88	0.36
ICOS	Inducible T-cell costimulator	0.36
KLRG1	Killer cell lectin-like receptor subfamily G member 1	0.36
LRP8	Low-density lipoprotein receptor-related protein 8	0.36
F2R	Proteinase-activated receptor 1	0.36
HM13	Minor histocompatibility antigen H13	0.36
EMR2	EGF-like module-containing mucin-like hormone receptor-like 2	0.36
TREML1	Trem-like transcript 1 protein	0.36
C17orf60	Allergin-1	0.36
GPR146	Probable G-protein coupled receptor 146	0.36
SLAMF6	SLAM family member 6	0.35
SLC7A6	Y+L amino acid transporter 2	0.35
RELL2	RELT-like protein 2	0.35
LGR6	Leucine-rich repeat-containing G-protein coupled receptor 6	0.35
PANX1	Pannexin-1	0.35
C18orf1	Low-density lipoprotein receptor class A domain-containing protein 4	0.35
SLMAP	Sarcolemmal membrane-associated protein	0.35
CCR5	C-C chemokine receptor type 5	0.35
MUC1	Mucin-1 subunit beta	0.35
EMR3	EGF-like module-containing mucin-like hormone receptor-like 3 subunit beta	0.35
COL23A1	Collagen alpha-1(XXIII) chain	0.35
OR2W3	Olfactory receptor 2W3	0.35
LNPEP	Leucyl-cystinyl aminopeptidase, pregnancy serum form	0.34
PRR7	Proline-rich protein 7	0.34
NOTCH1	Notch 1 intracellular domain	0.34
RFT1	Solute carrier family 52, riboflavin transporter, member 1	0.34
TNFRSF25	Tumor necrosis factor receptor superfamily member 25	0.34

ANO6	Anoctamin-6	0.34
AQP3	Aquaporin-3	0.34
ADAM9	Disintegrin and metalloproteinase domain-containing protein 9	0.34
INSR	Insulin receptor subunit beta	0.34
FZD5	Frizzled-5	0.34
ERG	Potassium voltage-gated channel subfamily H member 2	0.34
MME	Neprilysin	0.34
FCGR2B	Low affinity immunoglobulin gamma Fc region receptor II-b	0.33
LSR	Lipolysis-stimulated lipoprotein receptor	0.33
DDR1	Epithelial discoidin domain-containing receptor 1	0.33
CNR2	Cannabinoid receptor 2	0.33
ATR	Anthrax toxin receptor 1	0.33
P2RY14	P2Y purinoceptor 14	0.33
VEZT	Vezatin	0.33
ALG10B	Putative Dol-P-Glc:Glc(2)Man(9)GlcNAc(2)-PP-Dol alpha-1,2-glucosyltransferase	0.33
PAQR7	Membrane progesterin receptor alpha	0.33
FLT3LG	Fms-related tyrosine kinase 3 ligand	0.33
CD40LG	CD40 ligand, soluble form	0.33
FCGR2A	Low affinity immunoglobulin gamma Fc region receptor II-a	0.33
CLDN12	Claudin-12	0.33
GP6	Platelet glycoprotein VI	0.33
EPHB4	Ephrin type-B receptor 4	0.33
SEMA4C	Semaphorin-4C	0.33
CD300C	CMRF35-like molecule 6	0.33
PEAR1	Platelet endothelial aggregation receptor 1	0.33
FFAR2	Free fatty acid receptor 2	0.33
SLC2A6	Solute carrier family 2, facilitated glucose transporter member 6	0.32
TMEM150A	Transmembrane protein 150A	0.32
ANO8	Anoctamin-8	0.32
CD200R1	Cell surface glycoprotein CD200 receptor 1	0.32
FCER1A	High affinity immunoglobulin epsilon receptor subunit alpha	0.32
BEST1	Bestrophin-1	0.32
CLDN5	Claudin-5	0.32
SLC47A1	Multidrug and toxin extrusion protein 1	0.32
SLC5A10	Sodium/glucose cotransporter 5	0.32
CD40	Tumor necrosis factor receptor superfamily member 5	0.31
ANO9	Anoctamin-9	0.31
CLEC2D	C-type lectin domain family 2 member D	0.31
VIPR1	Vasoactive intestinal polypeptide receptor 1	0.31
SLC16A7	Monocarboxylate transporter 2	0.31
UTS2R	Urotensin-2 receptor	0.31
CLSTN3	Calsyntenin-3	0.31
GPR35	G-protein coupled receptor 35	0.31
SYT15	Synaptotagmin-15	0.31

FAM57A	Protein FAM57A	0.31
CD8B	T-cell surface glycoprotein CD8 beta chain	0.31
IL17RC	Interleukin-17 receptor C	0.31
GLDN	Gliomedin	0.31
FZD2	Frizzled-2	0.31
KCNA3	Potassium voltage-gated channel subfamily A member 3	0.3
MGA	Glucoamylase	0.3
GPR1	G-protein coupled receptor 1	0.3
IL6ST	Interleukin-6 receptor subunit beta	0.3
PCDHGB5	Protocadherin gamma-B5	0.3
OR1I1	Olfactory receptor 1I1	0.3
PTH1R	Parathyroid hormone/parathyroid hormone-related peptide receptor	0.3
NLGN2	Neurologin-2	0.3
MMP24	Processed matrix metalloproteinase-24	0.3
CDH22	Cadherin-22	0.3
TNFRSF8	Tumor necrosis factor receptor superfamily member 8	0.3
CHRNA2	Acetylcholine receptor subunit gamma	0.3
PSEN1	Presenilin-1 CTF12	0.3
GPR114	Probable G-protein coupled receptor 114	0.3
PLXNB2	Plexin-B2	0.3
CHRNA2	Neuronal acetylcholine receptor subunit alpha-2	0.3
GPR34	Probable G-protein coupled receptor 34	0.3
LPAR6	Lysophosphatidic acid receptor 6	0.3
ATP8A1	Probable phospholipid-transporting ATPase 1A	0.3
FZD1	Frizzled-1	0.3
CCR2	C-C chemokine receptor type 2	0.3
P2RY1	P2Y purinoceptor 1	0.3
SLC16A9	Monocarboxylate transporter 9	0.3
C20orf103	Lysosome-associated membrane glycoprotein 5	0.3
ADORA2B	Adenosine receptor A2b	0.3
CLEC12B	C-type lectin domain family 12 member B	0.3
FCRL3	Fc receptor-like protein 3	0.29
CD180	CD180 antigen	0.29
TIGIT	T-cell immunoreceptor with Ig and ITIM domains	0.29
PPAP2A	Lipid phosphate phosphohydrolase 1	0.29
ATP11C	Probable phospholipid-transporting ATPase 1G	0.29
TNFRSF17	Tumor necrosis factor receptor superfamily member 17	0.29
TNFSF12	Tumor necrosis factor ligand superfamily member 12, secreted form	0.29
TBXA2R	Thromboxane A2 receptor	0.29
OR3A3	Olfactory receptor 3A3	0.29
GPR153	Probable G-protein coupled receptor 153	0.29
ATP11A	Probable phospholipid-transporting ATPase 1H	0.29

LRFN1	Leucine-rich repeat and fibronectin type III domain-containing protein 1	0.29
OR51B2	Olfactory receptor 51B2	0.29
KCNS1	Potassium voltage-gated channel subfamily S member 1	0.29
OR12D2	Olfactory receptor 12D2	0.29
GRM4	Metabotropic glutamate receptor 4	0.29
NEO1	Neogenin	0.29
DRD5	D(1B) dopamine receptor	0.29
PLXDC1	Plexin domain-containing protein 1	0.29
GPR157	Probable G-protein coupled receptor 157	0.29
CD300LB	CMRF35-like molecule 7	0.29
MARVELD1	MARVEL domain-containing protein 1	0.29
MFAP3	Microfibril-associated glycoprotein 3	0.29
CHRNA1	Acetylcholine receptor subunit beta	0.29
PVRL2	Poliovirus receptor-related protein 2	0.29
F2RL1	Proteinase-activated receptor 2, alternate cleaved 2	0.29
GPR124	G-protein coupled receptor 124	0.29
BACE1	Beta-secretase 1	0.29
C6orf105	Androgen-dependent TFPI-regulating protein	0.28
CXCR3	C-X-C chemokine receptor type 3	0.28
IGSF8	Immunoglobulin superfamily member 8	0.28
ATP8B1	Probable phospholipid-transporting ATPase IC	0.28
TP53I13	Tumor protein p53-inducible protein 13	0.28
MC1R	Melanocyte-stimulating hormone receptor	0.28
CD84	SLAM family member 5	0.28
CALHM1	Calcium homeostasis modulator protein 1	0.28
CHRNA6	Neuronal acetylcholine receptor subunit alpha-6	0.28
CDH10	Cadherin-10	0.28
SLC16A1	Monocarboxylate transporter 1	0.28
GPRC5D	G-protein coupled receptor family C group 5 member D	0.28
AGER	Advanced glycosylation end product-specific receptor	0.28
FASLG	FasL intracellular domain	0.28
GPR56	GPR56 C-terminal fragment	0.28
SIGLEC1	Sialoadhesin	0.28
KIR2DL5A	Killer cell immunoglobulin-like receptor 2DL5A	0.28
PLB1	Lysophospholipase	0.28
CD200	OX-2 membrane glycoprotein	0.27
ADAM28	Disintegrin and metalloproteinase domain-containing protein 28	0.27
SIT1	Sodium- and chloride-dependent transporter XTRP3	0.27
SLC23A2	Solute carrier family 23 member 2	0.27
CCR10	C-C chemokine receptor type 10	0.27
PRR4	Processed poliovirus receptor-related protein 4	0.27
GJD2	Gap junction delta-2 protein	0.27
SLC2A8	Solute carrier family 2, facilitated glucose transporter member 8	0.27
CD209	CD209 antigen	0.27

CD274	Programmed cell death 1 ligand 1	0.27
PROM2	Prominin-2	0.27
ATP6V0A2	V-type proton ATPase 116 kDa subunit a isoform 2	0.27
MPZ	Myelin protein P0	0.27
TNFRSF18	Tumor necrosis factor receptor superfamily member 18	0.27
MFSD2A	Major facilitator superfamily domain-containing protein 2A	0.27
HEG1	Protein HEG homolog 1	0.27
OXTR	Oxytocin receptor	0.27
CD99L2	CD99 antigen-like protein 2	0.27
LILRB4	Leukocyte immunoglobulin-like receptor subfamily B member 4	0.27
SMAGP	Small cell adhesion glycoprotein	0.27
OR51I2	Olfactory receptor 51I2	0.27
LY6G6D	Lymphocyte antigen 6 complex locus protein G6f	0.27
KCNQ4	Potassium voltage-gated channel subfamily KQT member 4	0.27
HRH2	Histamine H2 receptor	0.27
SLC39A2	Zinc transporter ZIP2	0.27
CLDN10	Claudin-10	0.27
GPM6B	Neuronal membrane glycoprotein M6-b	0.27
STEAP4	Metalloreductase STEAP4	0.27
APOLD1	Apolipoprotein L domain-containing protein 1	0.27
S1PR3	Sphingosine 1-phosphate receptor 3	0.27
SGMS2	Phosphatidylcholine:ceramide cholinephosphotransferase 2	0.27
KIR2DS5	Killer cell immunoglobulin-like receptor 2DS5	0.27
STAR	Heat-stable enterotoxin receptor	0.27
NIPA1	Magnesium transporter NIPA1	0.26
CNNM4	Metal transporter CNNM4	0.26
SLAMF1	Signaling lymphocytic activation molecule	0.26
KIAA1919	Sodium-dependent glucose transporter 1	0.26
TLR6	Toll-like receptor 6	0.26
CRB3	Protein crumbs homolog 3	0.26
SLC12A9	Solute carrier family 12 member 9	0.26
GPR68	Ovarian cancer G-protein coupled receptor 1	0.26
OR51J1	Olfactory receptor 51J1	0.26
TREML2	Trem-like transcript 2 protein	0.26
GPR176	Probable G-protein coupled receptor 176	0.26
FLVCR2	Feline leukemia virus subgroup C receptor-related protein 2	0.26
LPAR1	Lysophosphatidic acid receptor 1	0.26
PANX2	Pannexin-2	0.26
SLC6A6	Sodium- and chloride-dependent taurine transporter	0.26
PROKR2	Prokineticin receptor 2	0.26
CLDN9	Claudin-9	0.26
MYOF	Myoferlin	0.26
LY6G6F	Lymphocyte antigen 6 complex locus protein G6f	0.26
ESAM	Endothelial cell-selective adhesion molecule	0.26

NCR3	Natural cytotoxicity triggering receptor 3	0.25
HLA-DQB2	HLA class II histocompatibility antigen, DQ beta 2 chain	0.25
SLC4A5	Electrogenic sodium bicarbonate cotransporter 4	0.25
P2RY4	P2Y purinoceptor 4	0.25
ABCB1	Multidrug resistance protein 1	0.25
SLC9A1	Sodium/hydrogen exchanger 1	0.25
CELSR2	Cadherin EGF LAG seven-pass G-type receptor 2	0.25
SYT8	Synaptotagmin-8	0.25
PCDHA9	Protocadherin alpha-9	0.25
TMEM204	Transmembrane protein 204	0.25
PTPRJ	Receptor-type tyrosine-protein phosphatase eta	0.25
GRPR	Gastrin-releasing peptide receptor	0.25
SEMA6B	Semaphorin-6B	0.25
CLCN5	H(+)/Cl(-) exchange transporter 5	0.25
GLRA2	Glycine receptor subunit alpha-2	0.25
PLVAP	Plasmalemma vesicle-associated protein	0.25
ACVR1B	Activin receptor type-1B	0.25
JAM3	Junctional adhesion molecule C	0.25
LDLRAD3	Low-density lipoprotein receptor class A domain-containing protein 3	0.25
XG	Glycoprotein Xg	0.25
SLC2A11	Solute carrier family 2, facilitated glucose transporter member 11	0.24
PCDH9	Protocadherin-9	0.24
VAMP5	Vesicle-associated membrane protein 5	0.24
CDHR2	Cadherin-related family member 2	0.24
DRD2	D(2) dopamine receptor	0.24
LRIG2	Leucine-rich repeats and immunoglobulin-like domains protein 2	0.24
RAMP3	Receptor activity-modifying protein 3	0.24
SLC39A14	Zinc transporter ZIP14	0.24
STRA6	Stimulated by retinoic acid gene 6 protein homolog	0.24
ADRA2C	Alpha-2C adrenergic receptor	0.24
CLDN19	Claudin-19	0.24
CX3CR1	CX3C chemokine receptor 1	0.24
CD79B	B-cell antigen receptor complex-associated protein beta chain	0.24
KIR2DL2	Killer cell immunoglobulin-like receptor 2DL2	0.24
CXCR7	Atypical chemokine receptor 3	0.24
OR5L2	Olfactory receptor 5L2	0.24
LRRC52	Leucine-rich repeat-containing protein 52	0.24
JPH1	Junctophilin-1	0.24
ADORA1	Adenosine receptor A1	0.24
GPRC5C	G-protein coupled receptor family C group 5 member C	0.24
RET	Extracellular cell-membrane anchored RET cadherin 120 kDa fragment	0.24
PVR	Poliovirus receptor	0.24
ITGB3	Integrin beta-3	0.24

PTGIR	Prostacyclin receptor	0.24
LPHN1	Latrophilin-1	0.24
OR10J1	Olfactory receptor 10J1	0.24
MFAP3L	Microfibrillar-associated protein 3-like	0.24
GNMB	Transmembrane glycoprotein NMB	0.24
CELSR3	Cadherin EGF LAG seven-pass G-type receptor 3	0.23
CCR6	C-C chemokine receptor-like 2	0.23
DMPK	Myotonin-protein kinase	0.23
UPK3B	Uroplakin-3b	0.23
OR1D2	Olfactory receptor 1D2	0.23
OR7D2	Olfactory receptor 7D2	0.23
ITGB1	Integrin beta-1	0.23
HRH3	Histamine H3 receptor	0.23
GRIN2C	Glutamate receptor ionotropic, NMDA 2C	0.23
KIR3DL1	Killer cell immunoglobulin-like receptor 3DL1	0.23
EPHB2	Ephrin type-B receptor 2	0.23
OR2S2	Olfactory receptor 2S2	0.23
KIR2DL4	Killer cell immunoglobulin-like receptor 2DL4	0.23
CNNM1	Metal transporter CNNM1	0.23
MARVELD2	MARVEL domain-containing protein 2	0.23
CXCR6	C-X-C chemokine receptor type 6	0.23
NOV	Plexin-A1	0.23
ATCB6	ATP-binding cassette sub-family B member 6, mitochondrial	0.23
PVRL1	Poliovirus receptor-related protein 1	0.23
SLC46A2	Thymic stromal cotransporter homolog	0.23
ADORA3	Adenosine receptor A3	0.23
GPR125	Probable G-protein coupled receptor 125	0.23
CD22	B-cell receptor CD22	0.22
FZD3	Frizzled-3	0.22
LPAR5	Lysophosphatidic acid receptor 5	0.22
TMEM8B	Transmembrane protein 8B	0.22
PLXNA1	Plexin-A1	0.22
NPFFR1	Neuropeptide FF receptor 1	0.22
SEZ6L2	Seizure 6-like protein 2	0.22
LRRTM2	Leucine-rich repeat transmembrane neuronal protein 2	0.22
SLC16A11	Monocarboxylate transporter 11	0.22
GRIK5	Glutamate receptor ionotropic, kainate 5	0.22
SYT6	Synaptotagmin-6	0.22
TMEM102	Transmembrane protein 102	0.22
OR8B8	Olfactory receptor 8B8	0.22
GJB1	Gap junction beta-1 protein	0.22
GRM6	Metabotropic glutamate receptor 6	0.22
C20orf54	Solute carrier family 52, riboflavin transporter, member 3	0.22
OR52D1	Olfactory receptor 52D1	0.22

SLC46A1	Proton-coupled folate transporter	0.22
DSC2	Desmocollin-2	0.22
FAT1	Protocadherin Fat 1, nuclear form	0.22
GCGR	Glucagon receptor	0.22
POP1	Blood vessel epicardial substance	0.22
CXADR	Coxsackievirus and adenovirus receptor	0.22
ABCC6	Multidrug resistance-associated protein 6	0.22
GJA1	Gap junction alpha-1 protein	0.22
CXCR5	C-X-C chemokine receptor type 5	0.21
ABCB4	Multidrug resistance protein 3	0.21
CTLA4	Cytotoxic T-lymphocyte protein 4	0.21
TRPV1	Transient receptor potential cation channel subfamily V member 1	0.21
MRGPRX4	Mas-related G-protein coupled receptor member X4	0.21
SIGLEC6	Sialic acid-binding Ig-like lectin 6	0.21
IL9R	Interleukin-9 receptor	0.21
CHRNA2	Neuronal acetylcholine receptor subunit beta-2	0.21
PDGFRB	Platelet-derived growth factor receptor beta	0.21
TMPRSS11D	Transmembrane protease serine 11D catalytic chain	0.21
CDH24	Cadherin-24	0.21
PRRT2	Proline-rich transmembrane protein 2	0.21
GALR3	Galanin receptor type 3	0.21
OR51I1	Olfactory receptor 51I1	0.21
PTPRU	Receptor-type tyrosine-protein phosphatase U	0.21
LPAR4	Lysophosphatidic acid receptor 4	0.21
ZNRF3	E3 ubiquitin-protein ligase ZNRF3	0.21
P2RY6	P2Y purinoceptor 6	0.21
AGTR1	Type-1 angiotensin II receptor	0.21
GPR182	G-protein coupled receptor 182	0.21
PODXL	Podocalyxin	0.21
BDKRB1	B1 bradykinin receptor	0.21
DCHS1	Protocadherin-16	0.21
GRIN3B	Glutamate receptor ionotropic, NMDA 3B	0.21
PTGDR	Prostaglandin D2 receptor	0.21
PVRL4	Processed poliovirus receptor-related protein 4	0.21
GPR77	C5a anaphylatoxin chemotactic receptor 2	0.21
PARM1	Prostate androgen-regulated mucin-like protein 1	0.21
OR10H1	Olfactory receptor 10H1	0.21
OR10D3	Putative olfactory receptor 10D3	0.21
TNFSF14	Tumor necrosis factor ligand superfamily member 14, soluble form	0.21
FCRL5	Fc receptor-like protein 5	0.2
RNF43	E3 ubiquitin-protein ligase RNF43	0.2
AMIGO1	Amphotericin-induced protein 1	0.2

OR1F1	Olfactory receptor 1F1	0.2
SLCO4A1	Solute carrier organic anion transporter family member 4A1	0.2
TTYH2	Protein tweety homolog 2	0.2
GABRR2	Gamma-aminobutyric acid receptor subunit rho-2	0.2
GJD3	Gap junction delta-3 protein	0.2
GRID1	Glutamate receptor ionotropic, delta-1	0.2
CLDN1	Claudin-1	0.2
SLC6A13	Sodium- and chloride-dependent GABA transporter 2	0.2
SLC30A8	Zinc transporter 8	0.2
KIR2DL3	Killer cell immunoglobulin-like receptor 2DL3	0.2
GPR78	G-protein coupled receptor 78	0.2
UPK2	Uroplakin-2	0.2
CLDN14	Claudin-14	0.2
EDA	Ectodysplasin-A, secreted form	0.2
PTGER1	Prostaglandin E2 receptor EP1 subtype	0.2
TRPV5	Transient receptor potential cation channel subfamily V member 5	0.2
PRIMA1	Proline-rich membrane anchor 1	0.2
GJA9	Gap junction alpha-9 protein	0.2
SLC7A3	Cationic amino acid transporter 3	0.2
SSTR2	Somatostatin receptor type 2	0.2
CD1A	T-cell surface glycoprotein CD1a	0.2
SLC7A8	Large neutral amino acids transporter small subunit 2	0.2
CLIC6	Chloride intracellular channel protein 6	0.2
EPHA8	Ephrin type-A receptor 8	0.2
SLC20A2	Sodium-dependent phosphate transporter 2	0.2
SCNN1A	Amiloride-sensitive sodium channel subunit alpha	0.2
OR51B6	Olfactory receptor 51B6	0.2
OR14J1	Olfactory receptor 14J1	0.2
OR10C1	Olfactory receptor 10C1	0.2
OPRL1	Nociceptin receptor	0.2
CCR9	C-C chemokine receptor type 9	0.2
JPH4	Junctophilin-4	0.2
HTR1E	5-hydroxytryptamine receptor 1E	0.2
MC3R	Melanocortin receptor 3	0.2
CD163L1	Scavenger receptor cysteine-rich type 1 protein M160	0.2
SEZ6	Seizure protein 6 homolog	0.2
PRSS8	Prostasin heavy chain	0.2
CDH26	Cadherin-like protein 26	0.2
ODZ1	Teneurin C-terminal-associated peptide	0.2
FGFR3	Fibroblast growth factor receptor 3	0.2

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CLAIMS

- 1) A combination comprising immune cells comprising at least one inactivated gene coding a cell surface antigen X, with X being expressed on both immune cells and pathological cells and a therapeutic antibody specific for said cell surface antigen X.
- 2) The combination according to claim 1 wherein said immune cell is an immune T cell, a hematopoietic stem cell, preferably a TCR negative immune T cell, a TCR negative hematopoietic stem cell.
- 3) The combination according to claim 1 or 2 wherein said immune cell is an immune T cell, immune NK T cell, immune CD8 T cell, immune CD4 T cells, inflammatory T-cell cytotoxic T-cell, regulatory T-cell or helper T-cell. Cytotoxic CD4 T- cell, Cytotoxic CD8 T- lymphocyte.
- 4) The combination according to anyone of claims 1 to 3 wherein said gene is selected from any one of the genes in Table 4 to14.
- 5) The combination according to anyone of claims 1 to 4 wherein said genetic modification comprises a mutation, an insertion or a deletion generated using a rare cutting endonuclease.
- 6) The combination according to any one of claims 1 to 5 wherein said genetic modification is generated using a rare cutting endonuclease selected from a Meganuclease, a transcription activator-like (TAL)--nuclease, a zing-finger nuclease (ZFN), or a RNA/DNA guided endonucleases, preferably a transcription activator-like (TAL)—nuclease.
- 7) The combination according to any one of claims 1 to 6 wherein said genetic modification is generated by a TAL-Nuclease, preferably a mRNA encoding a TAL-Nuclease.
- 8) The combination according to any one of claims 1 to 7 wherein said genetic modification is an insertion and is generated by a TAL-Nuclease.
- 9) The combination according to any one of claims 1 to 8 wherein said immune T cell express a recombinant TCR, a chimeric antigen receptor (CAR), a single chain CAR or a multichain CAR.
- 10) The combination according to any one of claims 1 to 9 wherein said immune T cell express at least one CAR specific for a cell surface marker selected from any cluster of differentiation molecules (e.g. CD16, CD19, CD20, CD22, CD30, CD38, CD40, CD64, CD70, CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, 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), disialoganglioside GD2, o-acethyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, Flt3, WT1, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGF1 receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD123, HSP70, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, a cytokine receptor, 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, NEF, gp41); 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

- 11) The combination according to any one of claims 1 to 10 wherein said immune T cell express a CAR which target specifically a cell surface marker selected from CD19, CD38, HSP70, CD30, FAP, HER2, CD79a, CD79b, CD123, CD22, CLL-1, MUC-1, GD2, O-acetyl-GD2, and CS1.
- 12) The combination according to any one of claims 1 to 11 wherein said immune T cell express at least one CAR specific for a cell surface marker selected from any cluster of differentiation molecules (e.g. CD16, CD20, CD22, CD30, CD38, CD40, CD64, CD78, CD79a CD79b, CD96, CLL1, CD116, CD117, CD71, CD45, CD123 and CD138), CS1, a tumor-associated surface antigen, such as ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), disialoganglioside GD2, o-acethyl GD2, GD3, mesothelin, 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, RUI, RU2 (AS), intestinal carboxyl esterase, hsp70-2, HSP70, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ES0-1, LAGA-Ia, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil

elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGF1 receptor, mesothelin, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, RORI, 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, CD34, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), GM-CSF, CD123, FAP, HER2, CD79a, CD79b, CD123, MUC-1, and CS1, cytokine receptors, endoglin, a major histocompatibility complex (MHC) molecule, 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. an endogenous small antigen presented by HLA class I at the surface of the cells.

- 13) The combination according to any one of claims 1 to 10 wherein said immune T cell express a CAR which target specifically a cell surface marker selected from BCMA, CD33, EGFRVIII, Flt3, WT1, CD70, MUC16, PRAME, TSPAN10, CLAUDIN18.2, DLL3, LY6G6D, Liv-1, CHRNA2, ADAM10.
- 14) The combination according to any one of claims 1 to 13 wherein said CAR comprises an extracellular binding domain, said extracellular binding domain comprising two, three or more than 3 mAb-specific epitopes specifically recognized by ibrutumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10 and ustekinumab, preferably 2 mAb-specific epitopes specifically recognized by rituximab, or 1 mAb-specific epitopes specifically recognized by QBEND-10 and 3 mAb-specific epitopes specifically recognized by rituximab.
- 15) The combination according to any one of claims 1 to 14 wherein said immune T cell is further engineered by genetically inactivating at least one gene encoding a components of the T-cell receptor (TCR) and/or affecting the expression of molecule of the HLA complex.
- 16) The combination according to any one of claims 1 to 15 wherein a sequence encoding a CAR is inserted into the constant region of the TCR alpha gene or into the at least one gene coding a cell surface antigen comprising a genetic modification affecting cell surface expression of said cell surface antigen.

- 17) The combination according to any one of claims 1 to 16 wherein said immune T cell is further engineered to be less alloreactive as compared to non-engineered cells, by genetically inactivating the alpha TCR, beta1 TCR, beta2 TCR.
- 18) The combination according to any one of claims 1 to 16 wherein said immune T cell is further engineered by genetically inactivating a gene selected from beta2microglobulin, regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X-associated protein (RFXAP), and class II transactivator (CIITA), TAP-1, a combination thereof.
- 19) The combination according to any one of claims 1 to 18 further engineered to resist hypoxia, preferably wherein said CAR comprises an intracellular domain conferring resistance to hypoxia such as at least one HIF 1 alpha domain.
- 20) The combination according to any one of claims 1 to 19 further engineered to resist tumor-inducing inhibition of immune cells.
- 21) The combination according to any one of claims 1 to 20 further engineered to resist tumor-inducing inhibition anti-tumor activity of immune cells mediated by any one of the following molecules, Programmed Death 1 (PD-1), Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), LAG3 Tim3, BTLA, BY55, TIGIT, B7H5, LAIR1, SIGLEC10, and 2B4.
- 22) The combination according to any one of claims 1 to 21 comprising immune T cells with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody, preferably an anti-CD70 therapeutic antibody selected from Vorsetuzumab, Vorsetuzumab mafodotin, SGN CD70A, MDX 1203, ARGX 110, preferably an anti-CD70 therapeutic antibody ARGX-110 or MDX 1203.
- 23) The combination according to claim 22 wherein said genetic modification is generated using a transcription activator-like (TAL)-nuclease specific for CD70, comprising at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103, comprising at least 95% homology with SEQ ID NO 104 and SEQ ID NO 105, comprising at least 95% homology with SEQ ID NO 106 and SEQ ID NO 107 specific for the sequences SEQ ID NO 73 and SEQ ID NO 74; SEQ ID NO 76 and SEQ ID NO 77; SEQ ID NO 79 and SEQ ID NO 80, respectively.
- 24) The combination according to anyone of claims 1 to 21 comprising an immune T cell with a genetic modification of the CS-1 gene affecting cell surface expression of CS1 and an anti-CS-1 therapeutic antibody, preferably an anti-CS1 therapeutic antibody selected from Elotuzumab and ABBV838.
- 25) The combination according to claim 24 wherein said genetic modification is generated using a transcription activator-like (TAL)-nuclease specific for CS1, comprising at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; SEQ ID NO 110 and SEQ ID

- NO 111; or SEQ ID NO 112 and SEQ ID NO 113 each specific for the sequence SEQ ID NO 64 and SEQ ID NO 65; SEQ ID NO 67 and SEQ ID NO 68; SEQ ID NO 70 and SEQ ID NO 71, respectively.
- 26) The combination according to claim 24 wherein said genetic modification is generated using a transcription activator-like (TAL)–nuclease specific for CS1, comprising at least 95% homology with SEQ ID NO 171 and SEQ ID NO 172; SEQ ID NO 173 and SEQ ID NO 174; SEQ ID NO 175 and SEQ ID NO 176; or SEQ ID NO 177 and SEQ ID NO 178.
- 27) The combination according to anyone of claims 1 to 21 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody, preferably an anti-CD38 therapeutic antibody selected from Daratumumab HuMax-CD38;– Isatuximab HuMax-CD38, MOR202, MOR-03087, EDC8, and GBR 1342.
- 28) The combination according to claim 27 wherein said genetic modification is generated using a transcription activator-like (TAL)–nuclease specific for CD38, comprising at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; SEQ ID NO 116 and SEQ ID NO 117; or SEQ ID NO 118 and SEQ ID NO 119 specific for a sequence having at least 95% homology with SEQ ID NO 1, SEQ ID NO 4; SEQ ID NO 7, respectively.
- 29) A pharmaceutical composition comprising a combination according to any one of claim 1 or 28 and a pharmaceutically acceptable vehicle.
- 30) The combination according to any of the claims 1 to 28 or the pharmaceutical composition according to claim 28 for use in the treatment of a cancer, an infection or an auto-immune disease, preferably a cancer expressing or over expressing said cell surface antigen targeted by said therapeutic antibody, or relapse or refractory forms of said expressing or over expressing cancers.
- 31) The combination or the pharmaceutical composition for use according to claim 29 as a treatment in a lympho-depleted and/or irradiated patient.
- 32) The combination or the pharmaceutical composition for use according to claims 30 or 31 in the treatment or prophylaxis of cancer, wherein said cancer is selected from a hematopoietic or solid cancer, their relapse, refractory or metastatic forms.
- 33) The combination or the pharmaceutical composition for use according to any one of claims 30 to 32 for the treatment of Renal cell Carcinoma, Glioblastoma, glioma such as low grade glioma, Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), Waldenstrom's macroglobulinemia, Acute Myeloid Leukemia (AML), Multiple Myeloma (MM), diffuse large- cell lymphoma, follicular lymphoma and Non-Small Cell Lung Cancer.

- 34) The combination or the pharmaceutical composition for use according to any one of claims 30 to 32 in the treatment or prophylaxis of Multiple myeloma (MM), Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Acute lymphoblastic leukemia (ALL), Hodgkin lymphoma (HL) (relapsed, refractory), Non-Hodgkin lymphoma (NHL) (relapsed, refractory), Neuroblastoma, Ewing sarcoma, Myelodysplastic syndromes, BPDCN.
- 35) The combination or the pharmaceutical composition for use according to any one of claims 30 to 32 in the treatment or prophylaxis of Gliomas, pancreatic cancer, lung cancer, bladder cancer, colon cancer, breast cancer.
- 36) The combination or the pharmaceutical composition for use according to any one of claims 30 to 32 for the treatment of Non-Hodgkin's Lymphoma (indolent NHLs, follicular NHLs, small lymphocytic lymphoma, lymphoplasmacytic NHL, or marginal zone NHL); Hodgkin's disease (e.g., Reed-Sternberg cells); a cancer of the B-cell lineage, including, e.g., diffuse large B-cell lymphoma, follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, Cutaneous T cell lymphoma, B-cell lymphocytic leukemia (e.g., acute lymphocytic leukemia, chronic lymphocytic leukemia); Epstein Barr Virus positive B cell lymphoma; renal cell carcinoma (e.g., clear cell and papillary); nasopharyngeal carcinoma; thymic carcinoma; glioma; glioblastoma; neuroblastoma; astrocytoma; meningioma; Waldenstrom macroglobulinemia; multiple myeloma; colon cancer, stomach cancer, and rectal carcinoma.
- 37) A combination according to any one of claims 1 to 23 comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody ARGX 110, for use in the treatment of Cutaneous T cell lymphoma.
- 38) The combination to any one of claims 1 to 23 comprising an immune T cell with a genetic modification of the CD70 gene affecting cell surface expression of CD70 and an anti-CD70 therapeutic antibody MDX 1203 for use in the treatment of Renal Cell Carcinoma or Non-hodgkin's Lymphoma.
- 39) The combination according to any one of claims 1 to 21, 24, 25 and 26 for the treatment of carcinoma, blastoma, and sarcoma, preferably MM, leukemia, melanoma, relapse or refractory CS-1 expressing MM or a complication related to CS-1 expressing MM.
- 40) The combination according to any one of claims 1 to 21, 24, 25 and 26 for the treatment of any one of the following CS1 expressing cancers: Multiple Myeloma, Acute Myeloid Leukemia, Myelodysplastic Syndrome, Smoldering Multiple Myeloma, monoclonal gammopathy of unknown significance (MGUS), plasma cell leukemia, Non-Hodgkin's Lymphoma.

- 41) The combination according to any one of claims 1 to 21, 24, 25 and 26 for the treatment of any one of the following CS1 expressing cancers: Multiple Myeloma, Non-Hodgkin Lymphoma, Diffuse Large B Cell Lymphoma, Mantle-Cell Lymphoma, Follicular Lymphoma, Indolent B Cell Lymphoma, Primary Mediastinal Lymphoma, Lymphoplasmacytic Lymphoma.
- 42) The combination according to any one of claims 1 to 21, 24, 25 and 26 for the treatment of any one of the following CS1 expressing cancers : NK cell lymphoma, NK or T cell lymphoma, angioimmunoblastic T-cell lymphoma (AITL), or peripheral T cell lymphoma not otherwise specified (PTCL-NOS).
- 43) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Isatuximab for the treatment of Prostate Cancer, Non-small Cell Lung Cancer, Plasma Cell Myeloma, MM, T-cell Type Acute Leukemia, Precursor T-lymphoblastic Lymphoma or Leukaemia, prostate cancer, Non-small Cell Lung Cancer.
- 44) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and an anti-CD38 therapeutic antibody HuMax-CD38 – Daratumumab for the treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer, Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma, Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.
- 45) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of T-cell Type Acute Leukemia-Precursor T, lymphoblastic Lymphoma, Leukaemia.
- 46) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of a CD38-expressing or CD 38 over expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).

- 47) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of hematologic cancer selected from the group of leukemia, lymphoma and multiple myeloma (MM).
- 48) The combination according to any one of claims 1 to 21, 27 and 28 comprising an immune T cell with a genetic modification of the CD38 gene affecting cell surface expression of CD38 and Isatuximab, for the treatment of hematologic cancer selected from the group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic myelogenous leukemia (CML-BP).
- 49) The combination according to any one of claims 1 to 21, 27 and 28 for the treatment of Microsatellite Unstable Colorectal Cancer, Microsatellite Stable Colorectal Cancer, Mismatch Repair Proficient Colorectal Cancer, Mismatch Repair Deficient Colorectal Cancer, Waldenström Macroglobulinemia, Malignant Neoplasms of Male Genital Organs, Prostate Cancer, Hematopoietic Cancer, Acute Myelogenous Leukemia, High-Risk Myelodysplastic Syndrome, Plasma cell myeloma, Monoclonal Gammopathy, Smoldering Multiple Myeloma, Membranoproliferative Glomerulonephritis, Multiple Myeloma.
- 50) The combination according to any one of claims 1 to 21, 27 and 28 for the treatment of T-cell Type Acute Leukemia-Precursor T, lymphoblastic Lymphoma, Leukaemia.
- 51) The combination according to any one of claims 1 to 21, 27 and 28 for the treatment of a CD38-expressing or CD 38 over expressing hematologic cancer selected from the group of Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Acute Myelogenous Leukemia (AML), and Acute Lymphocytic Leukemia (ALL), multiple myeloma (MM).
- 52) The combination according to any one of claims 1 to 21, 27 and 28 for the treatment of hematologic cancer selected from the group of leukemia, lymphoma and multiple myeloma (MM).
- 53) The combination according to any one of claims 1 to 21, 27 and 28 for the treatment of hematologic cancer selected from the group of B-cell chronic lymphocytic leukemia (B-CLL), acute lymphoblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia or chronic myeloid leukemia (CML), acute myelogenous leukemia or acute myeloid leukemia (AML), acute lymphocytic

leukemia (ALL), hairy cell leukemia (HCL), myelodysplasia syndromes (MDS) or chronic myelogenous leukemia (CML-BP) and any subtypes of chronic myelogenous leukemia (CML-BP), leukemia, lymphoma and multiple myeloma.

- 54) A method for treating a patient comprising:
- Preparing a combination of a therapeutic antibody specific for an antigen marker expressed at the cell surface of pathological cells in said patient and a population of genetically modified immune cells wherein the gene(s) encoding said antigen marker is inactivated according to any of the claim 1 to 27 or a pharmaceutical composition of claim 28,
 - Administering said genetically modified cells to said patient, before, after and/or concomitantly to said therapeutic antibody.
55. A TAL- protein comprising a sequence having at least 95% homology with SEQ ID NO 114 and SEQ ID NO 115; comprising a sequence having at least 95% homology with SEQ ID NO 116 and SEQ ID NO 117; comprising a sequence having at least 95% homology with SEQ ID NO 118 and SEQ ID NO 119; comprising a sequence having at least 95% homology with SEQ ID NO 102 and SEQ ID NO 103; comprising a sequence having at least 95% homology with SEQ ID NO 104 and SEQ ID NO 105; comprising a sequence having at least 95% homology with SEQ ID NO 106 and SEQ ID NO 107; comprising a sequence having at least 95% homology with SEQ ID NO 108 and SEQ ID NO 109; comprising a sequence having at least 95% homology with SEQ ID NO 110 and SEQ ID NO 111; comprising a sequence having at least 95% homology with SEQ ID NO 112 and SEQ ID NO 113; comprising a sequence having at least 95% homology with SEQ ID NO 171 and SEQ ID NO 172; comprising a sequence having at least 95% homology with SEQ ID NO 173 and SEQ ID NO 174; comprising a sequence having at least 95% homology with SEQ ID NO 175 and SEQ ID NO 176; or comprising a sequence having at least 95% homology with SEQ ID NO 177 and SEQ ID NO 178.
56. A polynucleotide or a vector encoding a protein comprising any one of the TAL-protein of claim 55.
57. A vector coding a protein comprising any one of the TAL protein of claim 55 for use in the manufacturing of CART cells.
58. A vector coding any one of the TAL protein of SEQ ID NO 102 and SEQ ID NO 103; SEQ ID NO 104 and SEQ ID NO 105; SEQ ID NO 106 and SEQ ID NO 107; SEQ ID NO 108 and SEQ ID NO 109; SEQ ID NO 110 and SEQ ID NO 111; SEQ ID NO 112 and SEQ

ID NO 113; SEQ ID NO 114 and SEQ ID NO 115; SEQ ID NO 116 and SEQ ID NO 117; SEQ ID NO 118 and SEQ ID NO 119, SEQ ID NO 171 and SEQ ID NO 172; SEQ ID NO 173 and SEQ ID NO 174; SEQ ID NO 175 and SEQ ID NO 176; or SEQ ID NO 176 and SEQ ID NO 177; for use in the manufacturing of a combination according to any one of claim 1 to 28.

59. A method of preparing a combination of engineered T-cells, said engineered T cells comprising an inactivated gene coding an antigen marker, with a therapeutic antibody specific for said antigen marker and expressed at the cell surface of a pathological cells comprising the step of:
- (a) Genetically engineering a gene in a T-cell, which is involved in the expression or presentation of an antigen marker, said antigen marker being present both on the surface of said T-cell and the pathological cell; to inactivate said antigen marker and /or alter its binding to its receptor,
 - (b) Expressing into said T-cells a transgene encoding a chimeric antigen receptor directed against said antigen marker present at the surface of said pathological cell,
 - (c) Combining said T-cells with a therapeutic antibody specific for said antigen marker.
- 60) The method according to claim 59, wherein said antigen marker is selected from one listed in Table 4 to 14, or an immuno-reactive fragment thereof.
- 61) The method according to claim 59, wherein said antigen marker is CD38 or an immuno-reactive fragment thereof.
- 62) The method according to claim 59, wherein said antigen marker is CD70 or an immuno-reactive fragment thereof.
- 63) The method according to claim 59, wherein said antigen marker is CS1 or an immuno-reactive fragment thereof.
- 64) The method according to any one of claims 59 to 63, wherein said method includes a further step of activating and expanding the T-cells.
- 65) The method according to any one of claims 59 to 64, wherein said method includes a further step of purifying the resulting T-cells by excluding the cells presenting said marker antigen at their surface.

- 66) The method according to any one of claims 59 to 65, wherein said method includes a previous step of procuring the T-cells from a donor.
- 67) The method according to any one of claims 59 to 66, wherein said method includes a previous step of procuring the T-cells from a patient who is affected by the development of said pathological cells.
- 68) The method according to any one of claims 59 to 67, wherein said T-cell is derived from a primary stem cell, iPS or hES cell.
- 69) The method according to claim 68, wherein said T-cell is derived from iPS cell derived from said patient affected by the development of said pathological cells.
- 70) The method according to any one of claims 59 to 69, wherein step a) is performed using a rare-cutting endonuclease.
- 71) The method according to claim 70, wherein step a) is performed using a TAL-nuclease.
- 72) The method according to claim 71, wherein step a) is performed using a RNA-guided endonuclease
- 73) The method according to claim 72, wherein the RNA-guided endonuclease is Cas9.
- 74) The method according to claim 72, wherein RNA-guided endonuclease is split into at least 2 polypeptides, one comprising RuvC and another comprising HNH.
- 75) The method according to claim 71, wherein said endonuclease is expressed from transfected mRNA.
- 76) The method according to any one of claims 1 to 75, wherein said method includes a further step of inactivating a gene encoding a component of the T-cell receptor (TCR).
- 77) The method according to claim 78, wherein said component of the T-cell receptor is TCR α .
- 78) The method according to any one of claims 59 to 77, wherein said method includes a further step of inactivating a gene encoding a component of HLA.
- 79) The method according to any one of claims 59 to 78, wherein said method includes a further step of inactivating a gene encoding β 2m.

- 80) The method according to any one of claims 59 to 79, 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, TGFBR2, TGFBR1, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, GUCY1A2, GUCY1A3, GUCY1B2 and GUCY1B3.
- 81) The method according to claim 80, wherein said gene locus is involved into the expression of PD1 or CTLA-4 genes.
- 82) The method according to any one of claims 59 to 81, wherein said method includes a further step of inactivating a gene conferring sensitivity of the T-cells to chemotherapy or immunosuppressive drugs.
- 83) The method according to claim 82, wherein said further gene encodes CD52.
- 84) The method according to claim 82, wherein said further gene is hypoxanthine-guanine phosphoribosyltransferase (HPRT).
- 85) The method according to claim 82, wherein said further gene encodes a glucocorticoid receptor (GR).
- 86) The method according to claim 82, wherein said further gene is involved in the DCK regulatory pathway, in particular DCK expression.
- 87) The method according to any one of claims 59 to 86, wherein said T-cells in step a) are inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.
- 88) The method according to claim 87, wherein said T-cells are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.
- 89) The method according to any one of claims 59 to 88, wherein said transformed T-cells are expanded in-vitro.
- 90) The method according to any one of claims 59 to 89, wherein said transformed T-cells are expanded in-vivo.

- 91) The method according to any one of claims 59 to 90, wherein said pathological cells are selected from malignant cells or infected cells.
- 92) The method according to any one of claims 59 to 91, wherein said pathological cells are B-cells.
- 93) The method according to any one of claims 59 to 92, wherein said pathological cells are solid tumor cells.
- 94) The method according to any one of claims 59 to 93, for preparing T-cells to be used as a medicament.
- 95) The method according to claim 94 for preparing T-cells for treating a cancer, an immune disease or an infection in a patient in need thereof.
- 96) The method according to claim 95 for treating lymphoma.
- 97) The method according to claim 95 for treating leukemia.
- 98) The method according to claim 95 for treating chronic lymphocytic leukemia (CLL).
- 99) An engineered T-cell obtainable according to the method of any one of claims 59 to 93.
- 100) A method for treating a patient comprising:
 - (a) Diagnosing a patient for the presence of pathological cells expressing at the cell surface an antigen marker in common with T-cells;
 - (b) preparing a population of engineered T-cells wherein cell surface expression of said antigen marker is undetectable,
 - (c) administering said engineered T-cells to said patient diagnosed for said pathological cells, concomitantly to the therapeutic antibody, before or after said therapeutic antibody.

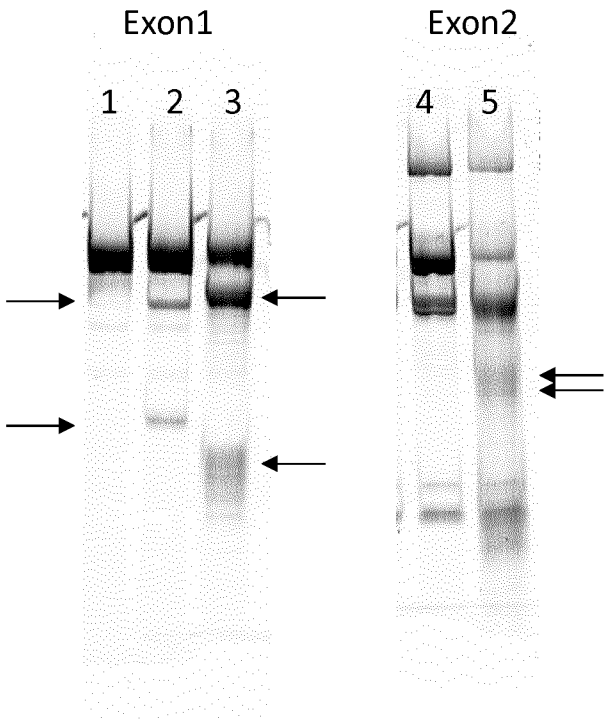


Figure 1

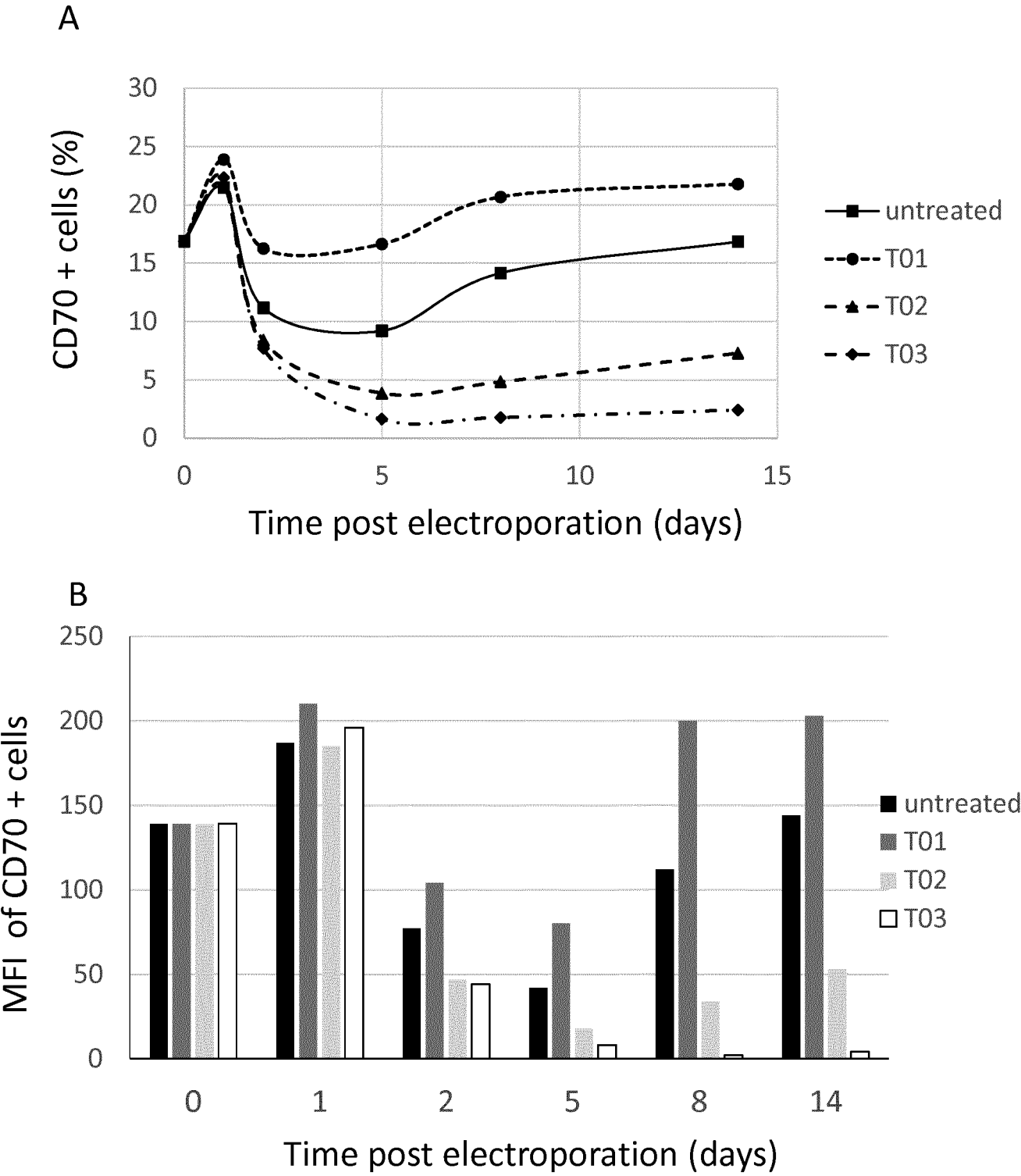


Figure 2

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/052229

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/00

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAURENT POIROT ET AL: "Multiplex Genome-Edited T-cell Manufacturing Platform for "Off-the-Shelf" Adoptive T-cell Immunotherapies", CANCER RESEARCH, vol. 75, no. 18, 16 July 2015 (2015-07-16), pages 3853-3864, XP055568648, Proceedings: AACR Annual Meeting 2014; April 5-9, 2014; San Diego, CA ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-14-3321	1-17, 27-36, 39-54, 59-61, 64-71, 76,77, 87-98, 100
Y	abstract page 3853, right-hand column, paragraph 3 - page 3854, left-hand column, paragraph 6 page 3854, right-hand column, paragraph 6 page 3857, right-hand column, paragraph 1 - page 3858, right-hand column, paragraph 1 page 3861, left-hand column, paragraph 2 - -/--	18-26, 37,38, 62,63, 72-75, 78-86



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

9 April 2019

Date of mailing of the international search report

11/06/2019

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/052229

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-54, 59-98, 100

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2019/052229

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	page 3861, right-hand column, paragraph 2 page 3862, left-hand column, paragraph 2 - page 3862, right-hand column, last paragraph ----- WO 2015/121454 A1 (CELLECTIS [FR]) 20 August 2015 (2015-08-20) page 1, line 6 - line 18 page 4, line 1 - page 5, line 3 page 5, line 10 - page 6, line 13; figures 4-8 page 10, line 1 - page 11, line 32 page 17, line 1 - page 18, line 20 page 28, line 22 - page 34, line 12 page 37, line 18 - page 40, line 33 page 43, line 1 - page 44, line 24; examples 1-3 -----	20-26, 37,38, 62,63, 72-75, 78,80-85
Y	WO 2017/025323 A1 (CELLECTIS [FR]) 16 February 2017 (2017-02-16) page 1, line 5 - line 16 page 3, line 26 - page 5, line 3 page 21, line 23 - page 23, line 11 page 44, line 3 - page 50, line 8 page 55, line 15 - line 16 page 62, line 12 - page 64, line 20 page 87, line 5 - page 92, line 17; examples 1-6 -----	18,19, 79,86
A	BRIDE K L: "Targetting CD38 in T cell acute lymphoblastic leukemia", BLOOD 20171201 AMERICAN SOCIETY OF HEMATOLOGY NLD , vol. 130, no. Supplement 1 1 December 2017 (2017-12-01), XP002785384, ISSN: 1528-0020 Retrieved from the Internet: URL:http://www.bloodjournal.org/content/130/Suppl_1/3888 [retrieved on 2018-10-05] the whole document ----- -/--	1-54, 59-98, 100

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/052229

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NIJHOF INGER S ET AL: "CD38 expression and complement inhibitors affect response and resistance to daratumumab therapy in myeloma.", BLOOD 18 08 2016, vol. 128, no. 7, 18 August 2016 (2016-08-18), pages 959-970, XP002785385, ISSN: 1528-0020 abstract page 968, right-hand column, paragraph 2 -----	1-54, 59-98, 100

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2019/052229

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2015216875 A1	28-07-2016
		BR 112016017806 A2	10-10-2017
		CA 2937711 A1	22-07-2016
		CN 106029875 A	12-10-2016
		DK 3105317 T3	14-01-2019
		EP 3105317 A1	21-12-2016
		EP 3505623 A1	03-07-2019
		ES 2711498 T3	06-05-2019
		HU E041497 T2	28-05-2019
		JP 2017506636 A	09-03-2017
		KR 20160138404 A	05-12-2016
		PL 3105317 T3	28-02-2019
		RU 2016136702 A	19-03-2018
		US 2018201901 A1	19-07-2018
		WO 2015121454 A1	20-08-2015

WO 2017025323	A1	16-02-2017	
		AU 2016307050 A1	15-02-2018
		BR 112018002600 A2	23-10-2018
		CA 2994746 A1	16-02-2017
		CN 108495641 A	04-09-2018
		EP 3334442 A1	20-06-2018
		JP 2018522907 A	16-08-2018
		KR 20180041152 A	23-04-2018
		US 2018236053 A1	23-08-2018
		WO 2017025323 A1	16-02-2017

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-54, 59-98, 100

A combination of immune cells comprising at least one inactivated cell surface antigen expressed on immune cells and pathological cells, and a therapeutic antibody specific for the cell surface antigen, compositions and medical uses thereof, as well as a method for preparing the combination

2. claims: 55-58

Tal-protein comprising a sequence having at least 95% homology with either one of sequences SEQ ID NOs:114-119, 102-113; encoding polynucleotide and vector

3. claim: 99

engineered T cell obtainable by a method comprising genetically engineering in a T cell a gene involved in the expression or presentation of an antigen marker present on the surface of said T cell and a pathological cell, inactivating said antigen marker and/or altering its binding to its receptor, and expressing in the T cell a transgene encoding a chimeric antigen receptor directed against said antigen marker
