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(54) **ANTI-HSP70 SPECIFIC CHIMERIC ANTIGEN RECEPTORS (CARS) FOR CANCER IMMUNOTHERAPY**

(71) Applicant: **Cellectis**, Paris (FR)

(72) Inventors: **Julien VALTON**, New York, NY (US); **Philippe DUCHATEAU**, Draveil (FR); **Alexandre JUILLERAT**, New York, NY (US); **Arvind RAJPAL**, San Francisco, CA (US); **Barbra Johnson SASU**, San Francisco, CA (US); **Julianne SMITH**, New York, NY (US)

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(57) **ABSTRACT**

The present invention relates to Chimeric Antigen Receptors (CAR) that are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward selected membrane antigens, and more particularly in which extracellular ligand binding is a scFV derived from an anti-HSP70 monoclonal antibody, conferring specific immunity against HSP70 positive cells. The engineered immune cells endowed with such CARs are particularly suited for treating in particular leukemia.

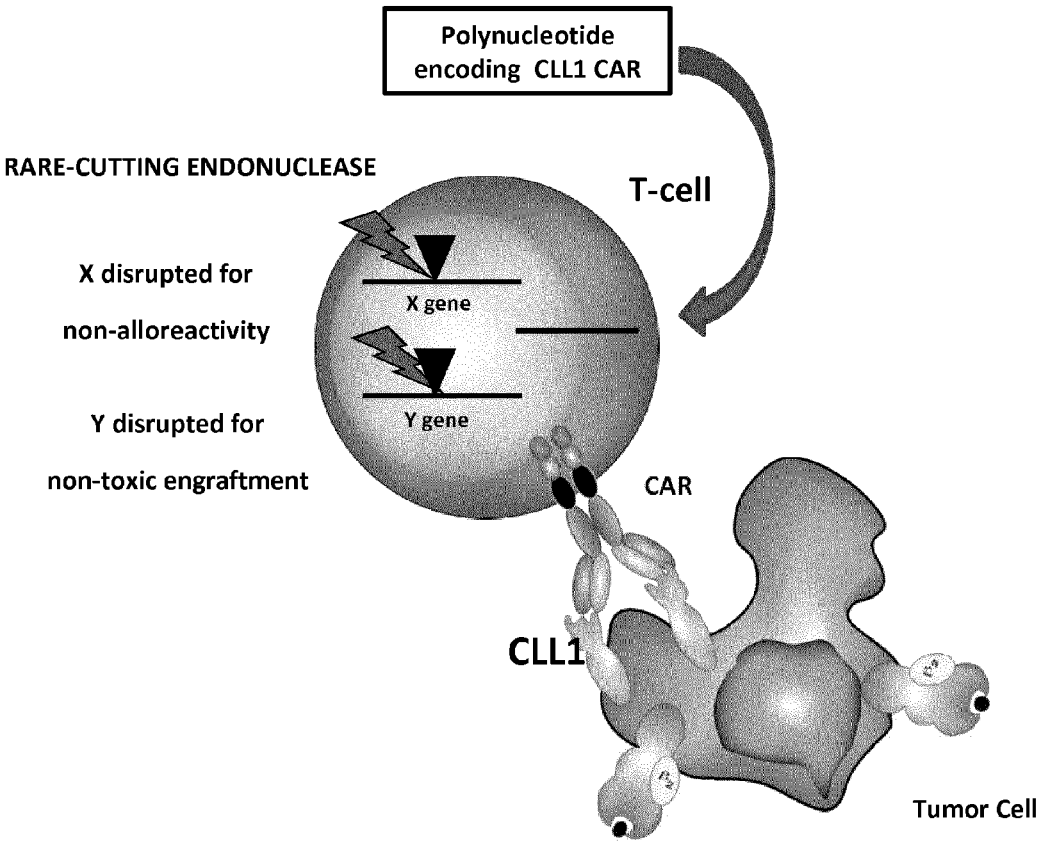
Polynucleotide
encoding CLL1 CAR
RARE-CUTTING ENDONUCLEASE
T-cell
X disrupted for
non-alloreactivity
X gene
Y gene
Y disrupted for
non-toxic engraftment
CAR
CLL1
Tumor Cell

Figure 1

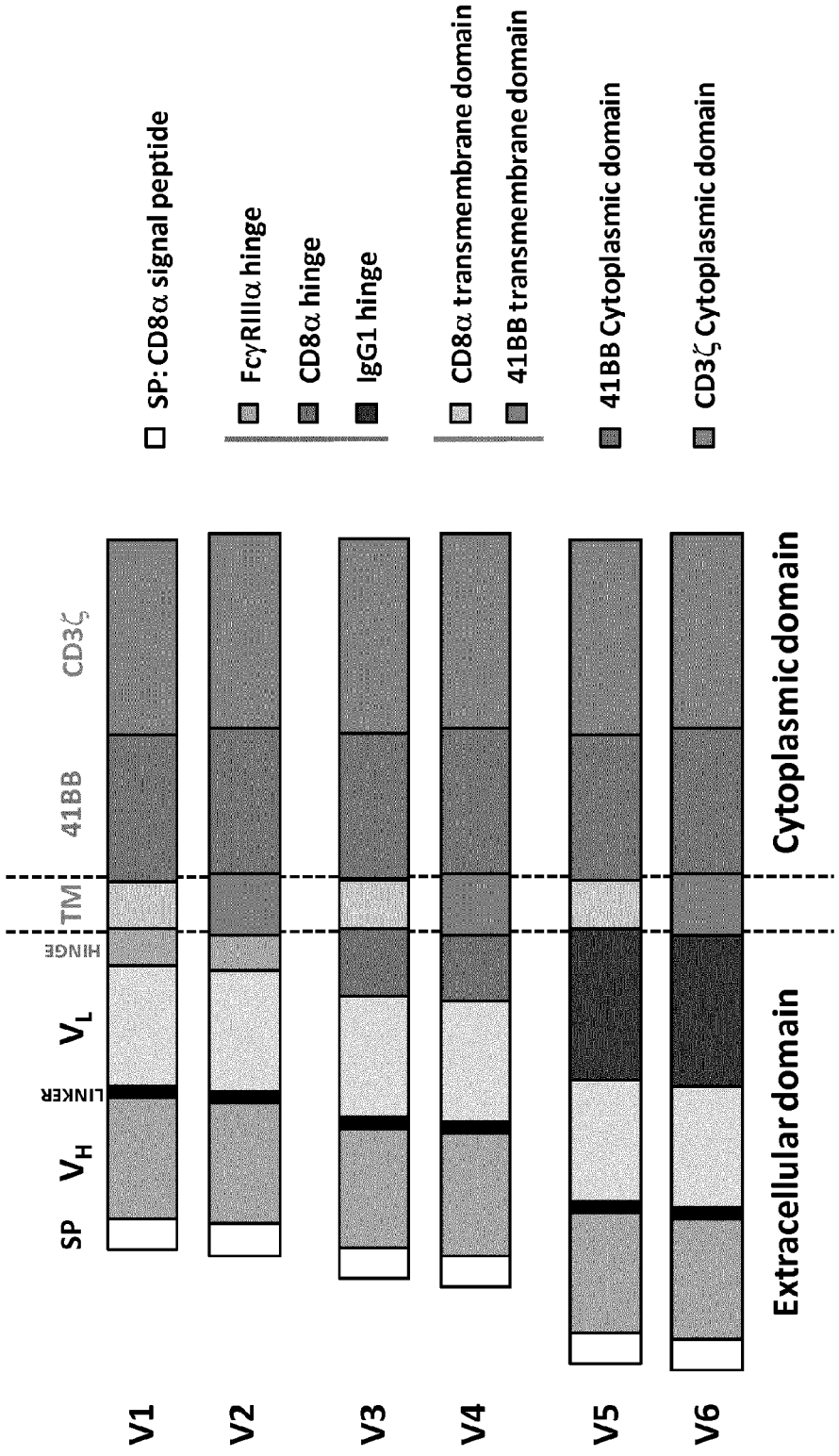
SP: CD8α signal peptide
FcγRIIIα hinge
CD8α hinge
IgG1 hinge
CD8α transmembrane domain
41BB transmembrane domain
41BB Cytoplasmic domain
CD3ζ Cytoplasmic domain
SP
$V_H$
LINKER
$V_L$
HINGE
TM
41BB
CD3ζ
Extracellular domain
Cytoplasmic domain
V1
V2
V3
V4
V5
V6

Figure 2

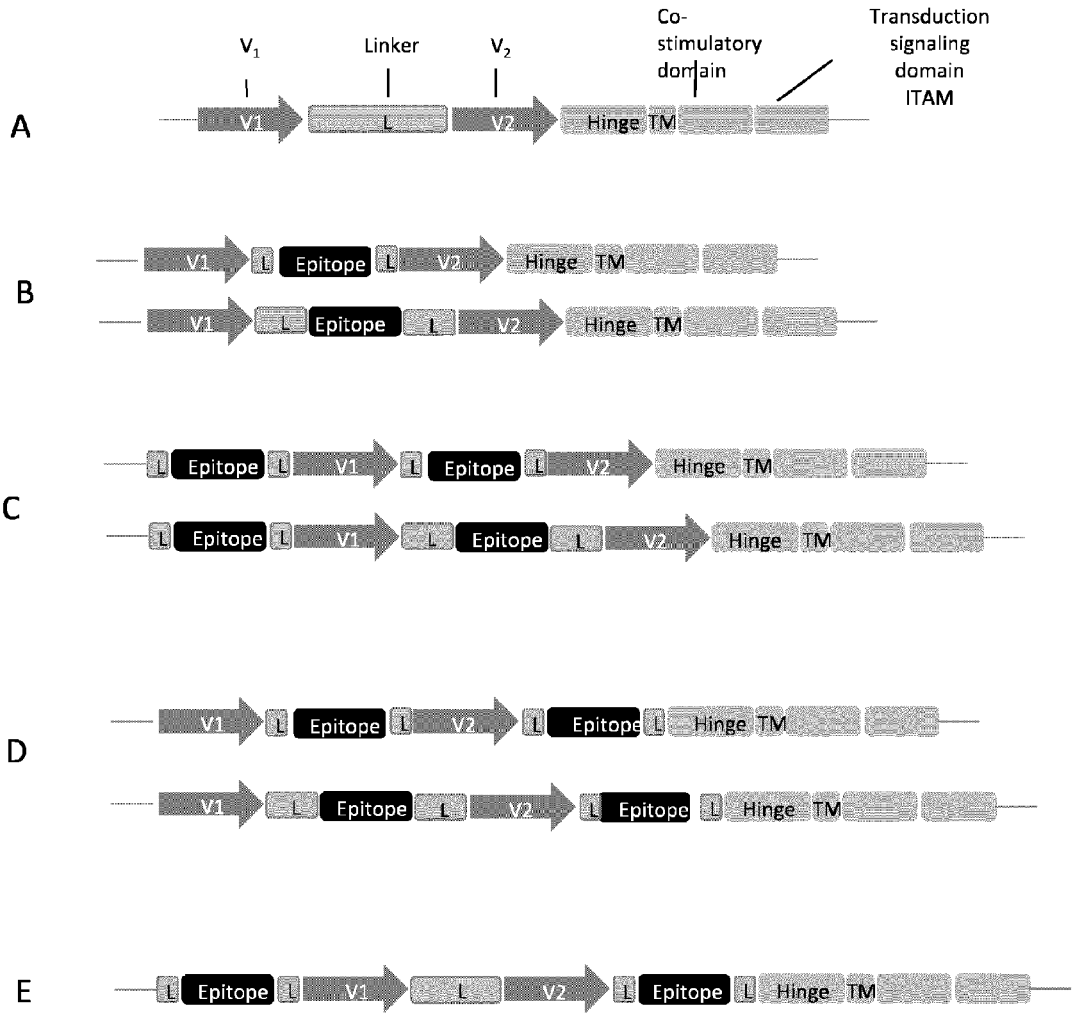
A
V1
Linker
V2
Co-stimulatory domain
Transduction signaling domain ITAM
L
Hinge
TM
B
Epitope
C
D
E

Figure 3A

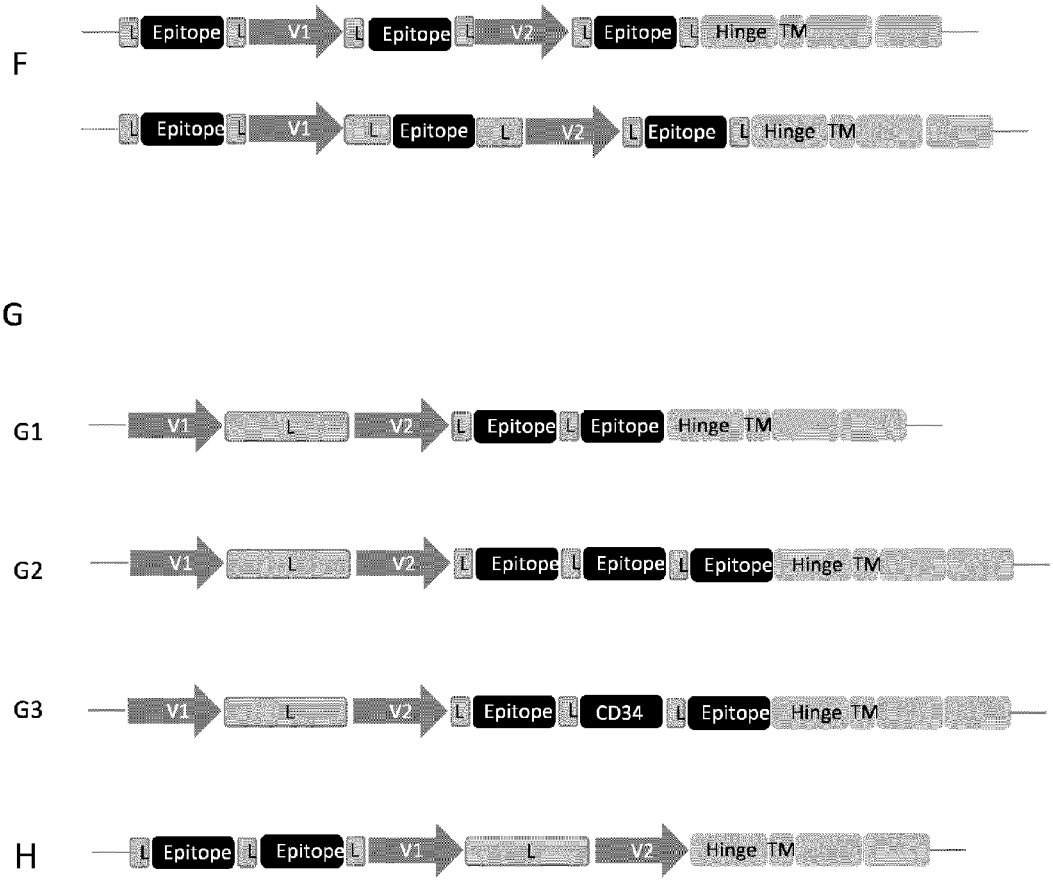
F
L Epitope L V1 L Epitope L V2 L Epitope L Hinge TM
L Epitope L V1 L Epitope L V2 L Epitope L Hinge TM
G
G1
V1 L V2 L Epitope L Epitope Hinge TM
G2
V1 L V2 L Epitope L Epitope L Epitope Hinge TM
G3
V1 L V2 L Epitope L CD34 L Epitope Hinge TM
H
L Epitope L Epitope L V1 L V2 Hinge TM

Figure 3B

# ANTI-HSP70 SPECIFIC CHIMERIC ANTIGEN RECEPTORS (CARS) FOR CANCER IMMUNOTHERAPY

## FIELD OF THE INVENTION

**[0001]** Heat-shock protein 70 (Hsp70) has been identified as being frequently over-expressed in patients affected by leukemia such as acute myeloid leukemia (AML) or by solid tumors such as colorectal, lung, neuronal, pancreatic carcinomas, liver metastases. The present invention relates to methods to target HSP70 positive malignant cells using Chimeric Antigen Receptors (anti HSP70-CAR), which are recombinant chimeric proteins able to redirect immune cell specificity and reactivity toward selected membrane antigen HSP70. These anti-HSP70 CAR more particularly comprise an extracellular ligand binding comprising a scFV derived from some specific anti-HSP70 monoclonal antibodies. The engineered immune cells endowed with such CARs confer adoptive immunity against HSP70 positive cell as part as various cell therapies for treating cancer, in particular hematologic cancers, with higher efficiency.

## BACKGROUND OF THE INVENTION

**[0002]** 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). Transfer of viral antigen specific T cells is a well-established procedure used for the treatment of transplant associated viral infections and rare viral-related malignancies. Similarly, isolation and transfer of tumor specific T cells has been shown to be successful in treating melanoma.

**[0003]** Novel specificities in T cells have been successfully generated through the genetic transfer of transgenic T cell receptors or chimeric antigen receptors (scCARs) (Jena, Dotti et al. 2010). scCARs 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 scCAR 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 scCARs are derived from the cytoplasmic region of the CD3zeta or the Fc receptor gamma chains. First generation scCARs 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 scCAR modified T cells. scCARs 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).

**[0004]** Meanwhile, induction treatments for acute myeloid leukemia (AML) have remained largely unchanged for nearly 50 years and AML remains a disease of poor prognosis. AML is a disease characterized by the rapid proliferation of immature myeloid cells in the bone marrow resulting in dysfunctional hematopoiesis. Although standard induction chemotherapy can induce complete remissions, many patients eventually relapse and succumb to the disease, calling for the development of novel therapeutics for AML. Recent advances in the immunophenotyping of AML cells have revealed several AML associated cell surface antigens that may act as targets for future therapies. Besides their intracellular chaperoning functions, heat shock proteins (Hsps) have been found to play key roles in cancer immunity. Among them, the 70 kilodalton heat shock proteins (such as Hsp70s, Uniprot ref: PODMV8 for the human protein, encoded by gene ref GeneID #3303) are a family of conserved ubiquitously expressed heat shock proteins. Heat shock 70 kDa protein has also as alternative names: HSP70. 1, HSPA1A or HSX70.

**[0005]** Proteins with similar structure exist in virtually all living organisms. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress (Tamura Y et al, 1993). Overexpressed inside cells, Hsp70 is transported to the cell membrane and also exported into the extracellular space. Generally, believed inducible Hsp70 is a protein inside the cell, but the researchers found that in peripheral circulation normal and disease states is able to detect some soluble Hsp70 and Hsp70 antibody, Hsp70 cells can be released outside the cells, causing the body to produce Hsp70 antibodies (Muthoff G et al, 2007).

**[0006]** Hsp70, the major heat-inducible member of the Hsp70 group, has been detected on the cell surface of tumor cells but not on normal cells (Multhoff G et al., 1995). With the exception of mammary carcinomas, an Hsp70 plasma membrane expression was found on freshly isolated human biopsy material of colorectal, lung, neuronal, and pancreas carcinomas, liver metastases, and leukemic blasts of patients with acute myelogenous leukemia (Hantschel M et al. 2000). Moreover, Hsp70 was qualified as "tumor marker" for detection of acute myeloid leukemia (AML) (K Steiner et al., 2006). Hsp70 membrane expression was shown to be a target for natural killer (NK) cells on tumor material and control tissues of head-and-neck cancer patients (Kleinjung T et al., 2003).

**[0007]** So far, all the immunotherapy Hsp70-based therapy in clinical trial relies on vaccination using a recombinant Hsp70 protein as antigen for treating leukemia such as Chronic Myelogenous Leukemia (CML) or in melanoma. Hsp70 DNA vaccine has also been tested to treat cervical cancer precancerous condition.

**[0008]** In view of the above, the inventors have pursued a new approach to target Hsp70 using immune cells endowed with specific chimeric antigen receptors based on anti-Hsp70 monoclonal antibodies, which redirect immune cell specificity towards Hsp70 positive cells.

**[0009]** The engineered immune cells that they obtained using this approach have proven efficacy to eliminate Hsp70 positive malignant cells. In particular, they have appeared to be particularly useful in the context of the production of allogeneic TCR negative engineered immune cells, allowing a reduction of side effects, such as GvHD.

**[0010]** Thus, the present invention opens the way to treating patients affected with a condition characterized by an overabundance of Hsp70-expressing cells using adoptive immunotherapy. Even more, the present invention provides with engineered allogeneic immune cells that may be used

as "off-the-shelf" allogeneic therapeutic products. As a further advantage of the invention, the CAR positive engineered cells can be made compatible (i.e. resistant) with chemotherapy or immunodepleting treatments, thereby enabling synergistic effects between chemotherapy and immunotherapy. Another aspect of the invention is the development of further engineered immune cells which expressed a CAR which extracellular domain comprises at least one epitope tagging sequence such as a CD20 mimotope, allowing a depletion of said immune cells by the use of antibodies against such epitope, in case of occurrence of adverse event such as cytokine storm.

## SUMMARY OF THE INVENTION

**[0011]** The inventors have generated Hsp70 specific single-chain scCAR having different design and comprising different scFV derived from anti-Hsp70 specific antibodies.

**[0012]** In particular, the Inventors have developed anti-Hsp70 specific CAR, and in particular single-chain CAR (scCAR), comprising VL and VL chains derived from antibodies, with different architectures and identified highly specific and very selective scCARs constructions that bind to Hsp70 expressing cells and selectively destroy Hsp70 expressing cancer cells.

**[0013]** The present invention aims particularly to chimeric antigen receptors which target specifically membrane HSP70 (mHsp70) antigen, and preferably the membrane HSP70-1 antigen;

**[0014]** Following non-specific activation in vitro (e.g. with anti CD3/CD28 coated beads and recombinant IL2), primary T-cells from donors have been transformed with polynucleotides expressing these scCARs using viral transduction. In certain instances, the T-cells were further engineered to create less or non-alloreactive T-cells, more especially by disruption of a component of TCR ($\alpha\beta$-T-Cell receptors) to prevent Graft versus host reaction.

**[0015]** Another aspect of the invention is the development of further engineered immune cells which expressed a CAR which extracellular domain comprises at least one epitope tagging sequence such as a CD20 mimotope, allowing a depletion of said immune cells by the use of antibodies against such epitope, in case of need (i.e. occurrence of adverse event). T-cells were further engineered to create T cells resistant to anti-cancer drugs, to be used in combination with said classical anti-cancer drugs.

**[0016]** The resulting engineered T-cells displayed reactivity in-vitro against HSP70 positive cells to various extend, showing that the scCARs of the present invention contribute to antigen dependent activation, and also proliferation, of the T-cells, making them useful for immunotherapy.

**[0017]** The resulting engineered T-cells displayed reactivity in-vivo against HSP70 positive cells and significantly reduce the number of cancer cells in vivo.

**[0018]** The engineered T-cells of the invention are designed to display in-vivo reactivity against HSP70 positive cells, can be used in concomitance with anti-cancer drugs, are well tolerated. In a particular embodiment, the engineered T-cells of the invention remain efficient even after several administrations, making them useful for immunotherapy as a first treatment (induction), as a consolidation treatment, as a treatment in combination with classical anticancer chemotherapy. The polypeptides and polynucleotide sequences encoding the CARs of the present invention are detailed in the present specification.

**[0019]** The engineered immune cells of the present invention are particularly useful for therapeutic applications such as acute myeloma leukemia (AML) treatments, or for treating solid tumor such as colorectal, lung, neuronal, pancreas carcinomas, liver metastases or head-and-neck cancer.

**[0020]** Finally, the present invention encompasses a therapeutic combination for treating HSP70 overexpressing cells related-disease comprising the sequential administration of antibodies against soluble HSP70 and then of immune cells expressing anti-membrane HSP70 chimeric antigen receptor.

## BRIEF DESCRIPTION OF THE FIGURES

**[0021]** FIG. **1**: Schematic representation of an engineered immune cell according to the invention. The engineered immune cell presented in this figure is a T-cell transduced with a retroviral vector encoding HSP70-scCAR. This T-cell was further engineered to allow a better and safer engraftment into the patient, which is optional within the frame of the present invention. X gene may be for instance a gene expressing a component of TCR (TCRalpha or TCRbeta), Y may be a gene involved into the sensitivity of T-cells to immune-suppressive drugs like CD52 (with respect to Campath) or HPRT (with respect to 6-Thioguanine).

**[0022]** FIG. **2**: Schematic representation of the different scCAR Architecture (V1 to V6) of the invention (anti-HSP70 scCAR) with the components presented in the following Table 1.

**[0023]** FIG. **3**A and FIG. **3**B: Schematic representation of different strategies based mAb-epitope tagging using for instance the CD20 mimotope for T cell depletion designed to mitigate possible side effects associated with CAR positive cells injection: V1 and v2 represents either VH or VL chain respectively, TM: transmembrane domain, L: linker.

**[0024]** (A) extracellular anti-Hsp70 ligand binding domain part of the multi-chain architecture according to the present invention, which does not include an epitope tagging sequence for sorting or depleting cells; V1: anti-Hsp70 monoclonal antibody VH; L: GS linker; V2: anti-Hsp70 monoclonal antibody VH; Hinge: preferably CD8 hinge; TM: preferably Fc$\epsilon$R$\gamma$-TM-IC.

**[0025]** (B) extracellular anti-Hsp70 domain of the multi-chain architectures according to the invention including at least one epitope inserted in the extracellular ligand binding domain of the CAR, wherein said epitope is inserted between the VH and VL chains; said epitope being bordered by different linkers.;

**[0026]** (C): both architectures presented here correspond to examples where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VH and VL chains, said $2^{nd}$epitope being also bordered by 2 at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the $2^{nd}$ epitope.

**[0027]** (D): both architectures presented here correspond to examples where two epitopes are inserted in the extracellular ligand binding domain of the CAR, one is inserted between the VH and VL chains; the other epitope is inserted between the VL chain and the hinge, each said epitope being also bordered by at least one or two linkers. The architectures illustrated herein differ by the linkers used bordering the $1^{st}$ epitope.

**[0028]** (E): one architecture is presented where two epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VL chain and the hinge, said $2^{nd}$epitope being also bordered by such linkers.

**[0029]** (F): both architectures presented here correspond to examples, where three epitopes are inserted in the extracellular domain of the CAR, one is inserted between the N-terminal end of the CAR and the VH chain, said epitope being bordered by at least one or two linkers; the second epitope is inserted between the VH and VL chains, said epitope being also bordered by such linkers, and the third epitope being inserted between the VL chain et the hinge. These two architectures differ by the linkers used bordering the $2^{nd}$ epitope.

**[0030]** (G): extracellular anti-Hsp70 domains of the multi-chain architectures according to the invention, where at least two epitopes (preferably CD20 epitopes) are inserted in the extracellular ligand binding domain between the hinge and the anti CLL1 VH and VL chains. In the third exemplary architecture, one CD34 epitope is included between two CD20 epitopes. Further architectures can be considered where CD34 replaces any other previous CD20 epitopes.

**[0031]** (H): extracellular anti-Hsp70 domains of the multi-chain architectures according to the invention, where at least two epitopes are inserted at the extremity of in the extracellular ligand binding domain.

TABLE 1

Sequence of the different scCAR components

| Functional domains | SEQ ID # | Raw amino acid sequence |
|---|---|---|
| CD8α signal peptide | SEQ ID NO. 1 | MALPVTALLLPLALLLHAARP |
| Alternative signal peptide | SEQ ID NO. 2 | METDTLLLWVLLLWVPGSTG |
| FcεRIIIα hinge | SEQ ID NO. 3 | GLAVSTISSFFPPGYQ |
| CD8α hinge | SEQ ID NO. 4 | TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD |
| IgG1 hinge | SEQ ID NO. 5 | EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK |
| CD8α transmembrane domain | SEQ ID NO. 6 | IYIWAPLAGTCGVLLLSLVITLYC |
| 41BB transmembrane domain | SEQ ID NO. 7 | IISFFLALTSTALLFLLFFLTLRFSVV |
| 41BB intracellular domain | SEQ ID NO. 8 | KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL |
| CD3ζ intracellular domain | SEQ ID NO. 9 | RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTAT KDTYDALHMQALPPR |
| Linker | SEQ ID NO. 10 | GGGGSGGGGSGGGGS |

TABLE 2

Sequence of variable regions of exemplary murine and humanized anti-HSP70 VH and VL chains and their respective CDRs

| ScFv sequences | SEQ ID # | Raw amino acid sequence |
|---|---|---|
| Murine cmHsp70.1 heavy chain variable region | SEQ ID NO. 11 | EVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLE WLGMIWGGGSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDT AMYFCARNGGYDVFHYWGQGTTVTVSS |
| Humanized cmHsp70.1 heavy chain variable region | SEQ ID NO. 12 | EVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKG LEWLGMIWGGGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAED TAVYYCARNGGYDVFHYWGQGTTVTVSS |

TABLE 2-continued

Sequence of variable regions of exemplary murine and humanized anti-HSP70 VH and VL chains and their respective CDRs

| ScFv sequences | SEQ ID # | Raw amino acid sequence |
|---|---|---|
| CDR1 | SEQ ID NO. 13 | GFSLSRNSVH |
| CDR2 | SEQ ID NO. 14 | WLGMIWGGGSTDYNSALKS |
| CDR3 | SEQ ID NO. 15 | NGGYDVFHY |
| Murine cmHsp70.1 light chain variable region | SEQ ID NO. 16 | QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLF TGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWY SNHLVFGGGTKLTVLG |
| Humanized cmHsp70.1 light chain variable region | SEQ ID NO. 17 | QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQA PRGLIGGTNNRAPWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCAL WYSNHLVFGGGTKLTVLG |
| CDR1 | SEQ ID NO. 18 | RSSTGAVTTSNYANWV |
| CDR2 | SEQ ID NO. 19 | GLIGGTNNRAP |
| CDR3 | SEQ ID NO. 20 | ALWYSNHLV |

TABLE 3

scCAR of structure V-1

| | scCAR Structure | | | | | | |
|---|---|---|---|---|---|---|---|
| scCAR Designation V-1 | signal peptide | VH | VL | FcεRIIIα hinge | CD8α TM | 41BB - IC | CD3ζ CD |
| V1 mouse cmHsp70.1 scCAR (SEQ ID NO. 21) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 3 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |
| V1 humanized cmHsp70.1 scCAR (SEQ ID NO. 27) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 3 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 4

scCAR of structure V-2

| | scCAR Structure | | | | | | |
|---|---|---|---|---|---|---|---|
| scCAR Designation V-2 | signal peptide | VH | VL | FcεRIIIα hinge | 41BB- TM | 41BB - IC | CD3ζ CD |
| V2-mouse cmHsp70.1 scCAR (SEQ ID NO. 22) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 3 | SEQ ID NO. 7 | SEQ ID NO. 8 | SEQ ID NO. 9 |
| V2-humanized cmHsp70.1 scCAR (SEQ ID NO. 28) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 3 | SEQ ID NO. 7 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 5

scCAR of structure V-3

| | scCAR Structure | | | | | | |
|---|---|---|---|---|---|---|---|
| scCAR Designation V-3 | signal peptide | VH | VL | CD8α hinge | CD8α TM | 41BB - IC | CD3ζ CD |
| V3-mouse cmHsp70.1 scCAR (SEQ ID NO. 23) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 4 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 5-continued

| scCAR of structure V-3 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | scCAR Structure | | | | | | |
| scCAR Designation V-3 | signal peptide | VH | VL | CD8α hinge | CD8α TM | 41BB - IC | CD3ζ CD |
| V3-humanized cmHsp70.1 scCAR (SEQ ID NO. 29) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 4 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 6

| scCAR of structure V-4 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | scCAR Structure | | | | | | |
| scCAR Designation V-4 | signal peptide | VH | VL | CD8α hinge | 41BB-TM | 41BB - IC | CD3ζCD |
| V4-mouse cmHsp70.1 scCAR (SEQ ID NO. 24) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 4 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |
| V4-humanized cmHsp70.1 scCAR (SEQ ID NO. 30) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 4 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 7

| scCAR of structure V-5 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | scCAR Structure | | | | | | |
| scCAR Designation V-5 | signal peptide | VH | VL | IgG1 hinge | CD8α TM | 41BB - IC | CD3ζCD |
| V5-mouse cmHsp70.1 scCAR (SEQ ID NO. 25) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 5 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |
| V5-humanized cmHsp70.1 scCAR (SEQ ID NO. 31) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 5 | SEQ ID NO. 6 | SEQ ID NO. 8 | SEQ ID NO. 9 |

TABLE 8

| scCAR of structure V-6 | | | | | | | |
|---|---|---|---|---|---|---|---|
| | scCAR Structure | | | | | | |
| scCAR Designation V-6 | signal peptide | VH | VL | IgG1 hinge | 41BB-TM | 41BB - IC | CD3ζ CD |
| V6-mouse cmHsp70.1 scCAR (SEQ ID NO. 26) | SEQ ID NO. 1 | SEQ ID NO. 11 | SEQ ID NO. 16 | SEQ ID NO. 5 | SEQ ID NO. 7 | SEQ ID NO. 8 | SEQ ID NO. 9 |
| V6-humanized cmHsp70.1 scCAR (SEQ ID NO. 33) | SEQ ID NO. 1 | SEQ ID NO. 12 | SEQ ID NO. 17 | SEQ ID NO. 5 | SEQ ID NO. 7 | SEQ ID NO. 8 | SEQ ID NO. 9 |

## DETAILED DESCRIPTION OF THE INVENTION

**[0032]** 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.

**[0033]** 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.

**[0034]** 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, N.Y.: 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). The present provides the following embodiments:

**[0035]** 1. A heat shock protein 70 (hHSP70) specific chimeric antigen receptor (anti-HSP70 CAR) comprising at least:

**[0036]** an extra cellular ligand binding-domain specific for-HSP70,

**[0037]** a transmembrane domain, and

**[0038]** a cytoplasmic signaling domain,

**[0039]** 2. A HSP70 specific chimeric antigen receptor (anti-HSP70 CAR) comprising at least:

**[0040]** an extra cellular ligand binding-domain specific forHSP70,

**[0041]** a transmembrane domain, and

**[0042]** a cytoplasmic signaling domain,

**[0043]** provided that said anti-HSP70 CAR does not bind to a "mut HSP70-2" antigen.

**[0044]** 3. A HSP70 specific chimeric antigen receptor (CAR) according to embodiment 1, wherein said CAR binds to a human membrane HSP70 antigen (mHSP70-1 antigen).

**[0045]** 4. A HSP70 specific chimeric antigen receptor (CAR) according to embodiment 1 or embodiment 2, wherein said CAR binds to human mHSP70-1 antigen.

**[0046]** 5. A HSP70 specific chimeric antigen receptor according to anyone of embodiment 1-3, further comprising a co-stimulatory domain.

**[0047]** 6. A HSP70 specific chimeric antigen receptor according to embodiment 1, further comprising a CD28 and/or a 4-1BB co-stimulatory domain.

**[0048]** 7. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 3, wherein said transmembrane domain comprises a CD8α transmembrane domain.

**[0049]** 8. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 4, further comprising a hinge.

**[0050]** 9. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 4, wherein said cytoplasmic signaling domain comprises a T-cell activating domain.

**[0051]** 10. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 6, wherein said chimeric antigen receptor is expressed under the form of a single polypeptide.

**[0052]** 11. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 7, wherein said extra cellular ligand binding-domain comprises domains from a monoclonal anti-HSP70 antibody.

**[0053]** 12. A HSP70 specific chimeric antigen receptor according to embodiment 8, wherein said extra cellular ligand binding-domain comprises a complementary determining region (CDR) from a VH domain and from a VL domain of at least one monoclonal anti-HSP70 antibody

**[0054]** 13. A HSP70 specific chimeric antigen receptor according to embodiment 9, wherein said CDRs are selected from SEQ ID NO. 13-15 and 18-20.

**[0055]** 14. A HSP70 specific scCAR according to any one of embodiment 1 to embodiment 10 having one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising a VH and a VL from a monoclonal anti-HSP70 antibody, a hinge transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain.

**[0056]** 15. A HSP70 specific scCAR according to any one of embodiment 1 to embodiment 11, wherein said structure V1 comprises a FcγRIIIa hinge and CD8α transmembrane domain.

**[0057]** 16. A HSP70 specific scCAR according to any one of embodiment 1 to embodiment 11, wherein said structure V3 comprises a CD8α hinge and a CD8α transmembrane domain.

**[0058]** 17. A HSP70 specific scCAR according any one of embodiment 1 to embodiment 11, wherein said structure V5 comprises an IgG1 hinge and a CD8α transmembrane domain.

**[0059]** 18. A HSP70 specific scCAR according to any one of embodiments 1 to 14, wherein said VH and VL have at least 80% identity with a polypeptide sequence selected from SEQ ID NO. 11-12 and SEQ ID NO. 16-17.

**[0060]** 19. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 15, wherein co-stimulatory domain from 4-1BB has at least 80% identity with SEQ ID NO. 8.

**[0061]** 20. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 16, wherein said CD3 zeta signaling domain has at least 80% identity with SEQ ID NO. 9.

**[0062]** 21. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1-12 or embodiment 15-17, wherein said FcγRIIIα hinge has at least 80% identity with SEQ ID NO. 3.

**[0063]** 22. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1-11, 13 or 15-17, wherein said CD8α hinge has at least 80% identity with SEQ ID NO. 4.

**[0064]** 23. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1-11 or 14-19, wherein said IgG1 hinge has at least 80% identity with SEQ ID NO. 5.

**[0065]** 24. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1-20, wherein said CD8α transmembrane domain has at least 80% identity with SEQ ID NO. 6.

**[0066]** 25. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 21 further

comprising another extracellular ligand binding domain which is not specific for HSP70.

**[0067]** 26. A HSP70 specific chimeric antigen receptor of structure V3 according to any one of embodiments embodiments 1-12 or embodiments 15-17, or embodiments or 21-22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 23 or 29.

**[0068]** 27. A HSP70 specific chimeric antigen receptor of structure V1 according to any one of embodiments 1-11, 13 or 15-17 or embodiments 21-22, which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 21 or 27.

**[0069]** 28. A HSP70 specific chimeric antigen receptor of structure V5 according to embodiments 1-11 or 14-19, or embodiments 21-22 which comprises a polypeptide sequence having at least 80% identity with SEQ ID NO. 25 or 31.

**[0070]** 29. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 25 further comprising a signal peptide.

**[0071]** 30. A HSP70 specific chimeric antigen receptor according to embodiment 26, wherein said signal peptide has at least 80% sequence identity with SEQ ID NO. 1 or SEQ ID NO. 2.

**[0072]** 31. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 30, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

**[0073]** 32. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 31, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.

**[0074]** 33. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 32, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes

**[0075]** 34. A HSP70 specific chimeric antigen receptor according to any one of embodiments 1 to 33, wherein the extracellular binding domain comprises the following sequence

**[0076]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$-;

**[0077]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-;

**[0078]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-;

**[0079]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0080]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0081]** Epitope1-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0082]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$;

**[0083]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$;

**[0084]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$-;

**[0085]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope3-$(L)_x$-;

**[0086]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$-;

**[0087]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$;

**[0088]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-;

**[0089]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$;

**[0090]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$;

**[0091]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$(L)_x$-Epitope2-$(L)_x$-$V_2$; or,

**[0092]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$(L)_x$-Epitope2-$(L)_x$-$V_2$-$(L)_x$-Epitope3-$(L)_x$;

**[0093]** wherein,

**[0094]** $V_1$ is $V_L$ and $V_2$ is $V_H$ or $V_1$ is $V_H$ and $V_2$ is $V_L$;

**[0095]** $L_1$ is a linker suitable to link the $V_H$ chain to the $V_L$ chain;

**[0096]** L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

**[0097]** x is 0 or 1 and each occurrence of x is selected independently from the others; and,

**[0098]** Epitope 1, Epitope 2 and Epitope 3 are mAb-specific epitopes and can be identical or differents.

**[0099]** 35. A HSP70 specific chimeric antigen receptor according to embodiment 34, wherein the extracellular binding domain comprises the following sequence

**[0100]** $V_1$-$L_1$-$V_2$-L-Epitope1; $V_1$-$L_1$-$V_2$-L-Epitope1-L; $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2; $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L; $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L-Epitope3; $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L-Epitope3-L; $V_1$-$L_1$-$V_2$-Epitope1; $V_1$-$L_1$-$V_2$-Epitope1-L; $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2; $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L; $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L-Epitope3; $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L-Epitope3-L; Epitope1-$V_1$-$L_1$-$V_2$; Epitope1-L-$V_1$-$L_1$-$V_2$; L-Epitope1-$V_1$-$L_1$-$V_2$; L-Epitope1-L-$V_1$-$L_1$-$V_2$; Epitope1-L-Epitope2-$V_1$-$L_1$-$V_2$; Epitope1-L-Epitope2-L-$V_1$-$L_1$-$V_2$; L-Epitope1-L-Epitope2-$V_1$-$L_1$-$V_2$; L-Epitope1-L-Epitope2-L-$V_1$-$L_1$-$V_2$; Epitope1-L-Epitope2-L-Epitope3-$V_1$-$L_1$-$V_2$; Epitope1-L-Epitope2-L-Epitope3-L-$V_1$-$L_1$-$V_2$; L-Epitope1-L-Epitope2-L-Epitope3-$V_1$-$L_1$-$V_2$; L-Epitope1-L-Epitope2-L-Epitope3-L-$V_1$-$L_1$-$V_2$; $V_1$-L-Epitope1-L-$V_2$; L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$; $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L; $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L-Epitope3; $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-Epitope3; $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L-Epitope3-Epitope4; L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3-L; Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3-L; L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3; L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L; L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L-Epitope3; L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-Epitope3, or Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L-Epitope3-Epitope4

**[0101]** wherein

**[0102]** $V_1$ is $V_L$ and $V_2$ is $V_H$ or $V_1$ is $V_H$ and $V_2$ is $V_L$;

**[0103]** $L_1$ is any linker suitable to link the $V_H$ chain to the $V_L$ chain;

**[0104]** L is a linker comprising glycine and serine residues, and each occurrence of L in the extracellular binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

**[0105]** epitope 1, epitope 2 and epitope 3 are mAb-specific epitopes and can be identical or differents.

**[0106]** 36. A HSP70 specific chimeric antigen receptor according to embodiment 34 or 35, wherein $L_1$ is a linker comprising Glycine and/or Serine.

**[0107]** 37. A HSP70 specific chimeric antigen receptor according to embodiment 33, wherein $L_1$ is a linker comprising the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, or $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is 1, 2, 3, 4 or 5.

**[0108]** 38. A HSP70 specific chimeric antigen receptor according to anyone embodiment 34 to embodiment 37, wherein $L_1$ is a linker comprising the amino acid sequence $(Gly_4Ser)_4$ or $(Gly_4Ser)_3$.

**[0109]** 39. A HSP70 specific chimeric antigen receptor according to embodiment 38, wherein L is a linker having an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGGS, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGGSGGGGS.

**[0110]** 40. A HSP70 specific chimeric antigen receptor according to embodiment 39, wherein L is a SGGGG, GGGGS or SGGGGS.

**[0111]** 41. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 38 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.

**[0112]** 42. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 41 wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes having an amino acid sequence of anyone of SEQ ID NO 33 to SEQ ID NO 42.

**[0113]** 43. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 42 wherein Epitope 1 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 33.

**[0114]** 44. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 43 wherein Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0115]** 45. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 44 wherein Epitope 3 is an mAb-specific epitope having an amino acid sequence of anyone of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0116]** 46. A HSP70 specific chimeric antigen receptor according to any one of embodiments 31 to 45 wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 41 or 42.47. A polynucleotide encoding a chimeric antigen receptor according to any one of embodiments 1 to 46.

**[0117]** 48. An expression vector comprising a nucleic acid of embodiment 47.

**[0118]** 49. An engineered lymphoid immune cell expressing at the cell surface membrane an anti-HSP70 CAR according to any one of embodiments 1 to 46.

**[0119]** 50. An engineered lymphoid immune cell according to embodiment 49 derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

**[0120]** 51. An engineered cell according to any one of embodiments 49 or 50 for use in therapy.

**[0121]** 52. An engineered cell according to any one of embodiments 49 to 51 for use in therapy, wherein the patient is a human.

**[0122]** 53. An engineered cell according to any one of embodiments 49 to 52 for use in therapy, wherein the condition is a pre-malignant or malignant cancer condition characterized by HSP70-expressing cells.

**[0123]** 54. An engineered cell according to any one of embodiments 49 to 53 for use in therapy, wherein the condition is a condition which is characterized by an overabundance of HSP70-expressing cells.

**[0124]** 55. An engineered cell according to any one of embodiments 49 to 54 for use in therapy, wherein the condition is a hematological cancer condition.

**[0125]** 56. An engineered cell according to any one of embodiments 49 to 55 for use in therapy, wherein the hematological cancer condition is leukemia.

**[0126]** 57. An engineered cell according to any one of embodiments 49 to 55 for use in therapy, wherein the leukemia is acute myelogenous leukemia (AML).

**[0127]** 58. An engineered cell according to any one of embodiments 49 to 57, wherein expression of TCR is suppressed in said immune cell.

**[0128]** 59. An engineered cell according to any one of embodiments 49 to 58, wherein expression of at least one MHC protein, preferably β2m or HLA, is repressed or suppressed in said immune cell.

**[0129]** 60. An engineered cell according to any one of embodiments 49 to 59, wherein said cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.

**[0130]** 61. 60. A Combination of at least an immune cell (e.g., T cell) modified to at least express an anti-mHsp70 CAR according to any one of embodiments 1 to 46, with an antibody directed against soluble Hsp70 A Combination of at least an immune cell (e.g., T cell) modified to at least express an anti-mHsp70 CAR according to any one of embodiments 1 to 46, with an antibody directed against soluble Hsp70 for use in a method of treating a disease associated with Hsp70.1 overexpressing cells.

**[0131]** 62. Combination according to embodiment 61 to be sequentially administered to the patient, said antibodies directed against soluble Hsp70 being administered first until the level of the soluble Hsp70 is reduced in the plasma of the patient by at least 50%, preferably 75%, and more preferably 90% compared to that before administration of said antibodies, said administration of soluble Hsp70 specific monoclonal antibodies being followed by the administration of aid an anti-mHsp70 CAR expressing immune cells.

**[0132]** 63. A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered

cell according to any one of embodiments 49 to 60 in an amount effective to cause impairment of said cancer cell.

**[0133]** 64. A Combination of at least an immune cell (e.g., T cell) modified to at least express an anti-mHsp70 CAR according to any one of embodiments 1 to 46, with a drug.

**[0134]** 65. A method of engineering an immune cell comprising:

**[0135]** (a) Providing an immune cell,

**[0136]** (b) Expressing at the surface of said cell at least one HSP70 single-chain specific chimeric antigen receptor according to any one of embodiments 1 to 46.

**[0137]** 66. The method of engineering an immune cell of embodiment 65 comprising:

**[0138]** (a) Providing an immune cell,

**[0139]** (b) Introducing into said cell at least one polynucleotide encoding said HSP70 single-chain specific chimeric antigen receptor,

**[0140]** (c) Expressing said polynucleotide into said cell.

**[0141]** 67. The method of engineering an immune cell of embodiment 66 comprising:

**[0142]** (a) Providing an immune cell,

**[0143]** (b) Introducing into said cell at least one polynucleotide encoding said anti-HSP70 single-chain specific chimeric antigen receptor,

**[0144]** (c) Introducing at least one other chimeric antigen receptor which is not specific for HSP70.

**[0145]** 68. A method of treating a subject in need thereof comprising:

**[0146]** (a) Providing an immune cell expressing at the surface an anti-HSP70 single-chain specific chimeric antigen receptor according to any one of embodiments 1 to 46;

**[0147]** (b) Administrating said immune cells to said patient.

**[0148]** 69. A method according to embodiment 68, wherein said immune cell is provided from a donor.

**[0149]** 70. A method according to embodiment 68, wherein said immune cell is provided from the patient himself.

**[0150]** 71. Method for depleting engineered lymphoid immune cell expressing a HSP70 specific CAR and at least one epitope according to any one of embodiments 31 to 46 in a patient, wherein an antibody, preferably monoclonal, specific to said epitope is administered to said patient in case of need.

HSP70 Single-Chain Specific Chimeric Antigen Receptors

**[0151]** The present invention relates to HSP70 specific chimeric antigen receptor comprising an extracellular ligand-binding domain specifically directed against one portion of the HSP70 antigen, a transmembrane domain and a signaling transducing domain.

**[0152]** By chimeric antigen receptor (CAR) is intended molecules that combine an extracellular binding domain directed against a component present on a target cell, for example an antibody-based specificity for a desired antigen (e.g., tumor antigen) with an immune cell receptor component to generate a chimeric protein that will transduce an activating or inhibitory signal toward cellular immune activity.

**[0153]** The present invention more particularly relates to a HSP70 specific chimeric antigen receptor (anti-HSP70 CAR) comprising at least:

**[0154]** an extracellular ligand binding-domain anti-HSP70,

**[0155]** a transmembrane domain, and

**[0156]** a cytoplasmic signaling domain,

**[0157]** provided that said anti-HSP70 CAR does not bind to the mut HSP70-2 antigen.

**[0158]** By "anti-HSP70 chimeric receptor" used throughout all the present application, it is meant all the chimeric antigen receptor (CAR) which can bind to any human HSP70 antigen, provided that said CAR does not bind to the human mut Hsp70-2 antigen.

**[0159]** By "mut Hsp70-2" (or mut HSP72) antigen which is not bound by the anti-HSP70 CAR of the present invention, it is meant a polypeptide under the Uniprot reference P54652 in which the aminoacid residue at the position 8 is mutated from isoleucine to aspartic acid (Gaudin C et al, 1999), or in which the aminoacid residue at the position 564 is mutated from lysine to alanine (Jakobsen M E et al. 2013)

**[0160]** The present invention encompasses anti-HSP70 CAR which can bind to human Hsp70.1, non-mutated Hsp70-2, Hsp70-3, Hsp70-4, Hsp70-6, Hsp70-7, Hsp70-8, Hsp70-9, Hsp70-13 or Hsp70-14 antigen. All these isoforms in humans are described in Daugaard M et al. (2007 or in Kabani M et al. (2008).

**[0161]** According to a preferred embodiment, the anti-HSP70 CAR of the invention binds to the human membrane HSP70-1 heat shock antigen (other names HSP70.1, HSPA1A or HSX70, protein which is encoded by the HSPA1A gene).

**[0162]** Hereafter, by the term "Hsp70" is meant more specifically to membrane Hsp70 (mHsp70), to be distinguished with extracellular Hsp70 (eHsp70) which is a secreted form of Hsp70 (Pockley A G et al. 1998).

**[0163]** Preferably, the HSP70 specific chimeric antigen receptor according to the invention further comprises a co-stimulatory domain, preferably a CD28 or a 4-1BB co-stimulatory domain, and more preferably a 4-1BB co-stimulatory domain as described for instance by Jena, B., G. Dotti, et al. (2010). It can also comprise a transmembrane domain which can be a CD8α transmembrane domain, as well as an optional hinge.

**[0164]** The signal transducing domain or "cytoplasmic signaling domain" of a CAR according to the present invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation or inhibition of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation or inactivation of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "cytoplasmic signaling domain" refers to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

**[0165]** The cytoplasmic signaling domain, which is preferably from a human protein involved in signal transduction pathway(s), determines whether anti-HSP70 CAR is a positive CAR (PCAR) or a negative CAR (NCAR) depending on the nature of the signaling. Respectively, the CAR is a

PCAR when the signaling domain, such as CD3zeta from human TCR receptor, has the effect of stimulating the cellular immune activity of the immune cell when the extracellular ligand binding-domain is bound to HSP70. Conversely, the anti-HSP70 CAR is a NCAR or inhibitory CAR (iCAR) when the signaling domain has the effect of reducing the cellular immune activity, such as signaling domains of human immunoinhibitory receptors CTLA-4 and PD-1 (Federov et al., Sci Transl Med. 2013 Dec. 11; 5 (215): 215ra172). Preferred examples of signal transducing domain for use in a anti-HSP70 CAR can be the cytoplasmic sequences of the T cell receptor and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivate or variant of these sequences and any synthetic sequence that has the same functional capability. Signal transduction domain comprises two distinct classes of cytoplasmic signaling sequence, those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequence can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non limiting examples those derived from TCRzeta, FcRgamma, FcRbeta, FcRepsilon, CD3gamma, CD3delta, CD3epsilon, CD5, CD22, CD79a, CD79b and CD66d. In a preferred embodiment, the signaling transducing domain of the anti-HSP70 CAR can comprise the CD3zeta signaling domain which has amino acid sequence with at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97% or 99% or 100% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 9.

**[0166]** In particular embodiment the signal transduction domain of the anti-HSP70 CAR of the present invention comprises a co-stimulatory signal molecule. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient immune response. "Co-stimulatory ligand" refers to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83. A "co-stimulatory molecule" refers to the cognate binding partner on a T-cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

**[0167]** In a preferred embodiment, the signal transduction domain of the anti-HSP70 CAR of the present invention comprises a part of co-stimulatory signal molecule selected from the group consisting of fragment of 4-1BB (GenBank: AAA53133.) and CD28 (NP_006130.1). In particular the signal transduction domain of the anti-HSP70 CAR of the present invention comprises amino acid sequence which comprises at least 70%, preferably at least 80%, more preferably at least 90%, 95% 97% or 99% sequence identity with amino acid sequence selected from the group consisting of SEQ ID NO: 8.

**[0168]** An anti-HSP70 CAR according to the present invention generally further comprises a transmembrane domain (TM). The distinguishing features of appropriate transmembrane domains comprise the ability to be expressed at the surface of a cell, preferably in the present invention an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and to interact together for directing cellular response of immune cell against a predefined target cell. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein. As non-limiting examples, the transmembrane polypeptide can be a subunit of the T-cell receptor such as α, β, γ or ζ, polypeptide constituting CD3 complex, IL2 receptor p55 (α chain), p75 (β chain) or γ chain, subunit chain of Fc receptors, in particular Fcγ receptor III or CD proteins. Alternatively the transmembrane domain can be synthetic and can comprise predominantly hydrophobic residues such as leucine and valine. In a preferred embodiment said transmembrane domain is derived from the human CD8 alpha chain (e.g. NP_001139345.1) The transmembrane domain can further comprise a hinge region between said extracellular ligand-binding domain and said transmembrane domain. The term "hinge region" used herein generally means any oligo- or polypeptide that functions to link the transmembrane domain to the extracellular ligand-binding domain. In particular, hinge region are used to provide more flexibility and accessibility for the extracellular ligand-binding domain. A hinge region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Hinge region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively, the hinge region may be a synthetic sequence that corresponds to a naturally occurring hinge sequence, or may be an entirely synthetic hinge sequence. In a preferred embodiment said hinge domain comprises a part of human CD8 alpha chain, FcγRIIIα receptor or IgG1 respectively referred to in this specification as SEQ ID NO. 3, SEQ ID NO. 4 and SEQ ID NO. 5, or hinge polypeptides which display preferably at least 80%, more preferably at least 90%, 95% 97% or 99% sequence identity with these polypeptides.

**[0169]** According to a preferred embodiment, the anti-HSP70 CAR according to the invention comprises a transmembrane domain more particularly selected from CD8α and 4-1BB, showing identity with the polypeptides of SEQ ID NO. 6 or 7.

**[0170]** An anti-HSP70 CAR according to the invention generally further comprises a transmembrane domain (TM) more particularly a TM selected from CD8α and 4-1BB, and even more particularly showing identity with the polypeptides of SEQ ID NO. 6 or 7.

**[0171]** In a preferred embodiment, an anti-HSP70 CAR according to the invention further comprises a TM domain from CD8α with SEQ ID NO. 6 or showing at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO. 6

**[0172]** Downregulation or mutation of target antigens is commonly observed in cancer cells, creating antigen-loss escape variants. Thus, to offset tumor escape and render immune cell more specific to target, the specific anti-HSP70 CAR according to the invention can comprise another extracellular ligand-binding domains, to simultaneously bind different elements in target thereby augmenting immune cell activation and function. In one embodiment, the extracellular ligand-binding domains can be placed in tandem on the same transmembrane polypeptide, and optionally can be separated by a linker. In another embodiment, said different extracellular ligand-binding domains can be placed on different transmembrane polypeptides composing the anti-HSP70 CAR. In another embodiment, the present invention relates to a population of anti-HSP70 CAR s 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 anti-HSP70 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 anti-HSP70 CAR each one comprising different extracellular ligand binding domains. By population of anti-HSP70 CARs, it is meant at least two, three, four, five, six or more anti-HSP70 CAR s 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 anti-HSP70 CAR s each one comprising different extracellular ligand binding domains.

**[0173]** HSP70 specific chimeric antigen receptors according to the invention can have different architectures, as they can be expressed, for instance, under a single-chain chimeric protein (scCAR) or under the form of several polypeptides (multi-chain) including at least one such chimeric protein. Such multi-chain CAR architectures are disclosed in WO2014/039523, especially in FIGS. **2** to **4**, and from page 14 to 21, which are herein incorporated by reference.

**[0174]** In general, anti-HSP70 CAR comprises an extracellular single chain antibody (scFv Fc) fused to the intracellular signaling domain of T-cell antigen receptor complex zeta chain (scFv Fc:ζ), which has the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity.

**[0175]** The present application discloses several anti-HSP70 single chain CAR directed against HSP70 antigen, which comprise as non-limiting example the amino acid sequences: SEQ ID NO: 21 to 32.

**[0176]** HSP70 CAR of the present invention can also be "multi-chain CARs" as previously mentioned, which means that the extracellular binding domain and the signaling domains are preferably located on different polypeptide chains, whereas co-stimulatory domains may be located on the same or a third polypeptide. Such multi-chain CARs can be derived from FcεRI (Ravetch et al, 1989), by replacing the high affinity IgE binding domain of FcεRI alpha chain by an extracellular ligand-binding domain such as scFv, whereas the N and/or C-termini tails of FcεRI beta and/or gamma chains are fused to signal transducing domains and co-stimulatory domains respectively. The extracellular ligand binding domain has the role of redirecting T-cell specificity towards cell targets, while the signal transducing domains activate or reduce the immune cell response. The fact that the different polypeptides derive from the alpha, beta and gamma polypeptides from FcεRI are transmembrane polypeptides sitting in juxtamembrane position provides a more flexible architecture to CARs, improving specificity towards the targeted molecule and reducing background activation of immune cells as described in WO2014/039523.

**[0177]** Extracellular Ligand-Binding Domain

**[0178]** The term "extracellular ligand-binding domain" as used herein is defined as an oligo- or polypeptide that is capable of binding a ligand. Preferably, the domain will be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state. It can be for instance binding domains derived from a ligand, a receptor, human or mice antibodies or antigen recognition domains derived from camels or cartilaginous fish.

**[0179]** In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising the light ($V_L$) and the heavy ($V_H$) variable fragment of a target antigen specific monoclonal anti HSP70 antibody joined by a flexible linker.

**[0180]** Said $V_L$ and $V_H$ are preferably selected from the antibodies referred to in the literature as the scFvs of the cmHsp70.1 antibodies disclosed in Zettlitz K A et al 2010. The target of cmHsp70.1 antibodies is the Heat shock 70 kDa protein 1A encoded HSPA1A (NP_005336.3) gene. According to this publication, cmHsp70.1 was humanized; both mouse and humanized antibodies recognizing the same region including amino acids 473-504 of the C-terminal substrate-binding domain (SBD) of mHsp70. Such cmHsp70.1 antibodies recognize a 14-mer peptide termed "TKD" (Stangl S et al, 2011). In one embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising heavy ($V_H$) and light ($V_L$) variable fragment of a target antigen specific monoclonal anti HSP70 antibody mouse cmHsp70.1 joined by a flexible linker, said VH and VL variable fragment having at least 80%, preferably 90%, more preferably 95% and even more preferably 99% of identity with respectively SEQ ID NO. 11 and 16.

**[0181]** In a preferred embodiment, said extracellular ligand-binding domain comprises a single chain antibody fragment (scFv) comprising heavy ($V_H$) and light ($V_L$) variable fragment of a target antigen specific monoclonal anti HSP70 antibody humanized cmHsp70.1 joined by a flexible linker, said VH and VL variable fragment having at least 80%, preferably 90%, more preferably 95% and even more preferably 99% of identity with respectively SEQ ID NO 12. and 17.

**[0182]** In another embodiment, said extracellular ligand-binding domain comprises CDRs from VH and VL domains of monoclonal anti-HSP70 mouse and humanized cmHsp70.1 antibodies selected from SEQ ID NO. 13 to 15 described below.

**[0183]** According to a preferred embodiment, the CDR sequences of VH chain from humanized monoclonal anti-HSP70 cmHsp70.1 antibody may be chosen among GFSLSRNSVH (SEQ ID NO 13), WLGMIWGGGSTDYNSALKS (SEQ ID NO 14), or NGGYDVFHY (SEQ ID NO 15).

**[0184]** According to a preferred embodiment, the CDR sequences of VL chain from mouse or humanized monoclonal cmHsp70.1 anti-HSP70 antibody may be chosen among RSSTGAVTTSNYANWV (SEQ ID NO 18), GLIGGTNNRAP (SEQ ID NO 19), or ALWYSNHLV (SEQ ID NO 20).

**[0185]** In one embodiment, said $V_L$ and $V_H$ are preferably selected from the antibodies referred to in EP2070947 (deposited with the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany on Nov. 14, 2003, and assigned Accession Number DSM ACC2629, or cmHsp70.2 as produced by hybridoma cmHsp70.2, deposited with the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH on Nov. 14, 2003, and assigned Accession Number DSM ACC2630.) or those in WO2002022656 or those disclosed in Juhasz K. et al., Cancers 2014 6 42-66 doi 10.3390/cancers6010042.

**[0186]** In a preferred embodiment, said antibody are humanized.

**[0187]** In another embodiment, said extracellular ligand-binding domain comprises CDRs from VH and VL domains of monoclonal anti-HSP70 mouse and humanized cmHsp70.1 antibodies as in EP2070947 or those in WO2002022656 (deposited with the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany on Nov. 14, 2003, and assigned Accession Number DSM ACC2629, or cmHsp70.2 as produced by hybridoma cmHsp70.2, deposited with the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH on Nov. 14, 2003, and assigned Accession Number DSM ACC2630.)

**[0188]** The extracellular domain and the transmembrane domain are preferably linked together by a flexible linker comprising the sequence SEQ ID NO. 10.

**[0189]** By the term "recombinant antibody" as used herein, is meant an antibody or antibody fragment which is generated using recombinant DNA technology, such as, for example, an antibody or antibody fragment expressed by a bacteriophage, a yeast expression system or a mammalian cell expression system. The term should also be construed to mean an antibody or antibody fragment which has been generated by the synthesis of a DNA molecule encoding the antibody or antibody fragment and which DNA molecule expresses an antibody or antibody fragment protein, or an amino acid sequence specifying the antibody or antibody fragment, wherein the DNA or amino acid sequence has been obtained using recombinant or synthetic DNA or amino acid sequence technology which is available and well known in the art.

**[0190]** The present invention discloses a HSP70 specific single-chain chimeric antigen receptor, preferably single-chain CAR (anti-HSP70 scCAR), as described above, wherein said extra cellular ligand binding-domain comprises VH and VL chains which are humanized.

**[0191]** By the term "humanized antibody" as used herein, is meant the polypeptides include a humanized heavy chain variable region and a humanized light chain variable region. For example, the polypeptides may include the framework (FR) regions of the light and heavy chain variable regions of a human antibody, while retaining substantially the antigen-binding specificity of a parental monoclonal antibody. The humanized heavy chain variable region and/or the humanized light chain variable region are at least about 87% humanized, at least about 90% humanized, at least about 95% humanized, at least about 98% humanized, or at least about 100% humanized, excluding the complementary-determining regions (CDRs). The antigen-binding polypeptides molecules may be derived from monoclonal antibody donors (e.g., mouse monoclonal antibody donors) and may include CDRs from the monoclonal antibodies (e.g., mouse monoclonal CDRs).

**[0192]** By the term "monoclonal antibody" as used herein, is meant antibody produced by a laboratory-grown cell clone, either of a hybridoma or a virus-transformed lymphocyte that is more abundant and uniform than natural antibody and is able to bind specifically to a single site on HSP70 antigen. They are monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell, in contrast to polyclonal antibodies which are made from several different immune cells. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope. Current methodology applied for humanization is according to Lefranc M P et al (Lefranc, M P, Ehrenmann F, Ginestoux C, Giudicelli V, Duroux P "Use of IMGT (®) databases and tools for antibody engineering and humanization", Methods Mol Biol. 2012; 907: 3-37). In these four alignments are indicated.

**[0193]** A humanized antibody can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., J. Immunol., 169: 1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et

al., J. Biol. Chem., 272(16): 10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8): 1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J. Mol. Biol., 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.).

**[0194]** Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within a anti-HSP70 CAR of the invention can be replaced with other amino acid residues from the same side chain family and the altered anti-HSP70 CAR can be tested for the ability to bind HSP70 using the functional assays described herein.

**[0195]** Anti-Hsp70 Single-Chain CAR (Sc CAR)

**[0196]** In a preferred embodiment, the present invention discloses an anti-HSP70 specific single-chain chimeric antigen receptor ("anti-HSP70 scCAR" or "scCAR") having one of the polypeptide structure selected from V1 to V6, and preferably form V1, V3 and V5 as illustrated in FIG. **2** and Tables 3-8, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a signaling domain and a co-stimulatory domain.

**[0197]** In a more preferred embodiment, the present invention discloses a HSP70 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises a FcγRIIIα, CD8 alpha or IgG1 hinge and a CD8 alpha transmembrane domain.

**[0198]** In another more preferred embodiment, said HSP70 specific scCAR comprises the co-stimulatory domain 4-1BB or the CD28, or more preferably the 4-1BB co-stimulatory domain.

**[0199]** The present invention discloses a HSP70 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises respectively a FcγRIIIα, CD8 alpha or IgG1 hinge and a 4-1BB transmembrane domain.

**[0200]** The present invention discloses a HSP70 specific scCAR as described above, wherein said structure V1, V3 or V5 comprises respectively a FcγRIIIα, CD8 alpha or IgG1, a 4-1BB cytoplasmic domain and a CD8 alpha transmembrane domain.

**[0201]** According to a preferred embodiment, the anti-HSP70 scCAR of the invention has one of the polypeptide structure selected from V1 to V6, and preferably versions V1, V3 and V5 as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain, said CD3 zeta signaling domain preferably having a sequence SEQ ID NO. 9.

**[0202]** According to another preferred embodiment, the anti-HSP70 scCAR of the invention has one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a hinge, a transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain, said 4-1BB co-stimulatory domain preferably having a sequence SEQ ID NO. 8.

**[0203]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge, a CD8α transmembrane domain, preferably having SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0204]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a CD8α hinge, a CD8α transmembrane domain, preferably having SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0205]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge, a CD8α transmembrane domain, preferably having SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0206]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO. 7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0207]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from

a monoclonal anti-HSP70 antibody, a CD8α hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO. 7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0208]** The present invention discloses anti-HSP70 scCAR having one of the polypeptide structure selected from V1 to V6 and preferably versions V1, V3 and V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge, a 4-1BB transmembrane domain, preferably having SEQ ID NO. 7, a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain.

**[0209]** In a particular aspect, the present invention discloses an anti-HSP70 specific scCAR having a V1 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge preferably with SEQ ID NO. 3, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8.

**[0210]** More specifically, the present invention discloses an anti-HSP70 specific scCAR having a V1 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge preferably with SEQ ID NO. 3, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL having at least 80% identity with SEQ ID NO. 16 or 17.

**[0211]** In another particular aspect, the present invention discloses an anti-HSP70 specific scCAR having a V3 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a CD8α hinge preferably with SEQ ID NO. 4, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8.

**[0212]** More specifically, the present invention discloses an anti-HSP70 specific scCAR having a V3 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a CD8α hinge preferably with SEQ ID NO. 4, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL chain having at least 80% identity with SEQ ID NO. 16 or 17.

**[0213]** In still another particular aspect, the present invention discloses an anti-HSP70 specific scCAR having a V5 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge preferably with SEQ ID NO. 5, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8.

**[0214]** More specifically, the present invention discloses an anti-HSP70 specific scCAR having a V5 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge preferably with SEQ ID NO. 5, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL chain having at least 80% identity with SEQ ID NO. 16 or 17.

**[0215]** The present invention discloses an anti-HSP70 specific scCAR having a V1 polypeptide structure, as illustrated in FIG. **2**, said polypeptide having at least 80% identity with SEQ ID NO. 21 or 27.

**[0216]** In particular, said anti-HSP70 specific scCAR having a V1 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge preferably with SEQ ID NO. 3, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL having at least 80% identity with SEQ ID NO. 16 or 17, and wherein said polypeptide has at least 80% identity with SEQ ID NO. 21 or 27.

**[0217]** The present invention discloses an anti-HSP70 specific scCAR of structure V3, as illustrated in FIG. **2**, said polypeptide having at least 80% identity with SEQ ID NO. 23 or 29.

**[0218]** More specifically, the present invention discloses an anti-HSP70 specific scCAR having a V3 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a CD8α hinge preferably with SEQ ID NO. 4, a CD8α transmembrane domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL chain having at least 80% identity with SEQ ID NO. 16 or 17, and wherein said polypeptide has at least 80% identity with SEQ ID NO. 23 or 29.

**[0219]** The present invention discloses an anti-HSP70 specific scCAR of structure V5, as illustrated in FIG. **2**, said polypeptide having at least 80% identity with SEQ ID NO. 25 or 31.

**[0220]** More specifically, the present invention discloses an anti-HSP70 specific scCAR having a V5 polypeptide structure, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge preferably with SEQ ID NO. 5, a CD8α transmembrane

domain, preferably with SEQ ID NO. 6, a cytoplasmic domain including a CD3 zeta signaling domain, preferably with SEQ ID NO. 9, and a 4-1BB co-stimulatory domain, preferably with SEQ ID NO. 8, wherein said VH chain having at least 80% identity with SEQ ID NO. 11 or 12 and said VL chain having at least 80% identity with SEQ ID NO. 16 or 17, and wherein said polypeptide having at least 80% identity with SEQ ID NO. 25 or 31.

**[0221]** The present invention more particularly discloses a HSP70 single-chain specific chimeric antigen receptor (scCAR) having a polypeptide structure V1, V3 or V5 as illustrated in FIG. **2**, and described above said structure comprising an extra cellular ligand binding-domain VH from a monoclonal anti-HSP70 antibody comprising the following CDR sequences: GFSLSRNSVH (SEQ ID NO 13), WLGMIWGGGSTDYNSALKS (SEQ ID NO 14) and NGGYDVFHY (SEQ ID NO 15),

**[0222]** and preferably, an extra cellular ligand binding-domain VL from a monoclonal anti-HSP70 antibody comprising the following CDR sequences: RSSTGAVTTSNYANWV (SEQ ID NO 18), GLIGGTNNRAP (SEQ ID NO 19), and ALWYSNHLV (SEQ ID NO 20),

**[0223]** and wherein said structure generally comprising:

**[0224]** a hinge, a transmembrane domain and a cytoplasmic domain including a CD3 zeta signaling domain and a co-stimulatory domain from 4-1BB.

**[0225]** The present invention discloses an anti-HSP70 single-chain specific chimeric antigen receptor (anti-HSP70 scCAR) as above, wherein said extra cellular ligand binding-domain VH and VL is humanized.

**[0226]** The present invention discloses a HSP70 single-chain specific chimeric antigen receptor (scCAR) as described above, wherein said extra cellular ligand binding-domain VH from a monoclonal anti-HSP70 antibody comprise at least one of the following sequences:

```
EVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGM

IWGGGSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGG

YDVFHYWGQGTTVTVSS
(corresponding to mouse cmHsp70.1),
or

EVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGM

IWGGGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGG

YDVFHYWGQGTTVTVSS
(corresponding to humanized cmHsp70.1),
```

**[0227]** and VL from a monoclonal anti-HSP70 antibody comprise at least one of the following sequences:

```
QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLI

GGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNHLVF

GGGTKLTVLG
(corresponding to mouse cmHsp70.1),

QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLI

GGTNNRAPWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNHLVF

GGGTKLTVLG
(corresponding to humanized cmHsp70.1),
```

**[0228]** The present invention also discloses a HSP70 specific scCAR as previously defined, further comprising another extracellular ligand binding domain which is not specific for HSP70, such as CD33 antigen, CD44 antigen, CD47 antigen, CD123 antigen, CD96 antigen and T-cell immunoglobulin mucin-3 (TIM-3).

**[0229]** The present invention discloses a HSP70 specific scCAR as above, further comprising a signal peptide, preferably of SEQ ID NO 1 or SEQ ID NO 2, in order to help the CAR polypeptide to reach the immune cell's membrane.

**[0230]** The present invention discloses a HSP70 specific scCAR as above, wherein a glycin-rich linker is inserted between VH and VL, such as the GS linker of SEQ ID NO 10.

**[0231]** Insertion of at Least One Epitope in the Extracellular Domain of the CAR

**[0232]** An anti-HSP70 CAR of the invention may include at least the insertion of at least one epitope in the extracellular domain of said CAR.

**[0233]** Said anti-HSP70 CAR in which at least one epitope is inserted in its extracellular domain may be single-chain CAR (scCAR) or multi-chain CAR (mcCAR), and preferably a scCAR.

**[0234]** This is intended to deplete the immune cells endowed with the CAR in the event these later would cause in vivo adverse effects such as cytokine storm. Moreover, such insertion of epitope or "epitope-tagging" may be useful to sort in vitro engineered immune cells for sake of purification. Said at least one epitope may be any antigenic peptide which is enough immunogenic to be bound by a specific antibody recognizing such peptide. For instance, this can be obtained, for instance, by inserting at least one, and preferably two copies of a CD20 mimotope, preferably of sequence CPYSNPSLCS (SEQ ID NO. 33), into the CAR polypeptide sequence. For purpose of simplication hereafter, the order of the scFvs from the N terminal end to the C terminal end is presented as follows: the VH chain and then the VL chain. However, it can be envisioned in the scope of the present invention that this order is inversed: VL chain and then the VL chain.

**[0235]** Different positions of the at least one CD20 mimotope are schematized in FIG. **3**. Said two copies of a CD20 mimotope can be linked to each other and also to the $V_L$ by a linker. They can also be inserted between the anti-HSP70 scFv and the hinge (such as CD8alpha), by using an optional linker. The CD20 mimotopes can be bound by anti-CD20 antibodies, such as Rituximab (McLaughlin P, et al. 1998).

**[0236]** The anti-HSP70 CAR of the present invention may thus comprise VH and a VL chains which are able to bind to HSP70 cell surface antigen, optionally humanized, a linker L, a suicide domain, a hinge or part of it, a transmembrane domain, a co-stimulatory domain and a stimulatory domain.

**[0237]** In a preferred embodiment, the epitope introduced within the chimeric scFv is the CD20 mimotope (SEQ ID NO. 33) and the infused mAb which is being used to target it—for sorting and/or depletion purpose(s) is rixutimab.

**[0238]** According to another embodiment, the epitope is a mimotope. As a macromolecule, often a peptide, which mimics the structure of an epitope, the mimotope has the advantage to be smaller than conventional epitope, and therefore may be beneficial for a non-conformational sequence and easier to reproduce in a long polypeptide such a CAR. Mimotopes are known for several pharmaceutically-

approved mAb such as two 10 amino acid peptides for cetuximab (Riemer et al., 2005), or a 24 AA for palivizumab (Arbiza et al, 1992). As these mimotopes can be identified by phage display, it is possible to try several of them in order to obtain a sequence which does not perturb the scFv for the same mAb. Furthermore, their use can enhance a complement-dependent cytotoxicity (CDC).

**[0239]** Several examples of such epitopes and mimotopes with their corresponding binding mAb are presented in the following Table 9.

TABLE 9

| Mimotopes and epitope with their corresponding mAb | | |
|---|---|---|
| **Rituximab** | | |
| Mimotope | SEQ ID NO 33 | CPYSNPSLC |
| **Palivizumab** | | |
| Epitope C | SEQ ID NO 34 | NSELLSLINDMPITNDQKKLMSNN |
| **Cetuximab** | | |
| Mimotope 1 | SEQ ID NO 35 | CQFDLSTRRLKC |
| Mimotope 2 | SEQ ID NO 36 | CQYNLSSRALKC |
| Mimotope 3 | SEQ ID NO 37 | CVWQRWQKSYVC |
| Mimotope 4 | SEQ ID NO 38 | CMWDRFSRWYKC |
| **Nivolumab** | | |
| Epitope A | SEQ ID NO 39 | SFVLNWYRMSPSNQTDKLAAFPEDR |
| Epitope B | SEQ ID NO 40 | SGTYLCGAISLAPKAQIKE |

**[0240]** In one embodiment, said at least one epitope is inserted between the VH and VL chains of the anti-Hsp70.1 CAR, optionally linked to said VH and VL chains by one linker.

**[0241]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one CD20 mimotope is inserted between the VH and VL chains of the anti-Hsp70.1 CAR, optionally linked to said VH and VL chains by one linker.

**[0242]** According to the invention, the linker which is used between the scFvs, epitope(s) and hinge within the extracellular domain of the anti-HSP70 CAR is preferably a glycin-rich linker such as GS linker (SEQ ID NO. 10) and may be of variable length. Such alternative linkers can be found in Table 1 in Priyanka V, Chichili R, Kumar V, and Sivaraman J (2013) "*Linkers in the structural biology of protein-protein interactions*" Protein Sci. 22(2): 153-167.

**[0243]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one CD20 mimotope is inserted between the VH and VL chains of the anti-Hsp70.1 CAR, optionally linked to said VH and VL chains by one linker.

**[0244]** In another embodiment, said at least one epitope is inserted at the N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N terminal end of the CAR by one linker.

**[0245]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted at the N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N terminal end of the CAR by one linker.

**[0246]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted at the N terminal end of the CAR -so upfront of the scFvs-, optionally linked to the VH chain and to the N terminal end of the CAR by one linker.

**[0247]** In another embodiment, said at least one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

**[0248]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

**[0249]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein one epitope is inserted between the scFvs and the hinge of the CAR, optionally linked to the VL chain and to the hinge by one linker.

**[0250]** In a preferred embodiment, at least two epitopes are inserted in the extracellular domain of the anti-Hsp70 CAR of the present invention.

**[0251]** In an embodiment, mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and two CD20 mimotopes,

**[0252]** said extra-binding domain comprising VH and VL chains directed against mHSP70 and a FcγRIIIα or CD8α or IgG1 hinge;

**[0253]** and said 2 epitopes being inserted in tandem between the scFvs and said hinge

**[0254]** a linker (SEQ ID NO. 10) interspaced between the 2 epitopes and between the VH and the 2 epitopes.

**[0255]** In an embodiment, mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and two CD20 mimotopes,

**[0256]** said extra-binding domain comprising VH and VL chains directed against mHSP70 and a FcγRIIIα or CD8α or IgG1 hinge;

**[0257]** and said 2 epitopes being inserted in tandem upfront the scFvs-N terminal end of the CAR-

**[0258]** a linker (SAQ ID NO. 10) interspaced between the 2 epitopes and at the N terminal end of the CAR.

**[0259]** According to one embodiment, at least two epitopes are inserted in the extracellular domain in such a way that the VH is located between them, all these components being optionally interspaced by at least one linker.

**[0260]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH is located between them, all these components being optionally interspaced by at least one linker.

**[0261]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

**[0262]** According to another embodiment, two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

**[0263]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

**[0264]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VL is located between them, all these components being optionally interspaced by at least one linker.

**[0265]** According to another embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) comprises an extracellular binding domain wherein at least two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0266]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0267]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein two epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0268]** In another embodiment, three epitopes are inserted in the extracellular domain of the anti-Hsp70 CAR of the present invention.

**[0269]** According to a particular embodiment, said mHSP70 specific CAR of the invention contains an extracellular binding domain wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0270]** In a preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0271]** In a more preferred embodiment, said mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure of version V3 as illustrated in FIG. **2**, said structure comprising at least an extracellular ligand binding-domain anti-mHSP70, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, and wherein three epitopes are inserted in the extracellular domain in such a way that the VH and VL chains ar located between them, all these components being optionally interspaced by at least one linker.

**[0272]** In another embodiment, mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling

domain, and three CD20 epitopes, said extra-binding domain comprising VH and VL chains directed against mHSP70 and a FcγRIIIα or CD8α or IgG1 hinge;

**[0273]** and said 3 epitopes being inserted in tandem between the scFvs and said hinge

**[0274]** a linker (SEQ ID NO. 10) interspaced between the 3 epitopes and between the VH and the 3 epitopes.

**[0275]** In another embodiment, mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) has one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain, two CD20 epitopes, and one CD34 epitope;

**[0276]** said extra-binding domain comprising VH and VL chains directed against mHSP70 and a FcγRIIIα or CD8α or IgG1 hinge;

**[0277]** said 2 epitopes being inserted in tandem between the scFvs and said hinge, and said CD34 epitope being inserted between the said 2 CD20 epitopes, all components being interspaced between them by a linker (SEQ ID NO. 10) and a linker between the epitope and and between the VH and the 3 epitopes.

**[0278]** Said CD34 epitope may be chosen among SEQ ID NO. 41 or SEQ ID NO. 42.

**[0279]** In all the above embodiments relating to the epitope-containing anti-mHSP70 CARs, the VH and VL chains which are used as extracellular binding domain are binding preferably to human membrane HSP70-1.

**[0280]** In a preferred embodiment, said above anti-mHSP70 CARs comprising at least an extra cellular ligand binding-domain including VH and VL chains derived from anti-mHSP70 monoclonal antibodies.

**[0281]** More specifically, the epitopes can be included into the CAR of the present invention can as follows:

**[0282]** In some embodiments, the extracellular binding domain comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

**[0283]** In some embodiments, the extracellular binding domain comprises at least 1, 2 or 3 mAb-specific epitopes.

**[0284]** In some embodiments, when the extracellular binding domain comprises several mAb-specific epitopes, all the mAb-specific epitopes are identical.

**[0285]** In some embodiments, when the extracellular binding domain comprises several mAb-specific epitopes, the mAb-specific epitopes are not identical. For example, the extracellular binding domain can comprises three mAb-specific epitopes, two of them being identical and the third one being different.

**[0286]** In some embodiments, the extracellular binding domain comprises a VH, a VL, one or more mAb-specific epitopes, preferably 1, 2 or 3, more preferably 2 or 3 mAb-specific epitopes.

**[0287]** In some embodiments, the extracellular binding domain comprises the following sequence (Nterm is located on the left hand side):

**[0288]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$;

**[0289]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$;

**[0290]** $V_1$-$L_1$-$V_2$-$(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$;

**[0291]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0292]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0293]** Epitope1-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-$V_1$-$L_1$-$V_2$;

**[0294]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$;

**[0295]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$;

**[0296]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$;

**[0297]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope3-$(L)_x$;

**[0298]** $(L)_x$-Epitope1-$(L)_x$-Epitope2-$(L)_x$-$V_1$-$L_1$-$V_2$-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$;

**[0299]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$;

**[0300]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$;

**[0301]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$;

**[0302]** $V_1$-$(L)_x$-Epitope1-$(L)_x$-$V_2$-$(L)_x$-Epitope2-$(L)_x$-Epitope3-$(L)_x$-Epitope4-$(L)_x$;

**[0303]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$(L)_x$-Epitope2-$(L)_x$-$V_2$;

**[0304]** $(L)_x$-Epitope1-$(L)_x$-$V_1$-$(L)_x$-Epitope2-$(L)_x$-$V_2$-$(L)_x$-Epitope3-$(L)_x$;

**[0305]** $V_1$-$L_1$-$V_2$-L-Epitope1;

**[0306]** $V_1$-$L_1$-$V_2$-L-Epitope1-L;

**[0307]** $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2;

**[0308]** $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L;

**[0309]** $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L-Epitope3;

**[0310]** $V_1$-$L_1$-$V_2$-L-Epitope1-L-Epitope2-L-Epitope3-L;

**[0311]** $V_1$-$L_1$-$V_2$-Epitope1;

**[0312]** $V_1$-$L_1$-$V_2$-Epitope1-L;

**[0313]** $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2;

**[0314]** $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L;

**[0315]** $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L-Epitope3;

**[0316]** $V_1$-$L_1$-$V_2$-Epitope1-L-Epitope2-L-Epitope3-L;

**[0317]** Epitope1-$V_1$-$L_1$-$V_2$;

**[0318]** Epitope1-L-$V_1$-$L_1$-$V_2$;

**[0319]** L-Epitope1-$V_1$-$L_1$-$V_2$;

**[0320]** L-Epitope1-L-$V_1$-$L_1$-$V_2$;

**[0321]** Epitope1-L-Epitope2-$V_1$-$L_1$-$V_2$;

**[0322]** Epitope1-L-Epitope2-L-$V_1$-$L_1$-$V_2$;

**[0323]** L-Epitope1-L-Epitope2-$V_1$-$L_1$-$V_2$;

**[0324]** L-Epitope1-L-Epitope2-L-$V_1$-$L_1$-$V_2$;

**[0325]** Epitope1-L-Epitope2-L-Epitope3-$V_1$-$L_1$-$V_2$;

**[0326]** Epitope1-L-Epitope2-L-Epitope3-L-$V_1$-$L_1$-$V_2$;

**[0327]** L-Epitope1-L-Epitope2-L-Epitope3-$V_1$-$L_1$-$V_2$;

**[0328]** L-Epitope1-L-Epitope2-L-Epitope3-L-$V_1$-$L_1$-$V_2$;

**[0329]** $V_1$-L-Epitope1-L-$V_2$;

**[0330]** L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$;

**[0331]** $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L;

**[0332]** $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L-Epitope3;

**[0333]** $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-Epitope3;

**[0334]** $V_1$-L-Epitope1-L-$V_2$-L-Epitope2-L-Epitope3-EpitoPe4;

**[0335]** L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3-L;

**[0336]** Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3-L;

**[0337]** L-Epitope1-L-$V_1$-L-Epitope2-L-$V_2$-L-Epitope3;

**[0338]** L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L;

**[0339]** L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L-Epitope3;

**[0340]** L-Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-Epitope3, or,

**[0341]** Epitope1-L-$V_1$-$L_1$-$V_2$-L-Epitope2-L-Epitope3-Epitope 4.

**[0342]** wherein,

**[0343]** $V_1$ and $V_2$ are $V_H$ and $V_L$ of an ScFv (i.e, $V_1$ is $V_L$ and $V_2$ is $V_H$ or $V_1$ is $V_H$ and $V_2$ is $V_L$);

**[0344]** $L_1$ is any linker suitable to link the VH chain to the VL chain in an ScFv;

**[0345]** L is a linker, preferably comprising glycine and serine residues, and each occurrence of L in the extracellular

binding domain can be identical or different to other occurrence of L in the same extracellular binding domain, and,

**[0346]** x is 0 or 1 and each occurrence of x is independently from the others; and,

**[0347]** epitope 1, epitope 2 and epitope 3 are mAb-specific epitopes and can be identical or different.

**[0348]** In some embodiments, the extracellular binding domain comprises the following sequence (Nterm is located on the left hand side):

**[0349]** $V_H$-$L_1$-$V_L$-L-Epitope1-L-Epitope2-L;

**[0350]** L-Epitope1-L-$V_H$-L-Epitope2-L-$V_L$-L-Epitope3-L;

**[0351]** $V_L$-$L^1$-$V_H$-L-Epitope1-L-Epitope2-L; or,

**[0352]** L-Epitope1-L-$V_L$-L-Epitope2-L-$V_H$-L-Epitope3-L.

**[0353]** wherein L, L1, epitope 1, epitope 2 and epitope 3 are as defined above.

**[0354]** In some embodiments, $L_1$ is a linker comprising Glycine and/or Serine. In some embodiment, $L_1$ is a linker comprising the amino acid sequence $(Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$ or $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$, where n is 1, 2, 3, 4 or 5. In some embodiments $L_1$ is $(Gly_4Ser)_4$ or $(Gly_4Ser)_3$.

**[0355]** In some embodiment, L is a flexible linker, preferably comprising Glycine and/or Serine. In some embodiments, L has an amino acid sequence selected from SGG, GGS, SGGS, SSGGS, GGGG, SGGGG, GGGGS, SGGGGS, GGGGGS, SGGGGGS, SGGGGG, GSGGGGS, GGGGGGGS, SGGGGGGG, SGGGGGGGS, or SGGGGSGGGGS preferably SGG, SGGS, SSGGS, GGGG, SGGGGS, SGGGGGS, SGGGGG, GSGGGGS or SGGGGSGGGGS. In some embodiment, when the extracellular binding domain comprises several occurrences of L, all the Ls are identical. In some embodiments, when the extracellular binding domain comprises several occurrences of L, the Ls are not all identical. In some embodiments, L is SGGGGS. In some embodiments, the extracellular binding domain comprises several occurrences of L and all the Ls are SGGGGS.

**[0356]** In some embodiments, Epitope 1, Epitope 2 and Epitope 3 are identical or different and are selected from mAb-specific epitopes having an amino acid sequence of anyone of SEQ ID NO 33 to SEQ ID NO 42.

**[0357]** In some embodiments, Epitope 1, Epitope 2 and Epitope 3 are identical or different and are selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.

**[0358]** In some embodiment, Epitope 1 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 33.

**[0359]** In some embodiment, Epitope 2 is a mAb-specific epitope having an amino acid sequence of anyone of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0360]** In some embodiment, Epitope 3 is a mAb-specific epitope having an amino acid sequence of anyone of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0361]** In some embodiment, Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0362]** In some embodiment, Epitope 2 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 33 and Epitope 3 is an mAb-specific epitope having an amino acid sequence of anyone of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**[0363]** In some embodiment, one of Epitope 1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID 41 or 42. In some embodiment, one of Epitope1, Epitope 2, Epitope 3 and Epitope 4 is a CD34 epitope, preferably an epitope of SEQ ID 41 or 42 and the other mAb specific epitopes are CD20 mimotopes, preferably mimotope of SEQ ID NO 33.

**[0364]** The present invention relates also to a method for depleting in a patient engineered lymphoid immune cell expressing a HSP70 specific scCAR and at least one epitope such as disclosed in this application, by administering in said patient an antibody—preferably monoclonal—specific to said epitope in case of need, i.e. to avoid adverse effects such as cytokine storm.

**[0365]** In a preferred embodiment, the monoclonal antibody rituximab specific to the at least one CD20 antigen inserted in the extracellular domain of the Hsp70 specific CAR is administered to the patient in order to deplete said engineered immune cells.

**[0366]** Production of Monoclonal Specific Membrane HSP70 Antibodies (Anti-mHsp70)

**[0367]** Another aspect of the present invention is related to de novo anti membrane Hsp70 (anti-mHsp70) antibodies which VH and VL chains may be used as extracellular binding domain in the architecture of the anti-mHsp70 CAR.

**[0368]** Said new anti-mHsp70 antibodies may be polyclonal or preferably monoclonal antibodies.

**[0369]** According to a preferred embodiment, anti-Hsp70 antibodies, which VH and VL chains are used as extra-binding domain in the architecture of the anti-Hsp70 CAR, are monoclonal anti-mHsp70 antibodies. Concerning monoclonal anti-mHsp70 in the prior art, said epitopes are localized in the extracellular part of the mHsp70. Exemplary epitopes may be found in the Table 1 in the publication of Multhoff et al., 2011: for instance epitopes located at amino-acids 450-461, 436-503, 383-447 respectively for the cmHsp70.1 and C92F3A antibodies. The discovery of said antibodies is helpful in improving the avidity and specificity against the target molecule compared to those known in the prior art. More importantly, in order to prevent the cross-reaction of scFvs inserted in the anti-HSP70 CAR of the invention with the soluble HSP70 antigen, it will be advantageous to raise monoclonal antibodies specifically against an epitope of the membrane HSP70 but not of the soluble HSP70.

**[0370]** Monoclonal antibodies are routinely produced such as described i.e. in Yokoyama W M et al, 2006.

**[0371]** According to one embodiment, HSP70 antigen is inserted within lipid bilayer of particles before animal immunization. Such insertion of HSP70 protein, preferably HSP70-1, or part thereof, it may increase the potential to raise monoclonal antibodies specific to the membrane.

**[0372]** Said lipid bilayer-containing particles are currently used as vehicle delivery system; they may be liposomes, nanoparticles, lipospheres and like such as reviewed in the book Domb A et al, 2014. As an example, self-assembled

lipid bilayer coat surrounding a PLGA core may be achieved by using lipids as the surfactant component of an emulsion/solvent evaporation-based PLGA particle synthesis. It is also possible to make stabilized Lipid-coated poly(lactide-co-glycolide) microparticles LCMPs either by the inclusion in the lipid bilayer of cholesterol or lipids with saturated carbon chains. Techniques to make those particles are well known in the prior art, such as reviewed in White S et al (2007), and by instance in U.S. Pat. No. 8,968,539, U.S. Pat. No. 7,939,270 or WO 2008102121.

**[0373]** Preferably, the immunization of animal by injection of such HSP70 antigen containing lipid bilayer particles will provide monoclonal anti-membrane Hsp70 antibodies which will be made by using myeloma fusion technique.

**[0374]** The present invention, in one aspect, provides a method for making anti-mHsp70, preferably anti-mHsp70-1, monoclonal antibodies wherein an animal is immunized with at least one mHsp70, preferably mHsp70.1 antigen, and monoclonal antibodies are made and identified which bind to said mHsp70, and preferably mHsp70.1 antigen.

**[0375]** According to one embodiment, the method of preparing at least one monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies is provided, said method comprising the steps of:

**[0376]** (a) providing a liquid, preferably a cell-free liquid containing at least one mHsp70, and preferably mHsp70.1 antigen,

**[0377]** (b) contacting a sample of said liquid with a monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies specific to said mHsp70, and preferably mHsp70.1 antigen to form complexes between them in said liquid;

**[0378]** (c) removing said complexes from said liquid to yield a partially purified liquid substantially free of mHsp70, and preferably mHsp70.1 antigens;

**[0379]** (d) immunizing an animal, preferably a mouse, with said partially purified liquid,

**[0380]** (e) fusing spleen cells from said immunized animal to myeloma cells to form hybridomas capable of producing monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies,

**[0381]** (f) culturing said hybridomas to produce said monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies,

**[0382]** (g) isolating one or more of said monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies,

**[0383]** (h) repeating steps (b) through (g) in sequence, at least once, using in steps (b) and (c) at least one monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies of step (g) in the previous sequence until said liquid contains one antigen.

**[0384]** In general a liquid is a medium, preferably a cell free medium.

**[0385]** As one embodiment, according to the monoclonal antibody production, different animal models may be used, such as mice, rats, hamsters, guinea pigs, goats and preferably rabbits, according to routine protocols such as described in i.e. Hanly W C et al, 1995 or Hau J et al, 2005.

**[0386]** In one embodiment, the inoculum injected to the animal comprises at least one antigen being immunogenic to the membrane Hsp70, and preferably membrane Hsp70-1.

**[0387]** According to the invention, immunogenic Hsp70 antigen, alone or preferably inserted within lipid bilayer, may be administrated to the animal for routine antibody production subcutaneously, intradermally intramuscularly or intraperitoneally.

**[0388]** According to the invention, a dose of 50-1000 μg of immunogenic Hsp70 antigen, alone or preferably inserted within lipid bilayer is administered to the rabbit; a dose of 10-50 μg is administered to the mouse; a dose of 50-500 μg is administered to the guinea pig; a dose of 250-5000 μg μg is administered to the goat.

**[0389]** Preferably, said injections are made in two to four sites per animal, generally on the back, away from the spine. Typically, recommended subcutaneous injection volumes and amounts in rabbits are 0.1-0.25 ml/site with 8-10 sites maximum, but less than 1.5 ml in total. Typically, recommended intramuscular injection volumes and amounts in rabbits are 0.25 ml/site with 2 sites maximum, but less than 0.5 ml in total. Typically, recommended intradermal injection volumes and amounts in rabbits are 0.025 ml/site with 5-8 sites maximum, but less than 0.5 ml in total.

**[0390]** Adjuvant such as Freund adjuvant may be used. Usually, 10% of blood volume can be removed without replacement at one time and repeated every 2 weeks. The response is evaluated e.g. immunoassay, western blot, immunofluorescence, etc.). When a good response is raised, a terminal bleed can be performed if an ongoing need for the antibody is required, rabbits are preferably not be maintained longer than 18 months for antibody production when adjuvants are utilized.

**[0391]** Moreover, the invention provides hybridoma cell lines that produce any of the monoclonal anti-mHsp70, and preferably mHsp70.1 antibodies disclosed herein.

**[0392]** The invention also relates to isolated nucleic acid comprising DNA encoding a monoclonal anti-mHsp70, and preferably mHsp70.1 antibody as herein disclosed; a vector comprising the nucleic acid; a host cell comprising the vector; a method of producing an monoclonal anti-mHsp70, and preferably anti mHsp70.1 antibody comprising culturing the host cell under conditions wherein the DNA is expressed and, optionally, further comprising recovering the antibody from the host cell culture.

**[0393]** Compositions

**[0394]** The present invention provides a composition for its use or a method for inhibiting the proliferation or reducing the population of cancer cells expressing Hsp70 in a patient, the methods comprising contacting the Hsp70-expressing cancer cell population with an anti-Hsp70 CART cell, and in particular scCART, of the invention that binds to the Hsp70-expressing cell, binding of an anti-Hsp70 CAR cell, and in particular scCART, of the invention to the HSP70-expressing cancer cell resulting in the destruction of the HSP70-expressing cancer cells

**[0395]** In certain aspects, the anti-HSP70 CART cell, and in particular scCART, of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% (to undetectable level) in a subject with or animal model for myeloid leukemia or another cancer associated with HSP70-expressing cells, relative to a negative control.

**[0396]** The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disorder or condition associated with HSP70-expressing cells (e.g., associated with a hematologic cancer), the methods comprising administering to a subject in need

an anti-HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with HSP70-expressing cells include inflammatory disorders (such as rheumatoid arthritis) and cancers (such as hematological cancers, in particular AML or AML complications).

**[0397]** The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disease associated with HSP70-expressing cells, the method comprising administering to a subject in need an anti-HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell. In one aspect, the subject is a human. Non-limiting examples of diseases associated with HSP70-expressing cells include in particular Acute Myeloid Leukemia (AML).

**[0398]** The present invention provides a composition for its use or a method for treating or preventing relapse of cancer associated with HSP70-expressing cells, the method comprising administering to a subject in need thereof an anti-HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell. In another aspect, the methods comprise administering to the subject in need thereof an effective amount of an anti HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell in combination with an effective amount of another therapy.

**[0399]** In particular embodiments, the present invention contemplates, in part, cells, CAR constructs, nucleic acid molecules and vectors that can administered either alone or in any combination using standard vectors and/or gene delivery systems, and in at least some aspects, together with a pharmaceutically acceptable carrier or excipient. In certain embodiments, subsequent to administration, said nucleic acid molecules or vectors may be stably integrated into the genome of the subject.

**[0400]** In specific embodiments, viral vectors may be used that are specific for certain cells or tissues and persist in said cells. Suitable pharmaceutical carriers and excipients are well known in the art. The compositions prepared according to the disclosure can be used for the prevention or treatment or delaying the above identified diseases.

**[0401]** In another aspect, the invention further provides a composition comprising a monoclonal anti-mHsp70, and preferably mHsp70.1 antibody as described herein and a carrier.

**[0402]** In addition, a method of treating mammalian cancer cells overexpressing mHsp70, and preferably mHsp70.1 is provided which comprises exposing said mammalian cancer cells to an effective amount of a monoclonal anti-mHsp70, and preferably mHsp70.1 antibody as disclosed herein.

**[0403]** The invention further pertains to an article of manufacture comprising a container and a composition contained within said container, wherein the composition includes a monoclonal anti-mHsp70, and preferably mHsp70.1 antibody as described herein.

**[0404]** Polynucleotides, Vectors:

**[0405]** The present invention also relates to polynucleotides and vectors allowing heterologous expression into cells of the anti-HSP70 CAR according to the invention, encoding the polypeptides sequences which have been previously detailed.

**[0406]** The polynucleotides may be included in an expression cassette or expression vector (e.g. a plasmid for introduction into a bacterial host cell, or a viral vector such as a baculovirus vector for transfection of an insect host cell, or a plasmid or viral vector such as a lentivirus for transfection of a mammalian host cell).

**[0407]** In a particular embodiment, the different nucleic acid sequences can be included in one polynucleotide or vector which comprises a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see (Donnelly and Elliott 2001; Atkins, Wills et al. 2007; Doronina, Wu et al. 2008)). By "codon" is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

**[0408]** To direct transmembrane polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in polynucleotide sequence or vector sequence. The secretory signal sequence is operably linked to the transmembrane nucleic acid sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the nucleic acid sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleic acid sequence of interest (see, e.g., Welch et al., U.S. Pat. No. 5,037,743; Holland et al., U.S. Pat. No. 5,143,830). In a preferred embodiment the signal peptide comprises the amino acid sequence SEQ ID NO: 1 and 2 or at least 90%, 95% 97% or 99% sequence identity with SEQ ID NO: 1 and/or 2.

**[0409]** Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. Preferably, the nucleic acid sequences of the present invention are codon-optimized for expression in mammalian cells, preferably for expression in human cells. Codon-optimization refers to the exchange in a sequence of interest of codons that are generally rare in highly expressed genes of a given species by codons that are generally frequent in highly expressed genes of such species, such codons encoding the amino acids as the codons that are being exchanged.

**[0410]** Delivery Methods

**[0411]** The present invention encompasses the different means to express the anti-HSP70 Chimeric Antigen Receptor (CAR) described herein in immune cells

**[0412]** Methods for introducing a polynucleotide construct into cells are known in the art and include as non-limiting examples stable transformation methods wherein the polynucleotide construct encoding said CAR is integrated into the genome of the cell, transient transformation methods

wherein the polynucleotide construct is not integrated into the genome of the cell and virus mediated methods.

**[0413]** Said polynucleotides may be introduced into a cell by for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, transient transformation methods include for example microinjection, electroporation or particle bombardment, cell fusion. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells. Said plasmid vector can comprise a selection marker which provides for identification and/or selection of cells which received said vector.

**[0414]** Different transgenes can be included in one vector. Said vector can comprise a nucleic acid sequence encoding ribosomal skip sequence such as a sequence encoding a 2A peptide. 2A peptides, which were identified in the Aphthovirus subgroup of picornaviruses, causes a ribosomal "skip" from one codon to the next without the formation of a peptide bond between the two amino acids encoded by the codons (see Donnelly et al., J. of General Virology 82: 1013-1025 (2001); Donnelly et al., J. of Gen. Virology 78: 13-21 (1997); Doronina et al., Mol. And. Cell. Biology 28(13): 4227-4239 (2008); Atkins et al., RNA 13: 803-810 (2007)).

**[0415]** By "codon" is meant three nucleotides on an mRNA (or on the sense strand of a DNA molecule) that are translated by a ribosome into one amino acid residue. Thus, two polypeptides can be synthesized from a single, contiguous open reading frame within an mRNA when the polypeptides are separated by a 2A oligopeptide sequence that is in frame. Such ribosomal skip mechanisms are well known in the art and are known to be used by several vectors for the expression of several proteins encoded by a single messenger RNA.

**[0416]** In a more preferred embodiment of the invention, polynucleotides encoding polypeptides according to the present invention can be mRNA which is introduced directly into the cells, for example by electroporation. The inventors determined the optimal condition for mRNA electroporation in T-cell. The inventor used the cytoPulse technology which allows, by the use of pulsed electric fields, to transiently permeabilize living cells for delivery of material into the cells. The technology, based on the use of PulseAgile (BTX Havard Apparatus, 84 October Hill Road, Holliston, Mass. 01746, USA) electroporation waveforms grants the precise control of pulse duration, intensity as well as the interval between pulses (U.S. Pat. No. 6,010,613 and International PCT application WO2004083379). All these parameters can be modified in order to reach the best conditions for high transfection efficiency with minimal mortality. Basically, the first high electric field pulses allow pore formation, while subsequent lower electric field pulses allow moving the polynucleotide into the cell.

**[0417]** The different methods described above involve introducing CAR, and in particular scCAR, into a cell. As non-limiting example, said CAR can be introduced as transgenes encoded by one plasmid vector. Said plasmid vector can also contain a selection marker which provides for identification and/or selection of cells which received said vector.

**[0418]** Polypeptides may be synthesized in situ in the cell as a result of the introduction of polynucleotides encoding said polypeptides into the cell. Alternatively, said polypeptides could be produced outside the cell and then introduced thereto. Methods for introducing a polynucleotide construct into cells are known in the art and including as non limiting examples stable transformation methods wherein the polynucleotide construct is integrated into the genome of the cell, transient transformation methods wherein the polynucleotide construct is not integrated into the genome of the cell and virus mediated methods. Said polynucleotides may be introduced into a cell by for example, recombinant viral vectors (e.g. retroviruses, adenoviruses), liposome and the like. For example, transient transformation methods include for example microinjection, electroporation or particle bombardment. Said polynucleotides may be included in vectors, more particularly plasmids or virus, in view of being expressed in cells.

**[0419]** Activation and Expansion of T Cells

**[0420]** Whether prior to or after genetic modification of the T cells, even if the genetically modified immune cells of the present invention are activated and proliferate independently of antigen binding mechanisms, the immune cells, particularly T-cells of the present invention can be further activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005. T cells can be expanded in vitro or in vivo.

**[0421]** Generally, the T cells of the invention are expanded by contact with an agent that stimulates a CD3 TCR complex and a co-stimulatory molecule on the surface of the T cells to create an activation signal for the T-cell. For example, chemicals such as calcium ionophore A23187, phorbol 12-myristate 13-acetate (PMA), or mitogenic lectins like phytohemagglutinin (PHA) can be used to create an activation signal for the T-cell.

**[0422]** As non-limiting examples, T cell populations may be stimulated in vitro such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. Conditions appropriate for T cell culture include an appropriate media (e.g., Minimal Essential Media or RPMI Media 1640 or, X-vivo 5, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (e.g., fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-□, IL-4, IL-7, GM-CSF, -10, -2, IL-15, TGF, and TNF- or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanoi. Media can include RPMI 1640, A1M-V, DMEM, MEM, a-MEM, F-12, X-Vivo 1, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, e.g., penicillin and streptomycin, are included only in experimental cultures, not in cultures of

cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37° C.) and atmosphere (e.g., air plus 5% C02). T cells that have been exposed to varied stimulation times may exhibit different characteristics

**[0423]** In another particular embodiment, said cells can be expanded by co-culturing with tissue or cells. Said cells can also be expanded in vivo, for example in the subject's blood after administrating said cell into the subject.

**[0424]** Engineered Immune Cells

**[0425]** A "Cell" according to the present invention generally refers to a cell of hematopoietic origin functionally involved in the initiation and/or execution of innate and/or adaptative immune response. Cell according to the present invention is preferably an isolated immune cell, and more preferably a T-cell obtained from a donor. Said immune cell according to the present invention can also be derived from a stem cell. The stem cells can be adult stem cells, non-human embryonic stem cells, more particularly non-human stem cells, cord blood stem cells, progenitor cells, bone marrow stem cells, induced pluripotent stem cells, totipotent stem cells or hematopoietic stem cells. Representative human cells are CD34+ cells. Said isolated cell can also be a dendritic cell, killer dendritic cell, a mast cell, a NK-cell, a B-cell or a T-cell selected from the group consisting of inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes. In another embodiment, said cell can be derived from the group consisting of CD4+ T-lymphocytes and CD8+ T-lymphocytes.

**[0426]** Prior to expansion and genetic modification of the cells of the invention, a source of cells can be obtained from a subject through a variety of non-limiting methods. Cells can be obtained from a number of non-limiting sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available and known to those skilled in the art, may be used.

**[0427]** In another embodiment, said cell can be derived from a healthy donor, from a patient diagnosed with cancer or from a patient diagnosed with an infection. In another embodiment, said cell is part of a mixed population of cells which present different phenotypic characteristics. In the scope of the present invention is also encompassed a cell line obtained from a transformed T-cell according to the method previously described. Modified cells resistant to an immunosuppressive treatment and susceptible to be obtained by the previous method are encompassed in the scope of the present invention.

**[0428]** As a preferred embodiment, the present invention provides T-cells or a population of primary T-cells, endowed with a HSP70 CAR as described above, that do not express functional TCR and that a reactive towards HSP70 positive cells, for their allogeneic transplantation into patients.

**[0429]** As a more preferred embodiment, the present invention provides T-cells or a population of T-cells endowed with a HSP70 CAR and that a reactive towards HSP70 positive cells as described above, that do not express a functional TCR and are resistant to a selected drug, for their allogeneic transplantation into patients treated with said selected drug. The present invention encompasses the method of preparing engineered immune cells for immunotherapy comprising introducing ex-vivo into said immune cells the polynucleotides or vectors encoding the HSP70 CAR according to transformation methods as previously described in WO2014/130635, WO2013176916, WO2013176915 and incorporated herein by reference.

**[0430]** In a preferred embodiment, said polynucleotides are introduced into the immune cells by means of retroviral vectors in view of being stably integrated into the cell genome.

**[0431]** Therapeutic Combinations of Anti-mHsp70 CAR with Antibodies Against Soluble Hsp70

**[0432]** The present invention encompasses a combination of at least immune cell (e.g., T cell) modified to at least express an anti-mHsp70 CAR and antibodies directed against soluble Hsp70, for use in a method of treating a disease associated with Hsp70 overexpressing cells.

**[0433]** By soluble Hsp70, it is meant extracellular HSP70 secreted into the plasma. According to several publications including Heck et al. 2011 and Krause et al, 2015, increased soluble Hsp70 is associated with inflammatory and oxidative stress conditions and serum soluble Hsp70 concentrations are positively correlated with markers of inflammation, such as C-reactive protein, monocyte count, and TNF-α.

**[0434]** According to a preferred embodiment, the present invention provides a therapeutic combination to be administrated to the patient for treating a disease associated with Hsp70 overexpressing cells comprising:

**[0435]** at least immune cell (e.g., T cell) modified to at least express an anti-mHsp70 CAR, and;

**[0436]** at least one antibody directed against soluble Hsp70;

**[0437]** said at least one antibody directed against soluble Hsp70 being administrated to reduce the level of soluble Hsp70 in the plasma of the patient by at least 50%, preferably 75%, and more preferably 90% compared to the level before administration,

**[0438]** said soluble specific Hsp70 monoclonal antibodies being administrated of mHsp70 CAR expressing immune cells in order to prevent the soluble Hsp70 from being bound by said mHsp70 CAR.

**[0439]** According to Zang X et al, 2010, the concentration of soluble Hsp70 of a population from a study varies from about 0.5 to 5 ng/ml in plasma. Thus, it is advantageous to monitor the level of soluble Hsp70 in the plasma of the patient before the administration of anti-soluble Hsp70 monoclonal antibodies by using i.e. Elisa test with anti-soluble Hsp70 monoclonal antibodies kits marketed by several companies. Then, the subsequent administration of the mHsp70 CAR expressing immune cells can be performed when the level of soluble Hsp70 in the plasma of the patient is reduced by at least 50%, preferably 75%, and more preferably 90% compared to the level before administration.

**[0440]** This embodiment is particularly adapted to the case when the scFvs of the mHsph70 CAR and of the soluble Hsp70 specific antibodies are binding respectively to the same (or overlapping) epitope of membrane HSP70 antigen and soluble HSP70 antigen.

**[0441]** The administration of the antibodies directed against soluble Hsp70 to the patient can be performed in one or several doses, preferably administered parenterally, generally by intravenous infusion. Administration may also be by intraperitoneal, oral, subcutaneous, or intramuscular routes. Antibodies are generally administered in the range of

about 0.1 to about 2 g/kg of patient weight, commonly about 0.5 to about 10 mg/kg, and often about 1 to about 5 mg/kg. In some cases it may be advantageous to administer a large loading dose followed by periodic (e.g., weekly) maintenance doses over the treatment period. Antibodies can also be delivered by slow-release delivery systems, pumps, and other known delivery systems for continuous infusion. Dosing regimens may be varied to provide the desired circulating levels of the particular antibody based on its pharmacokinetics.

**[0442]** Before administering the mHsp70 CAR expressing immune cells to the patients, it may be advantageous to monitor the level of soluble Hsp70 in a blood sample of the patient, by using for instance an ELISA test based on an soluble Hsp70 specific antibody.

**[0443]** The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermaly, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

**[0444]** The administration of the cells or population of cells can consist of the administration of $10^4$-$10^9$ cells per kg body weight, preferably $10^5$ to $10^6$ cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administrated will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

**[0445]** In another embodiment, said effective amount of cells or composition comprising those cells are administrated parenterally. Said administration can be an intravenous administration. Said administration can be directly done by injection within a tumor.

**[0446]** According to a preferred embodiment, the soluble Hsp70 specific antibody to be used in combination with the mHSP70 specific CAR is binding to an epitope which is different of that of the mHSP70 specific CAR. As example, such soluble Hsp70 specific antibodies may be chosen among those marketed by the Company Stressgen Biotechnologies Corp, or by the Company Abcam under the name ab133063.

**[0447]** Said CAR can be a single-chain CAR (scCAR) or a multi-chainCAR (mcCAR), and preferably a scCAR.

**[0448]** Said anti-mHsp70 CAR to be used in combination with antibodies directed against soluble Hsp70 may contain at least one epitope for depletion/sorting purpose(s) such as described in the present application.

**[0449]** In an embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) comprising at least an anti-mHSP70 extra cellular ligand binding-domain, a transmembrane domain, and a cytoplasmic signaling domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0450]** In a preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) comprising at least an anti-mHSP70 extra cellular ligand binding-domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain, CD3 zeta signaling domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0451]** In a more preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) having one of the polypeptide structure selected from V1, V3 or V5, as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a hinge, a CD8α transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0452]** In another preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) having one of the V1 polypeptide structure as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a FcγRIIIα hinge, a CD8α transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0453]** In another preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) having one of the V3 polypeptide structure as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a CD8α hinge, a CD8α transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0454]** In another preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) having one of the V5 polypeptide structure as illustrated in FIG. **2**, said structure comprising an extra cellular ligand binding-domain comprising VH and VL from a monoclonal anti-HSP70 antibody, a IgG1 hinge, a CD8α transmembrane domain, a cytoplasmic domain including a CD3 zeta signaling domain and a 4-1BB co-stimulatory domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0455]** In another embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) comprising at least an extra cellular ligand binding-domain including CDRs from VH and VL domains of monoclonal anti-HSP70 antibody(ies), a

transmembrane domain, and a cytoplasmic signaling domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0456]** In a particular embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR) comprising at least an extra cellular ligand binding-domain including VH and VL domains of monoclonal mHsp70, and preferably mHsp70.1 anti-HSP70 antibody, a transmembrane domain, and a cytoplasmic signaling domain; said CAR being associated with antibodies directed against soluble Hsp70.

**[0457]** In a preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR), said CAR being associated with monoclonal antibodies directed against soluble Hsp70.

**[0458]** In a more preferred embodiment, said therapeutic combination comprises at least a mHSP70 specific chimeric antigen receptor (anti-mHSP70 CAR), said CAR being associated with humanized monoclonal antibodies directed against soluble Hsp70.

**[0459]** According to the invention, said antibodies directed against soluble Hsp70 may be one available in the market, by instance by Enzo Life Sciences (kit ADI-EKS-715) or by Stressgen (kit EKS-700), both displaying a significant sensitivity to soluble Hsp70 in plasma and serum of human origin, or antibodies against soluble human membrane bound Hsp70 may be obtained de novo by making monoclonal antibodies against such protein, for instance by making an hybridoma.

**[0460]** Methods of Engineering Immune Cells Endowed with the CARs According to the Invention

**[0461]** The present invention also aims to produce immune cells endowed with anti HSP70 CAR, which are less or non-alloreactive, which can be used in allogeneic treatments (i.e. with reduced risk of inducing Graft versus host reaction) and/or made resistant to various standard of care treatments).

**[0462]** As further described in this specification, said methods may further comprise the step of genetically modifying said immune cell by using at least one endonuclease.

**[0463]** The term "endonuclease" refers to any wild-type or variant enzyme capable of catalyzing the hydrolysis (cleavage) of bonds between nucleic acids within a DNA or RNA molecule, preferably a DNA molecule. Endonucleases do not cleave the DNA or RNA molecule irrespective of its sequence, but recognize and cleave the DNA or RNA molecule at specific polynucleotide sequences, further referred to as "target sequences" or "target sites". Endonucleases can be classified as rare-cutting endonucleases when having typically a polynucleotide recognition site greater than 12 base pairs (bp) in length, more preferably of 14-55 bp.

**[0464]** Preferably, the methods according to the present invention involve a rare cutting endonuclease. Rare-cutting endonucleases can for example be a homing endonuclease (Paques and Duchateau 2007), a chimeric Zinc-Finger nuclease (ZFN) resulting from the fusion of engineered zinc-finger domains with the catalytic domain of a restriction enzyme such as FokI (Porteus and Carroll 2005), a TALE-nuclease, a Cas9 endonuclease from CRISPR system as described below (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) or a chemical endonuclease (Eisenschmidt, Lanio et al. 2005; Arimondo, Thomas et al. 2006). In chemical endonucleases, a chemical or peptidic cleaver is conjugated either to a polymer of nucleic acids or to another DNA recognizing a specific target sequence, thereby targeting the cleavage activity to a specific sequence. Chemical endonucleases also encompass synthetic nucleases like conjugates of orthophenanthroline, a DNA cleaving molecule, and triplex-forming oligonucleotides (TFOs), known to bind specific DNA sequences (Kalish and Glazer 2005). Rare-cutting endonucleases can be used for inactivating genes at a locus or to integrate transgenes by homologous recombination (HR) i.e. by inducing DNA double-strand breaks (DSBs) at a locus and insertion of exogeneous DNA at this locus by gene repair mechanism (Perrin, Buckle et al. 1993; Rouet, Smih et al. 1994; Choulika, Perrin et al. 1995; Pingoud and Silva 2007).

**[0465]** By "TALE-nuclease" (TALEN) is intended a fusion protein consisting of a nucleic acid-binding domain typically derived from a Transcription Activator Like Effector (TALE) and one nuclease catalytic domain to cleave a nucleic acid target sequence. The catalytic domain is preferably a nuclease domain and more preferably a domain having endonuclease activity, like for instance I-TevI, ColE7, NucA and Fok-1. In a particular embodiment, the TALE domain can be fused to a meganuclease like for instance I-CreI and I-OnuI or functional variant thereof. In a more preferred embodiment, said nuclease is a monomeric TALE-Nuclease. A monomeric TALE-Nuclease is a TALE-Nuclease that does not require dimerization for specific recognition and cleavage, such as the fusions of engineered TAL repeats with the catalytic domain of I-TevI described in WO2012138927. Transcription Activator like Effector (TALE) are proteins from the bacterial species *Xanthomonas* comprise a plurality of repeated sequences, each repeat comprising di-residues in position 12 and 13 (RVD) that are specific to each nucleotide base of the nucleic acid targeted sequence. Binding domains with similar modular base-per-base nucleic acid binding properties (MBBBD) can also be derived from new modular proteins recently discovered by the applicant in a different bacterial species. The new modular proteins have the advantage of displaying more sequence variability than TAL repeats. Preferably, RVDs associated with recognition of the different nucleotides are HD for recognizing C, NG for recognizing T, NI for recognizing A, NN for recognizing G or A, NS for recognizing A, C, G or T, HG for recognizing T, IG for recognizing T, NK for recognizing G, HA for recognizing C, ND for recognizing C, HI for recognizing C, HN for recognizing G, NA for recognizing G, SN for recognizing G or A and YG for recognizing T, TL for recognizing A, VT for recognizing A or G and SW for recognizing A. In another embodiment, critical amino acids 12 and 13 can be mutated towards other amino acid residues in order to modulate their specificity towards nucleotides A, T, C and G and in particular to enhance this specificity. TALE-nuclease have been already described and used to stimulate gene targeting and gene modifications (Boch, Scholze et al. 2009; Moscou and Bogdanove 2009; Christian, Cermak et al. 2010; Li, Huang et al. 2011). Engineered TAL-nucleases are available under

the trade name TALEN™ (Cellectis, 8 rue de la Croix Jarry, 75013 Paris, France) and can be ordered from manufacturers, such as Life Technologies (Carlsbad, Calif., USA).

**[0466]** Preferred TALE-nucleases recognizing and cleaving the target sequence are described in PCT/EP2014/075317. In particular, additional catalytic domain can be further introduced into the cell with said rare-cutting endonuclease to increase mutagenesis in order to enhance their capacity to inactivate targeted genes. More particularly, said additional catalytic domain is a DNA end processing enzyme. Non limiting examples of DNA end-processing enzymes include 5-3' exonucleases, 3-5' exonucleases, 5-3' alkaline exonucleases, 5' flap endonucleases, helicases, hosphatase, hydrolases and template-independent DNA polymerases. Non limiting examples of such catalytic domain comprise of a protein domain or catalytically active derivate of the protein domain selected from the group consisting of hExol (EXO1_HUMAN), Yeast Exol (EXO1_YEAST), *E. coli* Exol, Human TREX2, Mouse TREX1, Human TREX1, Bovine TREX1, Rat TREX1, TdT (terminal deoxynucleotidyl transferase) Human DNA2, Yeast DNA2 (DNA2_YEAST). In a preferred embodiment, said additional catalytic domain has a 3'-5'-exonuclease activity, and in a more preferred embodiment, said additional catalytic domain is TREX, more preferably TREX2 catalytic domain (WO2012/058458). In another preferred embodiment, said catalytic domain is encoded by a single chain TREX2 polypeptide. Said additional catalytic domain may be fused to a nuclease fusion protein or chimeric protein according to the invention optionally by a peptide linker.

**[0467]** By "Cas9 endonuclease". is meant any genome engineering tool developed based on the RNA-guided Cas9 nuclease (Gasiunas, Barrangou et al. 2012; Jinek, Chylinski et al. 2012; Cong, Ran et al. 2013; Mali, Yang et al. 2013) from the type II prokaryotic CRISPR (Clustered Regularly Interspaced Short palindromic Repeats) adaptive immune system (see for review (Sorek, Lawrence et al. 2013)). The CRISPR Associated (Cas) system was first discovered in bacteria and functions as a defense against foreign DNA, either viral or plasmid. CRISPR-mediated genome engineering first proceeds by the selection of target sequence often flanked by a short sequence motif, referred as the protospacer adjacent motif (PAM). Following target sequence selection, a specific crRNA, complementary to this target sequence is engineered. Trans-activating crRNA (tracrRNA) required in the CRISPR type II systems paired to the crRNA and bound to the provided Cas9 protein. Cas9 acts as a molecular anchor facilitating the base pairing of tracRNA with cRNA (Deltcheva, Chylinski et al. 2011). In this ternary complex, the dual tracrRNA:crRNA structure acts as guide RNA that directs the endonuclease Cas9 to the cognate target sequence. Target recognition by the Cas9-tracrRNA:crRNA complex is initiated by scanning the target sequence for homology between the target sequence and the crRNA. In addition to the target sequence-crRNA complementarity, DNA targeting requires the presence of a short motif adjacent to the protospacer (protospacer adjacent motif—PAM). Following pairing between the dual-RNA and the target sequence, Cas9 subsequently introduces a blunt double strand break 3 bases upstream of the PAM motif (Garneau, Dupuis et al. 2010). The use of Cas9 in immune cells, especially in T-Cells, has been previously described in WO2014191128.

**[0468]** Modifying T-Cell by Inactivating at Least One Gene Encoding a T-Cell Receptor (TCR) Component

**[0469]** According to one aspect, T-cell endowed with anti-HSP70 CAR of the present invention can be made less alloreactive, for instance, by inactivating at least one gene expressing one or more component of T-cell receptor (TCR) as described in WO 2013/176915. This inactivation can be combined with that of another gene, such as of a gene encoding or regulating HLA or 132m protein expression. Accordingly, the risk of graft versus host syndrome and graft rejection is significantly reduced.

**[0470]** Methods of making cells less allogenic can comprise the step of inactivating at least one gene encoding a T-Cell Receptor (TCR) component, in particular TCRalpha and/or TCRbeta genes.

**[0471]** Methods disclosed in WO2013/176915 to prepare CAR expressing immune cell suitable for allogeneic transplantation, by inactivating one or more component of T-cell receptor (TCR), are all incorporated herein by reference.

**[0472]** The present invention encompasses an anti-HSP70 CAR expressing immune cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) has been inactivated. Thus, the present invention provides an anti-HSP70 CAR expressing T cell wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated.

**[0473]** By inactivating a TCR gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiments, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. The nucleic acid strand breaks caused by the rare-cutting endonuclease are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). However, NHEJ is an imperfect repair process that often results in changes to the DNA sequence at the site of the cleavage. Mechanisms involve rejoining of what remains of the two DNA ends through direct re-ligation (Critchlow and Jackson 1998) or via the so-called microhomology-mediated end joining (Betts, Brenchley et al. 2003; Ma, Kim et al. 2003). Repair via non-homologous end joining (NHEJ) often results in small insertions or deletions and can be used for the creation of specific gene knockouts. Said modification may be a substitution, deletion, or addition of at least one nucleotide. Cells in which a cleavage-induced mutagenesis event, i.e. a mutagenesis event consecutive to an NHEJ event, has occurred can be identified and/or selected by well-known method in the art. In a particular embodiment, the step of inactivating at least a gene encoding a component of the T-cell receptor (TCR) into the cells of each individual sample comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one gene encoding a component of the T-cell receptor (TCR). In a more particular embodiment, said cells of each individual sample are transformed with nucleic acid encoding a rare-cutting endonuclease capable of disrupting at least one gene encoding a component of the T-cell receptor (TCR), and said rare-cutting endonuclease is expressed into said cells.—

**[0474]** In a preferred embodiment said method of further engineer the immune cells involves introducing into said T cells polynucleotides, in particular mRNAs, encoding specific rare-cutting endonuclease to selectively inactivate the genes mentioned above by DNA cleavage. In a more preferred embodiment said rare-cutting endonucleases are TALE-nucleases or Cas9 endonuclease. TAL-nucleases have so far proven higher specificity and cleavage efficiency over the other types of rare-cutting endonucleases, making them the endonucleases of choice for producing of the engineered immune cells on a large scale with a constant turn-over.

**[0475]** According to the invention, anti-HSP70 CAR immune cells with one or more component of T-cell receptor (TCR) inactivated are intended to be used as a medicament.

**[0476]** Drug Resistant T-Cells

**[0477]** According to another aspect, anti-HSP70 CAR expressing immune cells of the invention can be further genetically engineered to make them resistant to immunosuppressive drugs or chemotherapy treatments, which are used as standard care for treating cancer associated with HSP70 positive malignant cell, especially AML.

**[0478]** Several cytotoxic agents (anti-cancer drug)s) such as anti-metabolites, alkylating agents, anthracyclines, DNA methyltransferase inhibitors, platinum compounds and spindle poisons have been developed to kill cancer cells. However, the introduction of these agents with novel therapies, such as immunotherapies, is problematic. For example, chemotherapy agents can be detrimental to the establishment of robust anti-tumor immunocompetent cells due to the agents' non-specific toxicity profiles. Small molecule-based therapies targeting cell proliferation pathways may also hamper the establishment of anti-tumor immunity. If chemotherapy regimens that are transiently effective can be combined with novel immunocompetent cell therapies then significant improvement in anti-neoplastic therapy might be achieved (for review (Dasgupta, McCarty et al. 2011).

**[0479]** To improve cancer therapy and selective engraftment of allogeneic immune cells, drug resistance is conferred to said allogeneic cells to protect them from the toxic side-effects of chemotherapy agents. The drug resistance of immune 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.

**[0480]** Methods for engineering immune cells resistant to chemotherapeutic agents are disclosed in PCT/EP2014/075317 which is fully incorporated by reference herein.

**[0481]** In particular, the present invention relates to a method of engineering allogeneic cells suitable for immunotherapy wherein at least one gene encoding a T-cell receptor (TCR) component is inactivated and one gene is modified to confer drug resistance comprising:

**[0482]** Providing an anti-HSP70 CAR expressing T-cell; expressing T cell,

**[0483]** Modifying said anti-HSP70 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

**[0484]** Modifying said anti-HSP70 CAR expressing T-cell, preferably humanized HSP70 CAR, to confer drug resistance to said anti-HSP70 CAR expressing T-cell;

**[0485]** Expanding said engineered anti-HSP70 CAR expressing T-cell in the presence of said drug.

**[0486]** Alternatively, the present invention relates to a method comprising:

**[0487]** Providing an anti-HSP70 CAR expressing T-cell; preferably humanized HSP70 CAR;

**[0488]** Modifying said anti-HSP70 CAR expressing T-cell to confer drug resistance to said anti-HSP70 CAR expressing T-cell;

**[0489]** Modifying said anti-HSP70 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

**[0490]** Expanding said engineered anti-HSP70 CAR expressing T-cell in the presence of said drug.

**[0491]** In particular, the present invention also relates to a method of engineering allogeneic cells suitable for immunotherapy wherein at least one gene encoding a T-cell receptor (TCR) component is inactivated and one gene is modified to confer drug resistance comprising:

**[0492]** Providing an anti-HSP70 CAR expressing T-cell; preferably humanized HSP70 CAR;

**[0493]** Modifying said anti-HSP70 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

**[0494]** Modifying said anti-HSP70 CAR expressing T-cell to confer drug resistance to said anti-HSP70 CAR expressing T-cell;

**[0495]** Expanding said engineered anti-HSP70 CAR expressing T-cell in the presence of said drug.

**[0496]** Alternatively, the present invention relates to a method comprising:

**[0497]** Providing an anti-HSP70 CAR expressing T-cell; preferably humanized HSP70 CAR;

**[0498]** Modifying said anti-HSP70 CAR expressing T-cell to confer drug resistance to said anti-HSP70 CAR expressing T-cell;

**[0499]** Modifying said anti-HSP70 CAR expressing T-cell by inactivating at least one gene encoding a T-cell receptor (TCR) component;

**[0500]** Expanding said engineered anti-HSP70 CAR expressing T-cell in the presence of said drug.

**[0501]** Expression of Drug Resistance Genes in Anti-HSP70 CAR-Expressing Immune Cells

**[0502]** In a particular embodiment, said drug resistance can be conferred to the T-cell by the expression of at least one drug resistance gene. Said drug resistance gene refers to a nucleic acid sequence that encodes "resistance" to an agent, such as a chemotherapeutic agent (e.g. methotrexate). In other words, the expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent to a greater extent than the proliferation of a corresponding cell without the drug resistance gene. The expression of the drug resistance gene in a cell permits proliferation of the cells in the presence of the agent and does not affect its activity. A drug resistance gene of the invention can encode resistance to anti-metabolite, methotrexate, vinblastine, cisplatin, alkylating agents, anthracyclines, cytotoxic antibiotics, anti-immunophilins, their analogs or derivatives, and the like.

**[0503]** In one embodiment, a drug resistance gene of the invention can confer resistance to a drug (or an agent), in particular an anti-cancer drug selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine,

chlorambucil, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

**[0504]** Several 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, Lehmberg et al. 2005; Kushman, Kabler et al. 2007).

**[0505]** One example of drug resistance gene can also be a mutant or modified form of Dihydrofolate reductase (DHFR). 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 WO94/24277; US patent U.S. Pat. No. 6,642,043). In a particular embodiment, said DHFR mutant form comprises two mutated amino acids at position L22 and F31. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type DHFR polypeptide set forth in GenBank: AAH71996.1. In a particular embodiment, the serine residue at position 15 is preferably replaced with a tryptophan residue.

**[0506]** In another particular embodiment, the leucine residue at position 22 is preferably replaced with an amino acid which will disrupt binding of the mutant DHFR to antifolates, preferably with uncharged amino acid residues such as phenylalanine or tyrosine. In another particular embodiment, the phenylalanine residue at positions 31 or 34 is preferably replaced with a small hydrophilic amino acid such as alanine, serine or glycine.

**[0507]** 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 (C63717); 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).

**[0508]** Another example of drug resistance gene can also be a mutant or modified form of ionisine-5'-monophosphate dehydrogenase II (IMPDH2), a rate-limiting enzyme in the de novo synthesis of guanosine nucleotides. The mutant or modified form of IMPDH2 is an IMPDH inhibitor resistance gene. IMPDH inhibitors can be mycophenolic acid (MPA) or its prodrug mycophenolate mofetil (MMF). The mutant IMPDH2 can comprises at least one, preferably two mutations in the MAP binding site of the wild type human IMPDH2 (NP_000875.2) that lead to a significantly increased resistance to IMPDH inhibitor. The mutations are preferably at positions T333 and/or S351 (Yam, Jensen et al. 2006; Sangiolo, Lesnikova et al. 2007; Jonnalagadda, Brown et al. 2013). In a particular embodiment, the threonine residue at position 333 is replaced with an isoleucine residue and the serine residue at position 351 is replaced with a tyrosine residue. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human IMPDH2 polypeptide set forth in NP_000875.2.

**[0509]** Another drug resistance gene is the mutant form of calcineurin. Calcineurin (PP2B), an ubiquitously expressed serine/threonine protein phosphatase that is involved in many biological processes and which is central to T-cell activation. Calcineurin is a heterodimer composed of a catalytic subunit (CnA; three isoforms) and a regulatory subunit (CnB; two isoforms). After engagement of the T-cell receptor, calcineurin dephosphorylates the transcription factor NFAT, allowing it to translocate to the nucleus and active key target gene such as IL2. FK506 in complex with FKBP12, or cyclosporine A (CsA) in complex with CyPA block NFAT access to calcineurin's active site, preventing its dephosphorylation and thereby inhibiting T-cell activation (Brewin, Mancao et al. 2009). The drug resistance gene of the present invention can be a nucleic acid sequence encoding a mutant form of calcineurin resistant to calcineurin inhibitor such as FK506 and/or CsA. In a particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer a at positions: V314, Y341, M347, T351, W352, L354, K360, preferably double mutations at positions T351 and L354 or V314 and Y341. In a particular embodiment, the valine residue at position 341 can be replaced with a lysine or an arginine residue, the tyrosine residue at position 341 can be replaced with a phenylalanine residue; the methionine at position 347 can be replaced with the glutamic acid, arginine or tryptophane residue; the threonine at position 351 can be replaced with the glutamic acid residue; the tryptophane residue at position 352 can be replaced with a cysteine, glutamic acid or alanine residue, the serine at position 353 can be replaced with the histidine or asparagines residue, the leucine at position 354 can be replaced with an alanine residue; the lysine at position 360 can be replaced with an alanine or phenylalanine residue of a sequence corresponding to GenBank: ACX34092.1. Correspondence of amino acid positions described herein is frequently expressed in terms of the positions of the amino acids of the form of wild-type human calcineurin heterodimer a polypeptide set forth in (GenBank: ACX34092.1).

**[0510]** In another particular embodiment, said mutant form can comprise at least one mutated amino acid of the wild type calcineurin heterodimer b at positions: V120, N123, L124 or K125, preferably double mutations at positions L124 and K125. In a particular embodiment, the valine at position 120 can be replaced with a serine, an aspartic acid, phenylalanine or leucine residue; the asparagine at position 123 can be replaced with a tryptophan, lysine, phenylalanine, arginine, histidine or serine; the leucine at position 124 can be replaced with a threonine residue; the lysine at position 125 can be replaced with an alanine, a glutamic acid, tryptophan, or two residues such as leucine-arginine or isoleucine-glutamic acid can be added after the lysine at position 125 in the amino acid sequence coorresponding to GenBank: ACX34095.1. Correspondence of

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

**[0511]** Another drug resistance gene is 0(6)-methylguanine methyltransferase (MGMT) encoding human alkyl guanine transferase (hAGT). AGT is a DNA repair protein that confers resistance to the cytotoxic effects of alkylating agents, such as nitrosoureas and temozolomide (TMZ). 6-benzylguanine (6-BG) is an inhibitor of AGT that potentiates nitrosourea toxicity and is co-administered with TMZ to potentiate the cytotoxic effects of this agent. Several mutant forms of MGMT that encode variants of AGT are highly resistant to inactivation by 6-BG, but retain their ability to repair DNA damage (Maze, Kurpad et al. 1999). In a particular embodiment, AGT mutant form can comprise a mutated amino acid of the wild type AGT position P140, in the amino acid sequence according to the UniProt database under the reference P16455). In a preferred embodiment, said proline at position 140 is replaced with a lysine residue.

**[0512]** 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.

**[0513]** Overexpressing multidrug resistance protein 1 has been described to confer resistance to drugs such as Mitoxantrone (Charles S. Morrow, Christina Peklak-Scott, Bimjhana Bishwokarma, Timothy E. Kute, Pamela K. Smitherman, and Alan J. Townsend. Multidrug Resistance Protein 1 (MRP1, ABCC1) Mediates Resistance to Mitoxantrone via Glutathione-Dependent Drug Efflux *Mol Pharmacol* April 2006 69:1499-1505).

**[0514]** Thus, drug resistance can be conferred to cells by the expression of nucleic acid sequence that encodes MDR-1 (NP_000918).

**[0515]** Still another way of preparing drug resistant cells is to prepare cells with specific mutation (s) such as mutations at Arg486 and Glu571 in the Human Topoisomerase II gene, to confer resistance to amsacrine (S. PATEL, B. A. KELLER, and L. M. FISHER. 2000. MOLECULAR PHARMACOLOGY. Vol 57: p 784-791 (2000).

**[0516]** Still another way of preparing drug resistant cells is to prepare cells overexpressing microRNA-21 to confer resistance to Daunorubicine (Involvement of miR-21 in resistance to daunorubicin by regulating PTEN expression in the leukaemia K562 cell line Bai, Haitao et al. FEBS Letters, Volume 585, Issue 2, 402-408).

**[0517]** In a preferred embodiment, cells bearing such a drug resistance conferring mRNA or protein also comprise an inhibitory mRNA or a gene the expression of which is conditioned, allowing the selective destruction of said drug resistant cells in the presence of said drug or upon administration of said drug.

**[0518]** Drug resistance gene can also confer resistance to cytotoxic antibiotics, and can be ble gene or mcrA gene. Ectopic expression of ble gene or mcrA in an immune cell gives a selective advantage when exposed to the chemotherapeutic agent, respectively the bleomycine or the mitomycin C.

**[0519]** The most practical approach to gene therapy is the addition of a gene to engineer T-cell by using efficient gene delivery with vectors, preferably viral vector. Thus, in a particular embodiment, said drug resistance gene can be expressed in the cell by introducing a transgene preferably encoded by at least one vector into a cell.

**[0520]** In one embodiment, cells bearing a drug resistance gene or a modified gene conferring resistance to a drug also comprise an inducible suicide gene—the induction of which provokes cell death-allowing their selective destruction.

**[0521]** The random insertion of genes into the genome may lead to the inappropriate expression of the inserted gene or the gene near the insertion site. Specific gene therapy using homologous recombination of exogenous nucleic acid comprising endogenous sequences to target genes to specific sites within the genome can allow engineering secure T-cells. As described above, the genetic modification step of the method can comprise a step of introduction into cells of an exogeneous nucleic acid comprising at least a sequence encoding the drug resistance gene and a portion of an endogenous gene such that homologous recombination occurs between the endogenous gene and the exogeneous nucleic acid. In a particular embodiment, said endogenous gene can be the wild type "drug resistance" gene, such that after homologous recombination, the wild type gene is replaced by the mutant form of the gene which confers resistance to the drug.

**[0522]** Endonucleolytic breaks are known to stimulate the rate of homologous recombination. Thus, in a particular embodiment, the method of the invention further comprises the step of expressing in the cell a rare-cutting endonuclease which is able to cleave a target sequence within an endogenous gene. Said endogenous gene can encode for examples DHFR, IMPDH2, calcineurin or AGT. Said rare-cutting endonuclease can be a TALE-nuclease, a Zinc finger nuclease, a CRISPR/Cas9 endonuclease, a MBBBD-nuclease or a meganuclease.

**[0523]** Inactivation of Drug Sensitizing Genes in Anti-HSP70 CAR-Expressing Immune Cells

**[0524]** In another particular embodiment, said drug resistance can be conferred to the cell of the invention (anti-HSP70 CAR expressing immune cell,) by the inactivation of a drug sensitizing gene.

**[0525]** The inventor sought to inactivate potential drug sensitizing gene to engineer T-cell for immunotherapy, in particular to engineer anti-HSP70 CAR expressing immune cell that can be used in combination with a therapeutic agent (anti-cancer drug).

**[0526]** By inactivating a gene it is intended that the gene of interest is not expressed in a functional protein form. In particular embodiment, the genetic modification of the method relies on the expression, in provided cells to engineer, of one rare-cutting endonuclease such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. In a particular embodiment, the step of inactivating at least one drug sensitizing gene comprises introducing into the cell a rare-cutting endonuclease able to disrupt at least one drug sensitizing gene. In a more particular embodiment, said cells are transformed with nucleic acid encoding a rare-cutting endonuclease capable of disrupting a drug sensitizing gene, and said rare-cutting endonuclease is expressed into said cells. Said rare-cutting endonuclease can be a meganuclease, a Zinc finger nuclease, CRISPR/Cas9 nuclease, A MBBBD-nuclease or a TALE-nuclease. In a preferred embodiment, said rare-cutting endonuclease is a TALE-nuclease.

**[0527]** In a preferred embodiment, drug sensitizing gene which can be inactivated to confer drug resistance to the T-cell is the human deoxycytidine kinase (dCK) gene. This enzyme is required for the phosphorylation of the deoxyribonucleosides deoxycytidine (dC), deoxyguanosine (dG) and deoxyadenosine (dA). Purine nucleotide analogs (PNAs) are metabolized by dCK into mono-, di- and triphosphate PNA. Their triphosphate forms and particularly clofarabine triphosphate compete with ATP for DNA synthesis, acts as proapoptotic agent and are potent inhibitors of ribonucleotide reductase (RNR) which is involved in trinucleotide production.

**[0528]** Preferably, the inactivation of dCK in T cells is mediated by TALE nuclease. To achieve this goal, several pairs of dCK TALE-nuclease have been designed, assembled at the polynucleotide level and validated by sequencing. Examples of TALE-nuclease pairs which can be used according to the invention are depicted in PCT/EP2014/075317.

**[0529]** This dCK inactivation in T cells confers resistance to purine nucleoside analogs (PNAs) such as clofarabine, fludarabine or decitabine (Dacogen).

**[0530]** In another preferred embodiment, the dCK inactivation in T cells is combined with an inactivation of TRAC genes rendering these double knock out (KO) T cells both resistant to drug such as clofarabine and less allogeneic. This double features is particularly useful for a therapeutic goal, allowing "off-the-shelf" allogeneic cells for immunotherapy in conjunction with chemotherapy to treat patients with cancer. This double KO inactivation dCK/TRAC can be performed simultaneously or sequentially. One example of TALE-nuclease dCK/TRAC pairs which gave success in the invention is described in PCT/EP2014/075317, in particular, the target sequences in the 2 loci (dCK and TRAC).

**[0531]** Another example of enzyme which can be inactivated is human hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene (Genbank: M26434.1). In particular HPRT can be inactivated in engineered T-cells to confer resistance to a cytostatic metabolite, the 6-thioguanine (6TG) which is converted by HPRT to cytotoxic thioguanine nucleotide and which is currently used to treat patients with cancer, in particular leukemias (Hacke, Treger et al. 2013). Guanines analogs are metabolized by HPRT transferase that catalyzes addition of phosphoribosyl moiety and enables the formation of TGMP Guanine analogues including 6 mercapthopurine (6MP) and 6 thioguanine (6TG) are usually used as lymphodepleting drugs to treat leukemias. They are metabolized by HPRT (hypoxanthine phosphoribosyl transferase that catalyzes addition of phosphoribosyl moiety and enables formation TGMP. Their subsequent phosphorylations lead to the formation of their triphosphorylated forms that are eventually integrated into DNA. Once incorporated into DNA, thio GTP impairs fidelity of DNA replication via its thiolate groupment and generate random point mutation that are highly deleterious for cell integrity.

**[0532]** Thus, the present invention provides an anti-HSP70 CAR expressing cell, in particular an anti-HSP70 CAR expressing T cell wherein the CAR has a polypeptide sequence according to SEQ ID NO. 21 to 32, preferably the CAR in which the scFv are humanized, and wherein the dCK gene is inactivated.

**[0533]** In another embodiment, the inactivation of the CD3 normally expressed at the surface of the T-cell can confer resistance to anti-CD3 antibodies such as teplizumab.

**[0534]** Multiple Drug Resistance of Anti-HSP70 CAR-Expressing Immune Cells

**[0535]** In another particular embodiment, the inventors sought to develop an "off-the shelf" immunotherapy strategy, using allogeneic T-cells, in particular allogenic anti-HSP70 CAR expressing T-cell resistant to multiple drugs to mediate selection of engineered T-cells when the patient is treated with different drugs. The therapeutic efficiency can be significantly enhanced by genetically engineering multiple drug resistance allogeneic T-cells. Such a strategy can be particularly effective in treating tumors that respond to drug combinations that exhibit synergistic effects. Moreover multiple resistant engineered T-cells can expand and be selected using minimal dose of drug agents.

**[0536]** Thus, the method according to the present invention can comprise modifying T-cell to confer multiple drug resistance to said T-cell. Said multiple drug resistance can be conferred by either expressing more than one drug resistance gene or by inactivating more than one drug sensitizing gene. In another particular embodiment, the multiple drug resistance can be conferred to said T-cell by expressing at least one drug resistance gene and inactivating at least one drug sensitizing gene. In particular, the multiple drug resistance can be conferred to said T-cell by expressing at least one drug resistance gene such as mutant form of DHFR, mutant form of IMPDH2, mutant form of calcineurin, mutant form of MGMT, the ble gene, and the mcrA gene and inactivating at least one drug sensitizing gene such as HPRT gene. In a preferred embodiment, multiple drug resistance can be conferred by inactivating HPRT gene and expressing a mutant form of DHFR; or by inactivating HPRT gene and expressing a mutant form of IMPDH2; or by inactivating HPRT gene and expressing a mutant form of calcineurin; by inactivating HPRT gene and expressing a mutant form of MGMT; by inactivating HPRT gene and expressing the ble gene; by inactivating HPRT gene and expressing the mcrA gene.

**[0537]** In one embodiment, the present invention provides allogenic anti-HSP70 CAR expressing T-cell expressing more than one drug resistance gene or wherein more than one drug sensitizing gene is inactivated.

**[0538]** Suicide Genes in Anti-HSP70 CAR-Expressing Immune Cells

**[0539]** In some instances, since engineered T-cells can expand and persist for years after administration, it can be desirable to include a safety mechanism to allow selective deletion of administrated T-cells. Thus, in some embodiments, the method of the invention can comprises the transformation of said T-cells with a recombinant suicide gene. Said recombinant suicide gene is used to reduce the risk of direct toxicity and/or uncontrolled proliferation of said T-cells once administrated in a subject (Quintarelli C, Vera F, blood 2007; Tey S K, Dotti G., Rooney C M, boil blood marrow transplant 2007). Suicide genes enable selective deletion of transformed cells in vivo. In particular, the suicide gene has the ability to convert a non-toxic pro-drug into cytotoxic drug or to express the toxic gene expression product. In other words, "Suicide gene" is a nucleic acid coding for a product, wherein the product causes cell death by itself or in the presence of other compounds.

**[0540]** A representative example of such a suicide gene is one which codes for thymidine kinase of herpes simplex virus. Additional examples are thymidine kinase of varicella zoster virus and the bacterial gene cytosine deaminase which

can convert 5-fluorocytosine to the highly toxic compound 5-fluorouracil. Suicide genes also include as non limiting examples caspase-9 or caspase-8 or cytosine deaminase. Caspase-9 can be activated using a specific chemical inducer of dimerization (CID). Suicide genes can also be polypeptides that are expressed at the surface of the cell and can make the cells sensitive to therapeutic monoclonal antibodies. As used herein "prodrug" means any compound useful in the methods of the present invention that can be converted to a toxic product. The prodrug is converted to a toxic product by the gene product of the suicide gene in the method of the present invention. A representative example of such a prodrug is ganciclovir which is converted in vivo to a toxic compound by HSV-thymidine kinase. The ganciclovir derivative subsequently is toxic to tumor cells. Other representative examples of prodrugs include acyclovir, FIAU [1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil], 6-methoxypurine arabinoside for VZV-TK, and 5-fluorocytosine for cytosine deaminase.

**[0541]** One preferred suicide gene system employs a recombinant antigenic polypeptide comprising antigenic motif recognized by the anti-CD20 mAb Rituximab, especially QBen10, such as in the so-called RQR8 polypeptide described in WO2013153391, which is expressed independently from the anti-HSP70 CAR. Rituximab, an authorized antibody drug, can then be used for cell depletion when needed.

**[0542]** In one embodiment, the present invention provides allogenic anti-HSP70 CAR expressing T-cell expressing more than one drug resistance gene or wherein more than one drug sensitizing gene is inactivated, and a suicide gene allowing said cells to be destroyed.

**[0543]** In particular, the present invention relates to an allogeneic T-cell, in particular an allogeneic anti-HSP70 CAR expressing T-cell, and preferably an allogeneic anti-HSP70 CAR expressing T-cell comprising a peptide having 80% to 100% identity with scfv from cmHsp70.1 antibodies preferably humanized, said allogeneic anti-HSP70 CAR expressing T-cell comprising a peptide having 80% to 100% identity with scfv cmHps70.1 antibodies, preferably humanized is more particularly resistant to a drug, and specifically suitable for immunotherapy.

**[0544]** The resistance of a drug can be conferred by inactivation of drug sensitizing genes or by expression of drug resistance genes. Some examples of drugs which suit to the invention are the purine nucleoside analogues (PNAs) such as clofarabine or fludarabine, or other drugs such as 6-Mercaptopurine (6MP) and 6 thio-guanine (6TG).

**[0545]** In one aspect, the present invention provides methods for engineering immune cells to make them resistant to purine nucleotide analogs (PNA), such a clorofarabine or fludarabine, so that they can be used in cancer immunotherapy treatments in patients pre-treated with these conventional chemotherapies.

**[0546]** The resistance to drugs can be conferred to the T-cells by inactivating one or more gene(s) responsible for the cell's sensitivity to the drug (drug sensitizing gene(s)), such as the dcK and/or HPRT genes.

**[0547]** According to another aspect, the resistance to drugs can be conferred to a T-cell by expressing a drug resistance gene. 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 according to the invention.

**[0548]** For instance, CD52 and glucocorticoid receptors (GR), which are drug targets of Campath® (alemtuzumab) or rituximab and glucocorticoids treatments, can be inactivated to make the cells resistant to these treatments and give them a competitive advantage over patient's own T-cells not endowed with specific anti-HSP70 CARs. Expression of CD3 gene can also be suppressed or reduced to confer resistance to Teplizumab, which is another immune suppressive drug. Expression of HPRT can also be suppressed or reduced according to the invention to confer resistance to 6-thioguanine, a cytostatic agent commonly used in chemotherapy especially for the treatment of acute lymphoblasic leukemia.

**[0549]** Immune Checkpoints Engineered Cells

**[0550]** According to further aspect of the invention, the immune cells can be further manipulated to make them more active or limit exhaustion, by inactivating genes encoding proteins that act as "immune checkpoints" that act as regulators of T-cells activation, such as the following gene selected from CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, preferably, said gene is PDCD1 or CTLA-4. Examples of genes, which expression could be reduced or suppressed are also indicated in Table 10.

**[0551]** The present invention also provides allogeneic T-cells expressing an anti-HSP70 CAR, in particular an anti-HSP70, wherein at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated and/or one gene selected from the genes CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9, CD244, TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, CSK, PAG1, SIT1, FOXP3, PRDM1 (orblimp1), BATF, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, is inactivated as referred to in WO2014/184741.

**[0552]** In one embodiment said gene is a gene that acts as a regulator of T-cells activation coding the beta 2 microglobulin protein.

**[0553]** According to a further aspect of the invention, the anti-HSP70 CAR-immune cells of the invention can be further manipulated to make them resistant to a drug, in particular to a drug used during chemotherapy against cancer, in particular a HSP70-expressing cell-mediated cancer such as AML. This can be achieved by introducing a gene conferring resistance to said drug. This same gene may be turned on and off by using a gene inducible inhibition/expression system as previously described (Garcia E L, Mills A A (2002) Getting around lethality with inducible Cre-mediated excision. Semin Cell Dev Biol 13:151-8, Lewandoski M (2001) Conditional control of gene expression in the mouse. Nat Rev Genet 2:743-55; Scharfenberger L, Hennerici T, Kirly G et al. (2014) Transgenic mouse

technology in skin biology: Generation of complete or tissue-specific knockout mice. J Invest Dermatol 134:e16; Schwenk F, Kuhn R, Angrand P O et al. (1998) Temporally and spatially regulated somatic mutagenesis in mice. Nucleic Acids Res 26:1427-32

**[0554]** Thus, anti-HSP70 CAR-expressing, drug resistant immune cell, wherein (i) at least one gene expressing one or more component of T-cell receptor (TCR) is inactivated (ii) at least one gene conferring resistance to a drug is incorporated or a gene conferring sensitivity to said drug is deleted or mutated to be inactivated (iii) optionally another gene selected from the gene disclosed in the following table 9 is inactivated—is an object of the present invention.

**[0555]** The present invention encompasses the isolated anti-HSP70 CAR-immune cells or cell lines obtainable by the method of the invention, more particularly isolated cells comprising any of the proteins, polypeptides, allelic variants, altered or deleted genes or vectors described herein.

**[0556]** The immune cells of the present invention or cell lines can further comprise exogenous recombinant polynucleotides, in particular CARs or suicide genes or they can comprise altered or deleted genes coding for checkpoint proteins or ligands thereof that contribute to their efficiency as a therapeutic product, ideally as an "off the shelf" product. In another aspect, the present invention concerns the method for treating or preventing cancer in the patient by administrating at least once an engineered immune cell obtainable by the above methods.

**[0557]** HSP70+/Luc+ Drug Resistant Daudi Cells for Testing the Cytotoxicity of Drug Resistant Allogenic CAR T Cells

**[0558]** The present invention encompasses also a method for manufacturing target cells which express both a surface receptor specific to the CAR T cells and a resistance gene. These target cells are particularly useful for testing the cytotoxicity of CAR T cells. These cells are readily resistant to clinically relevant dose of clofarabine and harbor luciferase activity. This combination of features enable traking them in vivo in a mice model or destroy them when required.

**[0559]** More particularly, they can be used to assess the cytotoxicity properties drug resistant T cells in mice in the presence of clofarabine or other PNAs. Clofarabine resistant Daudi cells mimick the physiological state of acute myeloma leukemia (AML) patients relapsing form induction therapy, that harbor drug resistant B cell malignancies. Thus, these cells are of great interest to evaluate the reliability and cytotoxicity of drug resistant CAR T cells. Preferably, these target cells are HSP70+ Luciferase+ Daudi cells.

**[0560]** Isolated Cells

**[0561]** The resulting cells are engineered immune cell expressing at the cell surface membrane a HSP70 specific chimeric antigen receptor as previously described, in particular engineered immune cells derived from primary T-lymphocytes, optionally resistant to an anti-cancer drug, and bearing a deletion in a gene coding for an alpha TCR or a beta TCR.

TABLE 10

List of genes encoding immune checkpoint proteins.

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

**[0562]** The present invention discloses an engineered immune cell as above, wherein expression of TCR is suppressed.

**[0563]** The present invention discloses an engineered immune cell as above, wherein expression of at least one MHC protein, preferably β2m or HLA, is reduced or suppressed in said engineered immune cell. β2m stands for beta 2 microglobulin and HLA for human leukocyte antigen. The MHC protein is a MHC protein of Class I or of class II.

**[0564]** The present invention discloses an engineered immune cell as above, wherein said engineered immune cell is mutated to confer resistance to at least one immune suppressive drug, chemotherapy drug, or anti-cancer drug.

**[0565]** The present invention discloses an engineered immune cell as above for use in therapy.

**[0566]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the patient is a human.

**[0567]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the condition is a pre-malignant or malignant cancer condition characterized by HSP70-expressing cells.

**[0568]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the condition is a condition which is characterized by an over-abundance of HSP70-expressing cells.

**[0569]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the malignant cancer condition is a hematological cancer condition.

**[0570]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the hematological cancer condition is leukemia or malignant lymphoproliferative disorders.

**[0571]** The present invention discloses an engineered immune cell for use in therapy as above, wherein said leukemia is selected from the group consisting of acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, acute lymphoid leukemia, chronic lymphoid leukemia, and myelodysplastic syndrome.

**[0572]** The present invention discloses an engineered immune cell for use in therapy as above, wherein the leukemia is acute myelogenous leukemia (AML).

**[0573]** The present invention discloses an engineered immune cell for use in therapy as above, wherein said hematologic cancer is a malignant lymphoproliferative disorder.

**[0574]** The present invention discloses an engineered immune cell for use in therapy as above, wherein said malignant lymphoproliferative disorder is lymphoma.

**[0575]** The present invention discloses an engineered immune cell for use in therapy as above, wherein said lymphoma is selected from the group consisting of multiple myeloma, non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).

**[0576]** The present invention discloses a method of impairing a hematologic cancer cell comprising contacting said hematologic cancer cell with an engineered cell, which at least expresses anti-HSP70 CAR such as exposed above, in an amount effective to cause impairment of said cancer cell.

**[0577]** The present invention thus discloses a method of engineering an immune cell comprising:

**[0578]** (a) Providing an immune cell,

**[0579]** (b) Expressing at the surface of said cell at least one HSP70 single-chain specific chimeric antigen receptor such as previously exposed.

**[0580]** The present invention discloses a method of engineering an immune cell as above comprising:

**[0581]** (a) Providing an immune cell,

**[0582]** (b) Introducing into said cell at least one polynucleotide encoding said HSP70 single-chain specific chimeric antigen receptor,

**[0583]** (c) Expressing said polynucleotide into said cell.

**[0584]** The present invention discloses a method of engineering an immune cell as above comprising:

**[0585]** (a) Providing an immune cell,

**[0586]** (b) Introducing into said cell at least one polynucleotide encoding said HSP70 single-chain specific chimeric antigen receptor,

**[0587]** (c) Introducing at least one other chimeric antigen receptor which is not specific for HSP70.

**[0588]** The present invention discloses a method of treating a subject in need thereof comprising:

**[0589]** (a) Providing an immune cell expressing at the surface a HSP70 single-chain specific chimeric antigen receptor such as exposed above.

**[0590]** (b) Administrating said immune cells to said patient.

**[0591]** The present invention discloses a method of treating a subject in need thereof as above, wherein said immune cell is provided from a donor.

**[0592]** The present invention discloses a method of treating a subject in need thereof as above, wherein said immune cell is provided from the patient himself.

**[0593]** Pharmaceutical Composition

**[0594]** The present invention provides a pharmaceutical composition comprising a engineered immune cells of the invention and at least on acceptable carrier.

**[0595]** Therapeutic Applications

**[0596]** In another embodiment, isolated cell obtained by the different methods or cell line derived from said isolated cell as previously described can be used as a medicament.

**[0597]** In another embodiment, said medicament can be used for treating cancer, particularly for the treatment of leukemia in a patient in need thereof.

**[0598]** In another embodiment, said isolated cell according to the invention or cell line derived from said isolated cell can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

**[0599]** In a particular embodiment, an anti-HSP70 CAR expressing T cell is provided as a medicament for the treatment of AML, of an AML subtype, of an AML-related complication, of an AML-related condition.

**[0600]** In another embodiment, said medicament can be used for treating a HSP70-expressing cell-mediated pathological condition or a condition characterized by the direct or indirect activity of a HSP70-expressing cell.

**[0601]** In another aspect, the present invention relies on methods for treating patients in need thereof, said method comprising at least one of the following steps:

**[0602]** (a) providing an immune-cell obtainable by any one of the methods previously described;

**[0603]** (b) Administrating said transformed immune cells to said patient,

**[0604]** On one embodiment, said T cells of the invention can undergo robust in vivo T cell expansion and can persist for an extended amount of time.

**[0605]** 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.

**[0606]** Cells that can be used with the disclosed methods are described in the previous section. Said treatment can be used to treat patients diagnosed wherein a pre-malignant or malignant cancer condition characterized by HSP70-expressing cells, especially by an overabundance of HSP70-expressing cells. Such conditions are found in hematologic cancers, such as leukemia.

**[0607]** In one embodiment, the present invention provides a composition for its use in the treatment of a HSP70 expressing cells-mediated disease, in particular a HSP70 expressing cells—mediated hematologic cancer, said composition comprising said anti-HSP70 CAR expressing T cell of the invention.

**[0608]** Any other HSP70-mediating or HSP70-involving malignant lymphoproliferative disorders disclosed herein may be improved with the anti-HSP70 CAR-expressing cells of the present invention.

**[0609]** In a preferred embodiment, the cancer that may be treated using the anti-HSP70 CAR-expressing cells of the present invention is leukemia, a disease associated to leukemia or a complication thereof.

**[0610]** Leukemias that can be treated using the anti-HSP70 CAR-expressing cells of the present invention can be acute myelogenous leukemia (AML). AML or AML subtypes that may be treated using the anti-HSP70 CAR-expressing cells of the present invention may be in particular, acute myeloblastic leukemia, minimally differentiated acute myeloblastic leukemia, acute myeloblastic leukemia without maturation, acute myeloblastic leukemia with granulocytic maturation, promyelocytic or acute promyelocytic leukemia (APL), acute myelomonocytic leukemia, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia (M5a) or acute monocytic leukemia (M5b), acute erythroid leukemias, including erythroleukemia (M6a) and very rare pure erythroid leukemia (M6b), acute megakaryoblastic leukemia, acute basophilic leukemia, acute panmyelosis with myelofibrosis, whether involving HSP70-positive cells.

**[0611]** Subtypes of AML also include, hairy cell leukemia, philadelphia chromosome-positive acute lymphoblastic leukemia. AML may be classified as AML with specific genetic abnormalities. Classification is based on the ability of karyotype to predict response to induction therapy, relapse risk, survival.

**[0612]** Accordingly, AML that may be treated using the anti-HSP70 CAR-expressing cells of the present invention may be AML with a translocation between chromosomes 8 and 21, AML with a translocation or inversion in chromosome 16, AML with a translocation between chromosomes 9 and 11, APL (M3) with a translocation between chromosomes 15 and 17, AML with a translocation between chromosomes 6 and 9, AML with a translocation or inversion in chromosome 3, AML (megakaryoblastic) with a translocation between chromosomes 1 and 22.

**[0613]** The present invention is particularly useful for the treatment of AML associated with these particular cytogenetic markers.

**[0614]** The present invention also provides an anti-HSP70 CAR expressing T cell for the treatment of patients with specific cytogenetic subsets of AML, such as patients with t(15;17)(q22;q21) identified using all-trans retinoic acid (ATRA)16-19 and for the treatment of patients with t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) identified using repetitive doses of high-dose cytarabine.

**[0615]** Preferably, the present invention provides an anti-HSP70 CAR expressing T cell for the treatment of patients with aberrations, such as −5/del(5q), −7, abnormalities of 3q, or a complex karyotype, who have been shown to have inferior complete remission rates and survival.

**[0616]** The terms "therapeutic agent", "chemotherapeutic agent", or "drug" or "anti-cancer drug" as used herein refers to a medicament, preferably a compound or a derivative thereof that can interact with a cancer cell, thereby reducing the proliferative status of the cell and/or killing the cell. Examples of chemotherapeutic agents or "anti-cancer drug" include, but are not limited to, alkylating agents (e.g., busulfan, carboplatine, chlorambucil, cisplatine, cyclophosphamide, ifosfamide, melphalan, mechlorethamine, oxaliplatine, uramustine, temozolomide, fotemustine), metabolic antagonists (e.g., purine nucleoside antimetabolite such as clofarabine, fludarabine or 2'-deoxyadenosine, methotrexate (MTX), 5-fluorouracil or derivatives thereof, azathioprine, capecitabine, cytarabine, floxuridine, fluorouracile, gemcitabine, methotrexate, pemetrexed), antitumor antibiotics (e.g., mitomycin, adriamycin, bleomycine, daunorubicine, doxorubicine, epirubicine, hydroxyurea, idarubicine, mitomycin C, mitoxantrone), plant-derived antitumor agents (e.g., vincristine, vindesine, taxol, vinblastine, vinorelbine, docetaxel, paclitaxel), topoisomerase inhibitor (irinotecan, topotecan, etoposide).

**[0617]** In a preferred embodiment, a therapeutic agent, a chemotherapy drug as used herein refers to a compound or a derivative thereof that may be used to treat cancer, in particular to treat a hematopoietic cancer cell and more particularly AML, thereby reducing the proliferative status of the cancer cell and/or killing the cancer cell. Examples of chemotherapeutic agents include, but are not limited to aracytine, Cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof.

**[0618]** In other embodiments of the present invention, cells of the invention are administered to a patient in conjunction with a drug (or an agent) selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid, cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

**[0619]** Such agents may further include, but are not limited to, the anti-cancer agents TRIM ETHOTRIXATE™ (TMTX), TEMOZOLOMIDE™, RALTRITREXED™, S-(4-Nitrobenzyl)-6-thioinosine (NBMPR),6-benzyguanidine (6-BG), bis-chloronitrosourea (BCNU) and CAMPTOTHECIN™, or a therapeutic derivative of any thereof.

**[0620]** In a more preferred embodiment an anti-HSP70 CAR expressing T cell, is administered to a patient, in combination with at least one therapeutic agent selected from aracytine, Cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid and combination thereof.

**[0621]** As used herein, a cell which is "resistant or tolerant" to an agent means a cell which has been genetically modified so that the cell proliferates in the presence of an amount of an agent that inhibits or prevents proliferation of a cell without the modification.

**[0622]** Treatment of Chronic Myeloid Leukemia (CML)

**[0623]** In another embodiment, the anti-mHsp70.1 CAR of the present invention, alone or in combination with another molecule, is used for the treatment of Chronic myeloid leukemia (CML).

**[0624]** CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is found. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome. In Western countries it accounts for 15-20% of all adult leukemias and 14% of leukemias overall (including the pediatric population.

**[0625]** In one embodiment, the anti-mHsp70.1 CAR of the present invention, alone or in combination with another molecule, is used for the treatment of humans affected by the CML disease who are found to be associated with a chromosomal abnormality that involves a t(9;22)(q34;q11) translocation, resulting in the expression of the BCR/ABL fusion gene (Philadelphia Chromosome or Ph).

**[0626]** In one embodiment, the anti-mHsp70.1 CAR of the present invention, alone or in combination with another molecule, is used for the treatment of humans affected by the CML disease who are found to be associated with either a cryptic translocation that is invisible on G-banded chromosome preparations or a variant translocation involving another chromosome or chromosomes as well as chromosomes 9 and 22.

**[0627]** In still one embodiment, the anti-mHsp70.1 CAR of the present invention, alone or in combination with another molecule, is used for the treatment of humans affected by the CML disease who usually presents, in the so-called chronic phase, a clonal expansion of mature myeloid cells leads to an elevated white blood cell (WBC) count.

**[0628]** Treatment of Solid Tumor

**[0629]** In another embodiment, the anti-HSP70 CAR expressing immune cell of the invention is used for treating solid tumors.

**[0630]** In one particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating colorectal carcinoma.

**[0631]** In another particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating lung carcinoma.

**[0632]** In another particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating neuronal carcinoma.

**[0633]** In another particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating pancreatic carcinoma.

**[0634]** In another particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating liver metastases.

**[0635]** In another particular embodiment, the anti-HSP70 CAR expressing immune cell is used for treating head-and-neck cancer.

**[0636]** Group of Patients

**[0637]** In a preferred embodiment, the invention provides a treatment for AML in patients over 60 years or in patients of less than 20 years.

**[0638]** In a more preferred embodiment, the present invention provides a pediatric treatment, in particular a pediatric treatment against AML, or AML-related diseases or complications.

**[0639]** In still another preferred embodiment, the present invention is used as a treatment in AML patients with low, poor or unfavorable status that is to say with a predicted survival of less than 5 years survival rate. In this group, patients suffering AML with the following cytogenetic characteristics: −5; 5q; −7; 7q-;11q23; non t(9;11); inv(3); t(3;3); t(6;9); t(9;22) is associated with poor-risk status (Byrd J. C. et al., Dec. 15, 2002; Blood: 100 (13) and is especially contemplated to be treated according to the present invention or with an object of the present invention.

**[0640]** In one embodiment, the anti-HSP70 CAR expressing T cell of present invention may be used as induction therapy, as post remission therapy of AML or as a consolidation therapy in patient with AML.

**[0641]** In one embodiment, the anti-HSP70 CAR expressing T cell of the present invention may be used in case of AML relapse, or in case of refractory or resistant AML, and more preferably, in combination with at least one other anti-cancer drug

**[0642]** In another preferred embodiment, at least one anti-HSP70 CAR expressing cell of the invention is used for preventing cancer cells development occurring in particular after anti-cancer treatment, during bone marrow depletion or before bone marrow transplantation, after bone marrow destruction.

**[0643]** AML Complications

**[0644]** In one particular embodiment the invention provides a medicament that improves the health condition of a patient, in particular a patient undergoing a complication related to AML. More preferably, said engineered anti-HSP70 CAR expressing T cell of the invention is expressing at least one anti-HSP70 CAR of the invention and is used as a medicament for the treatment of a complication related to AML.

**[0645]** A complication or disease related to AML may include a preceding myelodysplasia phase, secondary leukemia, in particular secondary AML, high white blood cell count, and absence of Auer rods. Among others, leukostasis and involvement of the central nervous system (CNS), hyperleukocytosis, residual disease, are also considered as a complication or disease related to AML.

**[0646]** AML Associated Diseases

**[0647]** In one embodiment, the present invention also provides an anti-HSP70 CAR expressing T cell for the treatment of a pathological condition related to AML.

**[0648]** The present invention provides a therapy for AML related myeloid neoplasms, for acute myeloid leukemia and myelodysplastic syndrome, a treatment of relapsed or refractory acute myeloid leukemia, a treatment of relapsed or refractory acute promyelocytic leukemia in adults, a treat-

ment for acute promyeloid leukaemia, a treatment of acute myeloid leukemia in adults over 60 years.

**[0649]** According to another aspect, the present invention provides a composition for the treatment of AML associated diseases, in particular hematologic malignancy related to AML.

**[0650]** Hematologic malignancy related to AML conditions include myelodysplasia syndromes (MDS, formerly known as "preleukemia") which are a diverse collection of hematological conditions united by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to AML.

**[0651]** Other pathological conditions or genetic syndromes associated with the risk of AML can be improved with the adequate use of the present invention, said genetic syndromes include Down syndrome, trisomy, Fanconi anemia, Bloom syndrome, Ataxia-telangiectasia, Diamond-Blackfan anemia, Schwachman-Diamond syndrome, Li-Fraumeni syndrome, Neurofibromatosis type 1, Severe congenital neutropenia (also called Kostmann syndrome).

**[0652]** Compositions

**[0653]** The present invention also provides a composition comprising an engineered T cells according to the invention for its use or a method for treating a disease.

**[0654]** In one aspect, the disease is a hematologic cancer, in particular a stem cell cancer including but is not limited to leukemia (such as acute myelogenous leukemia (AML) or a complication thereof.

**[0655]** The present invention also provides a composition for its use or a method for inhibiting the proliferation or reducing a HSP70-expressing cell population or activity in a patient. An exemplary method includes contacting a population of cells comprising a HSP70-expressing cell with an anti-HSP70 CART cell, and in particular CART, of the invention that binds to the HSP70-expressing cell.

**[0656]** In a more specific aspect, the present invention provides a composition for its use or a method for inhibiting the proliferation or reducing the population of cancer cells expressing HSP70 in a patient, the methods comprising contacting the HSP70-expressing cancer cell population with an anti-HSP70 CART cell, and in particular CART, of the invention that binds to the HSP70-expressing cell, binding of an anti-HSP70 CAR cell, and in particular CART, of the invention to the HSP70-expressing cancer cell resulting in the destruction of the HSP70-expressing cancer cells

**[0657]** In certain aspects, the anti-HSP70 CART cell, and in particular CART, of the invention reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least 25%, at least 30%, at least 40%, at least 50%, at least 65%, at least 75%, at least 85%, at least 95%, or at least 99% (to undetectable level) in a subject with or animal model for myeloid leukemia or another cancer associated with HSP70-expressing cells, relative to a negative control.

**[0658]** The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disorder or condition associated with HSP70-expressing cells (e.g., associated with a hematologic cancer), the methods comprising administering to a subject in need an anti-HSP70 CART cell, and in particular CART, of the invention that binds to the HSP70-expressing cell. In one aspect, the subject is a human. Non-limiting examples of disorders associated with HSP70-expressing cells include inflammatory disorders (such as rheumatoid arthritis) and cancers (such as hematological cancers, in particular AML or AML complications).

**[0659]** The present invention also provides a composition for its use or a method for preventing, treating and/or managing a disease associated with HSP70-expressing cells, the method comprising administering to a subject in need an anti-HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell. In one aspect, the subject is a human. Non-limiting examples of diseases associated with HSP70-expressing cells include in particular Acute Myeloid Leukemia (AML).

**[0660]** The present invention provides a composition for its use or a method for treating or preventing relapse of cancer associated with HSP70-expressing cells, the method comprising administering to a subject in need thereof an anti-HSP70 CART cell, and in particular CART, of the invention that binds to the HSP70-expressing cell. In another aspect, the methods comprise administering to the subject in need thereof an effective amount of an anti HSP70 CART cell, and in particular scCART, of the invention that binds to the HSP70-expressing cell in combination with an effective amount of another therapy.

**[0661]** In one aspect, HSP70 is considered to be a "cancer stem cell" marker in AML. Therefore, an anti-HSP70 CART cell, and in particular scCART, of the invention can prevent relapse of AML, or even treat AML that is mostly HSP70-negative but with a "stem" population of HSP70+ cells (a HSP70-expressing cells).

**[0662]** In one aspect, the invention provides compositions and methods for treating subjects that have undergone treatment for a disease or disorder associated with elevated expression levels of CD 19, and exhibits a disease or disorder associated with elevated levels of HSP70.

**[0663]** The treatment with the engineered immune cells according to the invention may be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

**[0664]** Preferably, the treatment with the engineered immune cells according to the invention may be administered in combination (e.g., before, simultaneously or following) with one or more therapies against cancer selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof.

**[0665]** According to a preferred embodiment of the invention, said treatment can be administrated into patients undergoing an immunosuppressive treatment. Indeed, the present invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T-cells according to the invention within the patient.

**[0666]** The administration of the cells or population of cells according to the present invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplan-

tation. The compositions described herein may be administered to a patient subcutaneously, intradermaly, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

**[0667]** The administration of the cells or population of cells can consist of the administration of $10^4$-$10^9$ cells per kg body weight, preferably $10^5$ to $10^6$ cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administrated in one or more doses. In another embodiment, said effective amount of cells are administrated as a single dose. In another embodiment, said effective amount of cells are administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administrated will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

**[0668]** In another embodiment, said effective amount of cells or composition comprising those cells are administrated parenterally. Said administration can be an intravenous administration. Said administration can be directly done by injection within a tumor.

**[0669]** In certain embodiments of the present invention, cells are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizimab treatment for MS patients or efaliztimab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycoplienolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Henderson, Naya et al. 1991; Liu, Albers et al. 1992; Bierer, Hollander et al. 1993).

**[0670]** In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAM PATH.

**[0671]** 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.

**[0672]** In certain embodiments of the present invention, anti-HSP70 CAR expressing cells are administered to a patient in conjunction (e.g., before, simultaneously or following) with a drug selected from aracytine, cytosine arabinoside, amsacrine, daunorubicine, idarubicine, novantrone, mitoxantrone, vepeside, etoposide (VP16), arsenic trioxyde, transretinoic acid, combination of arsenic trioxyde, transretinoic acid, mechlorethamine, procarbazine, chlorambucil, and combination thereof. In these embodiments anti-HSP70 CAR expressing cells may be resistant to the particular drug or combination of drugs that is (are) administered in conjunction with anti-HSP70 CAR expressing cells.

**[0673]** In other embodiments of the present invention, anti-HSP70 CAR expressing cells are administered to a patient in conjunction with a drug selected from cytarabine, anthracyclines, 6-thioguanine, hydroxyurea, prednisone, and combination thereof.

Other Definitions

**[0674]** Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.—Amino acid residues in a polypeptide sequence are designated herein according to the one-letter code, in which, for example, Q means Gln or Glutamine residue, R means Arg or Arginine residue and D means Asp or Aspartic acid residue.

**[0675]** Amino acid substitution means the replacement of one amino acid residue with another, for instance the replacement of an Arginine residue with a Glutamine residue in a peptide sequence is an amino acid substitution.

**[0676]** Nucleotides are designated as follows: one-letter code is used for designating the base of a nucleoside: A is adenine, T is thymine, C is cytosine, and G is guanine. For the degenerated nucleotides, r represents g or a (purine nucleotides), k represents g or t, s represents g or c, w represents a or t, m represents a or c, y represents t or c (pyrimidine nucleotides), d represents g, a or t, v represents g, a or c, b represents g, t or c, h represents a, t or c, and n represents g, a, t or c.

**[0677]** "As used herein, "nucleic acid" or "polynucleotides" refers to nucleotides and/or polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (e.g., enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more

hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Nucleic acids can be either single stranded or double stranded.

**[0678]** By "delivery vector" or "delivery vectors" is intended any delivery vector which can be used in the present invention to put into cell contact (i.e "contacting") or deliver inside cells or subcellular compartments (i.e "introducing") agents/chemicals and molecules (proteins or nucleic acids) needed in the present invention. It includes, but is not limited to liposomal delivery vectors, viral delivery vectors, drug delivery vectors, chemical carriers, polymeric carriers, lipoplexes, polyplexes, dendrimers, microbubbles (ultrasound contrast agents), nanoparticles, emulsions or other appropriate transfer vectors. These delivery vectors allow delivery of molecules, chemicals, macromolecules (genes, proteins), or other vectors such as plasmids, peptides developed by Diatos. In these cases, delivery vectors are molecule carriers. By "delivery vector" or "delivery vectors" is also intended delivery methods to perform transfection.

**[0679]** The terms "vector" or "vectors" refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A "vector" in the present invention includes, but is not limited to, a viral vector, a plasmid, a RNA vector or a linear or circular DNA or RNA molecule which may consists of a chromosomal, non chromosomal, semi-synthetic or synthetic nucleic acids. Preferred vectors are those capable of autonomous replication (episomal vector) and/or expression of nucleic acids to which they are linked (expression vectors). Large numbers of suitable vectors are known to those of skill in the art and commercially available.

**[0680]** Viral vectors include retrovirus, adenovirus, parvovirus (e. g. adenoassociated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e. g., influenza virus), rhabdovirus (e. g., rabies and vesicular stomatitis virus), paramyxovirus (e. g. measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus, herpesvirus (e. g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomega-lovirus), and poxvirus (e. g., vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, In Fundamental Virology, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

**[0681]** By "lentiviral vector" is meant HIV-Based lentiviral vectors that are very promising for gene delivery because of their relatively large packaging capacity, reduced immunogenicity and their ability to stably transduce with high efficiency a large range of different cell types. Lentiviral vectors are usually generated following transient transfection of three (packaging, envelope and transfer) or more plasmids into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface glycoproteins with receptors on the cell surface. On entry, the viral RNA undergoes reverse transcription, which is mediated by the viral reverse transcriptase complex. The product of reverse transcription is a double-stranded linear viral DNA, which is the substrate for viral integration in the DNA of infected cells. By "integrative lentiviral vectors (or LV)", is meant such vectors as nonlimiting example, that are able to integrate the genome of a target cell. At the opposite by "non-integrative lentiviral vectors (or NILV)" is meant efficient gene delivery vectors that do not integrate the genome of a target cell through the action of the virus integrase.

**[0682]** Delivery vectors and vectors can be associated or combined with any cellular permeabilization techniques such as sonoporation or electroporation or derivatives of these techniques.

**[0683]** By cell or cells is intended any eukaryotic living cells, primary cells and cell lines derived from these organisms for in vitro cultures.

**[0684]** By "primary cell" or "primary cells" are intended cells taken directly from living tissue (i.e. biopsy material) and established for growth in vitro, that have undergone very few population doublings and are therefore more representative of the main functional components and characteristics of tissues from which they are derived from, in comparison to continuous tumorigenic or artificially immortalized cell lines.

**[0685]** As non-limiting examples cell lines can be selected from the group consisting of CHO-K1 cells; HEK293 cells; Caco2 cells; U2-OS cells; NIH 3T3 cells; NSO cells; SP2 cells; CHO-S cells; DG44 cells; K-562 cells, U-937 cells; MRCS cells; IMR90 cells; Jurkat cells; HepG2 cells; HeLa cells; HT-1080 cells; HCT-116 cells; Hu-h7 cells; Huvec cells; Molt 4 cells.

**[0686]** All these cell lines can be modified by the method of the present invention to provide cell line models to produce, express, quantify, detect, study a gene or a protein of interest; these models can also be used to screen biologically active molecules of interest in research and production and various fields such as chemical, biofuels, therapeutics and agronomy as non-limiting examples.

**[0687]** by "mutation" is intended the substitution, deletion, insertion of up to one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty five, thirty, fourty, fifty, or more nucleotides/amino acids in a polynucleotide (cDNA, gene) or a polypeptide sequence. The mutation can affect the coding sequence of a gene or its regulatory sequence. It may also affect the structure of the genomic sequence or the structure/stability of the encoded mRNA.

**[0688]** by "variant(s)", it is intended a repeat variant, a variant, a DNA binding variant, a TALE-nuclease variant, a polypeptide variant obtained by mutation or replacement of at least one residue in the amino acid sequence of the parent molecule.

**[0689]** by "functional variant" is intended a catalytically active mutant of a protein or a protein domain; such mutant may have the same activity compared to its parent protein or protein domain or additional properties, or higher or lower activity.

**[0690]** "identity" refers to sequence identity between two nucleic acid molecules or polypeptides. Identity can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base, then the molecules are identical at that position. A degree of similarity or identity between nucleic acid or amino acid sequences is a function of the number of identical or matching nucleotides at positions shared by the nucleic acid sequences. Various alignment algorithms and/or programs may be used to calculate the identity between two sequences, including FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default setting. For example, polypeptides having at least 70%, 85%, 90%, 95%, 98% or 99% identity to specific polypeptides described herein and preferably exhibiting substantially the same functions, as well as polynucleotide encoding such polypeptides, are contemplated.

**[0691]** "similarity" describes the relationship between the amino acid sequences of two or more polypeptides. BLASTP may also be used to identify an amino acid sequence having at least 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to a reference amino acid sequence using a similarity matrix such as BLOSUM45, BLOSUM62 or BLOSUM80. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity of similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. The polynucleotide sequences of similar polypeptides are deduced using the genetic code and may be obtained by conventional means. For example, a functional variant of pTalpha can have 70%, 75%, 80%, 85%, 87.5%, 90%, 92.5%, 95%, 97.5%, 98%, 99% sequence similarity to the amino acid sequence of SEQ ID NO: 107 disclosed in the application WO2013176916. A polynucleotide encoding such a functional variant would be produced by reverse translating its amino acid sequence using the genetic code.

**[0692]** The term "subject" or "patient" as used herein includes all members of the animal kingdom including non-human primates and humans.

**[0693]** The term "relapsed" refers to a situation where a subject or a mammal, who has had a remission of cancer after therapy has a return of cancer cells.

**[0694]** The term "refractory or resistant" refers to a circumstance where a subject or a mammal, even after intensive treatment, has residual cancer cells in his body.

**[0695]** The term "drug resistance" refers to the condition when a disease does not respond to the treatment of a drug or drugs. Drug resistance can be either intrinsic (or primary resistance), which means the disease has never been responsive to the drug or drugs, or it can be acquired, which means the disease ceases responding to a drug or drugs that the disease had previously responded to (secondary resistance). In certain embodiments, drug resistance is intrinsic. In certain embodiments, the drug resistance is acquired.

**[0696]** The term "hematologic malignancy" or "hematologic cancer" refers to a cancer of the body's blood-bone marrow and/or lymphatic tissue. Examples of hematological malignancies include, in particular, acute myeloid leukemia (AML), AML with trilineage myelodysplasia (AML/TMDS), mixed lineage leukemia (MLL), and other AM-related pathologies.

**[0697]** The term "leukemia" refers to malignant neoplasms of the blood-forming tissues, including, in particular to acute myeloid leukemia or acute myelogenous leukemia (AML).

**[0698]** The above written description of the invention provides a manner and process of making and using it such that any person skilled in this art is enabled to make and use the same, this enablement being provided in particular for the subject matter of the appended claims, which make up a part of the original description.

**[0699]** Where a numerical limit or range is stated herein, the endpoints are included. Also, all values and subranges within a numerical limit or range are specifically included as if explicitly written out.

**[0700]** The above description is presented to enable a person skilled in the art to make and use the invention, and is provided in the context of a particular application and its requirements. Various modifications to the preferred embodiments will be readily apparent to those skilled in the art, and the generic principles defined herein may be applied to other embodiments and applications without departing from the spirit and scope of the invention. Thus, this invention is not intended to be limited to the embodiments shown, but is to be accorded the widest scope consistent with the principles and features disclosed herein.

**[0701]** Having generally described this invention, a further understanding can be obtained by reference to certain specific examples, which are provided herein for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

## Examples

**[0702]** General Methods

**[0703]** Primary T-Cell Cultures

**[0704]** T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium. The PBMC layer was recovered and T cells were purified using a commercially available T-cell enrichment kit. Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20 ng/mL Human IL-2, 5% Human, and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies).

**[0705]** scCAR mRNA Transfection

**[0706]** Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15μg of mRNA encoding the different scCAR constructs. scCAR mRNAs were produced using T7 mRNA polymerase transfections done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4 cm gap cuvettes in a final volume of 200 μl of "Cytoporation buffer T" (BTX Harvard Apparatus). Cells were immediately diluted in X-Vivo™-15 media and incubated at 37° C. with 5% $CO_2$. IL-2 was added 2 h after electroporation at 20 ng/m L.

**[0707]** Degranulation Assay (CD107a Mobilization)

**[0708]** T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing various levels of the HSP70 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 6 hours at 37° C. with 5% $CO_2$. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody at the beginning of the co-culture, together with 1 μg/ml of anti-CD49d, 1 μg/ml of anti-CD28, and 1× Monensin solution. After the 6 h incubation period, cells were stained with a fixable viability dye and fluorochrome-conjugated anti-CD8 and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24 h after mRNA transfection.

**[0709]** IFN Gamma Release Assay

**[0710]** T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing various levels of the HSP70 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 24 hours at 37° C. with 5% $CO_2$. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFN gamma detection in the cell culture supernatants was done by ELISA assay. The IFN gamma release assays were carried by starting the cell co-cultures 24 h after mRNA transfection.

**[0711]** Cytotoxicity Assay

**[0712]** T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing HSP70) and 10,000 control (HSP70neg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet) before co-culturing them with scCAR+ T-cells. The co-cultures were incubated for 4 hours at 37° C. with 5% $CO_2$. After this incubation period, cells were labelled with a fixable viability dye and analyzed by flow cytometry. Viability of each cellular population (target cells or HSP70neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48 h after mRNA transfection.

**[0713]** T-Cell Transduction

**[0714]** Transduction of T-cells with recombinant lentiviral vectors expression the scCAR was carried out three days after T-cell purification/activation. scCAR detection at the surface of T-cells was done using a recombinant protein consisting on the fusion of the extracellular domain of the human HSP70 protein, together with a murine IgG1 Fc fragment. Binding of this protein to the scCAR molecule was detected with a fluorochrome-conjugated secondary antibody targeting the mouse Fc portion of the protein, and analyzed by flow cytometry.

**[0715]** Anti-Tumor Mouse Model

**[0716]** Immunodeficient NOG mice were intravenously (iv) injected with (HSP70 expressing_MOLM13-Luciferase cells as an AML xenograft mouse model. Optionally, mice received an anti-cancer treatment. Mice were then iv injected (either 2 or 7 days after injection of the tumor cell line) with different doses of scCAR+ T-cells to be tested, or with T-cells that were not transduced with the scCAR lentiviral vector. Bioluminescent signals were determined at the day of T-cell injection (DO), at D7, 14, 21, 28 and 40 after T-cell injection in order to follow tumoral progression on the different animals.

**[0717]** Production of Rabbit Polyclonal Anti-mHsp70.1 Antibodies

**[0718]** Standard rabbit immunization protocol for rabbit polyclonal antibody production may be performed as follows. mHsp70.1 antigen preparation, which can be conjugated, occurs before day 0. Injection amounts are given for a conjugated mHsp70.1 peptide antigen. A dose of 0.5 mg antigens is injected at 0.5 mg throughout the procedure. Protocol days are approximate (±2 days).

| Procedure | Protocol day | Description |
|---|---|---|
| Control serum collection | Day 0 | Pre-immune bleed (5 mL per rabbit) |
| Primary injection | Day 1 | Immunize with 0.25 mg antigen in CFA (Freund's Complete Adjuvant), SQ 4 sites |
| 1st booster | Day 14 | Boost with 0.10 mg antigen in incomplete Freund's adjuvant (IFA), 4 subcutaneous (SQ) sites |
| Serum collection | Day 28 | Bleed (~25 mL per rabbit) |
| 2nd booster | Day 42 | Boost with 0.10 mg antigen in IFA, 4 SQ sites |
| Serum collection | Day 56 | Bleed (~25 mL per rabbit) |
| 3rd Booster | Day 56 | Boost with 0.10 mg antigen in IFA, 4 SQ sites |
| Serum collection | Day 70, 72 | Two bleeds (~50 mL total per rabbit) |
| ELISA and shipping | Day 77 | ELISA titration |

**[0719]** Production of Mouse Monoclonal Anti-mHsp70.1 Antibodies

**[0720]** Standard rabbit immunization protocol for rabbit polyclonal antibody production may be performed as follows. Primary and first booster injections are IP as emulsions in Freund's Complete Adjuvant (CFA) or Incomplete Freund's Adjuvant (IFA); alternative adjuvants can be used if requested. Final boosts before fusion are intraperitoneal (IP) and intravenous (IV). Total development time is approximately 4-6 months. Depending on the initial ELISA Titration results, the mice may need additional boosts and bleeds in order to generate required titers for fusions.

| Procedure | Protocol day† | Description |
|---|---|---|
| Control serum collection | Day 0 | Pre-immune bleed (0.2-0.5 mL per mouse) |
| Primary Injection | Day 1 | Immunize with 0.1 mg antigen in CFA, IP |
| Booster injections | Days 14, 28 | Boost with 0.1 mg antigen in IFA, IP |
| Test bleeds | Day 42 | Test-bleed (0.2-0.5 mL per mouse) |
| ELISA titration | Day 43-60 | ELISA titration of pre-immune and test-bleeds; Data delivery and mouse selection |
| Pre-fusion booster | Day 62 | Boost with 0.1 mg antigen in saline, IP |
| Pre-fusion booster | Day 64 | Boost with 0.1 mg antigen in saline, IV |
| fusion | Day 66 | Fuse myeloma cells and spleen cells |
| ELISA and subcloning | Day 80 | Screen clones, then subclone to ensure monoclonal lines |
| ELISA screening | Day 94 | Screen clones, then freeze stocks; Test supernatants for evaluation |
| Expansion | Day 100 | Expansion and freeze-down of chosen parental stocks |

Example 1: Proliferation of TCRalpha Inactivated Cells Expressing a HSP70-scCAR

**[0721]** Heterodimeric TALE-nuclease targeting two 17-bp long sequences (called half targets) separated by an 15-bp spacer within T-cell receptor alpha constant chain region (TRAC) gene were designed and produced. Each half target is recognized by repeats of the half TALE-nucleases listed in Table 10.

TABLE 10

TAL-nucleases targeting TCRalpha gene

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

**[0722]** Each TALE-nuclease construct was subcloned using restriction enzyme digestion in a mammalian expression vector under the control of the T7 promoter. mRNA encoding TALE-nuclease cleaving TRAC genomic sequence were synthesized from plasmid carrying the coding sequence downstream from the T7 promoter.

**[0723]** Purified T cells preactivated during 72 hours with antiCD3/CD28 coated beads were transfected with each of the 2 mRNAs encoding both half TRAC_T01 TALE-nucleases. 48 hours post-transfection, different groups of T cells from the same donor were respectively transduced with a lentiviral vector encoding one of the anti-HSP70 scCAR previously described (SEQ ID NO: 21 to 32). 2 days post-transduction, $CD3_{NEG}$ cells were purified using anti-CD3 magnetic beads and 5 days post-transduction cells were reactivated with soluble anti-CD28 (5 μg/ml).

**[0724]** Cell proliferation was followed for up to 30 days after reactivation by counting cell 2 times per week. Increased proliferation in TCR alpha inactivated cells expressing the HSP70 scCARs, especially when reactivated with anti-CD28, was observed compared to non-transduced cells.

**[0725]** To investigate whether the human T cells expressing the HSP70-scCAR display activated state, the expression of the activation marker CD25 are analyzed by FACS 7 days post transduction. The purified cells transduced with the lentiviral vector encoding HSP70 scCAR assayed for CD25 expression at their surface in order to assess their activation in comparison with the non-transduced cells. Increased CD25 expression is expected both in CD28 reactivation or no reactivation conditions.

Example 2: Construction of HSP70 scCAR Using Various Anti-HSP70 Antibody Fragments

**[0726]** Primary T-Cell Cultures

**[0727]** T cells were purified from Buffy coat samples provided by EFS (Etablissement Français du Sang, Paris, France) using Ficoll gradient density medium (Ficoll Paque PLUS/GE Healthcare Life Sciences). The PBMC layer was recovered and T cells were purified using a commercially available T-cell enrichment kit (Stem Cell Technologies). Purified T cells were activated in X-Vivo™-15 medium (Lonza) supplemented with 20 ng/mL Human IL-2 (Miltenyi Biotech), 5% Human Serum (Sera Laboratories), and Dynabeads Human T activator CD3/CD28 at a bead:cell ratio 1:1 (Life Technologies). After activation cells were grown and maintained in X-Vivo™-15 medium (Lonza) supplemented with 20 ng/mL Human IL-2 (Miltenyi Biotec) and 5% Human Serum (Sera Laboratories)

**[0728]** scCAR mRNA Transfection

**[0729]** Transfections were done at Day 4 or Day 11 after T-cell purification and activation. 5 millions of cells were transfected with 15 μg of mRNA encoding the different scCAR constructs. scCAR mRNAs were produced using the mMESSAGE mMACHINE T7 Kit (Life Technologies) and purified using RNeasy Mini Spin Columns (Qiagen). Transfections were done using Cytopulse technology, by applying two 0.1 mS pulses at 3000V/cm followed by four 0.2 mS pulses at 325V/cm in 0.4 cm gap cuvettes in a final volume of 200 μl of "Cytoporation buffer T" (BTX Harvard Appa-

ratus). Cells were immediately diluted in X-Vivo™-15 media (Lonza) and incubated at 37° C. with 5% $CO_2$. IL-2 (from Miltenyi Biotec was added 2 h after electroporation at 20 ng/m L.

**[0730]** Degranulation Assay (CD107a Mobilization)

**[0731]** T-cells were incubated in 96-well plates (40,000 cells/well), together with an equal amount of cells expressing or not the HSP70 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 6 hours at 37° C. with 5% $CO_2$. CD107a staining was done during cell stimulation, by the addition of a fluorescent anti-CD107a antibody (APC conjugated, from Miltenyi Biotec) at the beginning of the co-culture, together with 1 μg/ml of anti-CD49d (BD Pharmingen), 1 μg/ml of anti-CD28 (Miltenyi Biotec), and 1× Monensin solution (eBioscience). After the 6 h incubation period, cells were stained with a fixable viability dye (eFluor 780, from eBioscience) and fluorochrome-conjugated anti-CD8 (PE conjugated Miltenyi Biotec) and analyzed by flow cytometry. The degranulation activity was determined as the % of CD8+/CD107a+ cells, and by determining the mean fluorescence intensity signal (MFI) for CD107a staining among CD8+ cells. Degranulation assays were carried out 24 h after mRNA transfection.

**[0732]** IFNgamma Release Assay

**[0733]** T-cells were incubated in 96-well plates (40,000 cells/well), together with cell lines expressing or not the HSP70 protein. Co-cultures were maintained in a final volume of 100 μl of X-Vivo™-15 medium (Lonza) for 24 hours at 37° C. with 5% $CO_2$. After this incubation period the plates were centrifuged at 1500 rpm for 5 minutes and the supernatants were recovered in a new plate. IFN gamma detection in the cell culture supernatants was done by ELISA assay (Human IFN-gamma Quantikine ELISA Kit, from R&D Systems). The IFN gamma release assays were carried by starting the cell co-cultures 24 h after mRNA transfection.

**[0734]** Cytotoxicity Assay

**[0735]** T-cells were incubated in 96-well plates (100,000 cells/well), together with 10,000 target cells (expressing HSP70) and 10,000 control (HSP70neg) cells in the same well. Target and control cells were labelled with fluorescent intracellular dyes (CFSE or Cell Trace Violet, from Life Technologies) before co-culturing them with scCAR+ T-cells. The co-cultures were incubated for 4 hours at 37° C. with 5% $CO_2$. After this incubation period, cells were labelled with a fixable viability dye (eFluor 780, from eBioscience) and analyzed by flow cytometry. Viability of each cellular population (target cells or HSP70neg control cells) was determined and the % of specific cell lysis was calculated. Cytotoxicity assays were carried out 48 h after mRNA transfection.

Exemplary anti-HSP70 single chain Chimeric Antigen Receptors

mouse cmHsp70.1-sc CAR-v1

(SEQ ID NO. 1 + SEQ ID NO. 21)

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITLYCKR

GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD

KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQAL

PPR

mouse cmHsp70.1-sc CAR-v2

(SEQ ID NO. 1 + SEQ ID NO. 22)

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRFSVVK

RGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL

DKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA

LPPR

mouse cmHsp70.1-sc CAR-v3

(SEQ ID NO. 1 + SEQ ID NO. 23)

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL

DFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSA

-continued

Exemplary anti-HSP70 single chain Chimeric Antigen Receptors

DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR

RGKGHDGLYQGLSTATKDTYDALHMQALPPR

mouse cmHsp70.1 sc CAR-v4

(SEQ ID NO. 1 + SEQ ID NO. 24)

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGL

DFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRS

ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER

RRGKGHDGLYQGLSTATKDTYDALHMQALPPR

mouse cmHsp70.1 sc CAR-v5

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

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART

PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA

PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK

LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFM

RPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKP

RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

mouse cmHsp70.1-sc CAR-v6

(SEQ ID NO. 1 + SEQ ID NO. 26)

MALPVTALLLPLALLLHAARPEVKLQESGPGLVAPSQSLSFTCTVSGFSLSRNSVHWVRQPPGKGLEWLGMIWGG

GSTDYNSALKSRLNISKDSSKSQVFLKMNSLQTDDTAMYFCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGSG

GGGSQAVVTQESALTTSPGETVTLTCRSSTGAVTTSNYANWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDK

AALTITGAQTEDEAIYFCALWYSNHLVFGGGTKLTVLGEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIART

PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA

PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK

LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPF

MRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG

KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

humanized cmHsp70.1 sc CAR-v1

(SEQ ID NO. 1 + SEQ ID NO. 27)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGGLAVSTISSFFPPGYQIYIWAPLAGTCGVLLLSLVITL

YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY

DVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR

-continued

Exemplary anti-HSP70 single chain Chimeric Antigen Receptors

humanized cmHsp70.1 sc CAR-v2

(SEQ ID NO. 1 + SEQ ID NO. 28)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGGLAVSTISSFFPPGYQIISFFLALTSTALLFLLFFLTLRF

SVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE

YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH

MQALPPR

humanized cmHsp70.1 scCAR-v3

(SEQ ID NO. 1 + SEQ ID NO. 29)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV

HTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVK

FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM

KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

humanized cmHsp70.1 sc CAR-v4

(SEQ ID NO. 1 + SEQ ID NO. 30)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAV

HTRGLDFACDIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV

KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG

MKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

humanized cmHsp70.1 sc CAR-v5

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

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL

MIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN

KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS

FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIF

KQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE

MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

humanized cmHsp70.1 sc CAR-v6

(SEQ ID NO. 1 + SEQ ID NO. 32)

MALPVTALLLPLALLLHAARPEVQLVESGGGLVQPGGSLRLSCAASGFSLSRNSVHWVRQAPGKGLEWLGMIWG

GGSTDYNSALKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARNGGYDVFHYWGQGTTVTVSSGGGGSGGGGS

GGGGSQAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNNRAPWTPARFSGSLL

-continued

| Exemplary anti-HSP70 single chain Chimeric Antigen Receptors |
|---|
| GGKAALTLSGVQPEDEAEYYCALWYSNHLVFGGGTKLTVLGEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTL |
| MIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN |
| KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS |
| FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYI |
| FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP |
| EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR |

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**[0825]** Zielske, S. P., J. S. Reese, et al. (2003). "In vivo selection of MGMT(P140K) lentivirus-transduced human NOD/SCID repopulating cells without pretransplant irradiation conditioning." J Clin Invest 112(10)1561-70.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 61

<210> SEQ ID NO 1
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 1

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly
20

<210> SEQ ID NO 2
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: signal peptide

<400> SEQUENCE: 2

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1 5 10 15

His Ala Ala Arg Pro
20

<210> SEQ ID NO 3
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: FcgRIIIa hinge

<400> SEQUENCE: 3

Gly Leu Ala Val Ser Thr Ile Ser Ser Phe Phe Pro Pro Gly Tyr Gln
1 5 10 15

<210> SEQ ID NO 4
<211> LENGTH: 45
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: CD8alpha hinge

<400> SEQUENCE: 4

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
35 40 45

<210> SEQ ID NO 5
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: IgG1 hinge

<400> SEQUENCE: 5

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```
Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                   10                  15

Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            20                  25                  30

Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val Val Val
        35                  40                  45

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    50                  55                  60

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
65                  70                  75                  80

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                85                  90                  95

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            100                 105                 110

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        115                 120                 125

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
    130                 135                 140

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
145                 150                 155                 160

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                165                 170                 175

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            180                 185                 190

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        195                 200                 205

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    210                 215                 220

Leu Ser Leu Ser Pro Gly Lys
225                 230


<210> SEQ ID NO 6
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: CD8alpha transmembrane domain

<400> SEQUENCE: 6

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

Ser Leu Val Ile Thr Leu Tyr Cys
            20


<210> SEQ ID NO 7
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: 41BB transmembrane domain

<400> SEQUENCE: 7

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

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

-continued

<210> SEQ ID NO 8
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Fragment of 4-1BB (residues 214-255)

<400> SEQUENCE: 8

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

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

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

<210> SEQ ID NO 9
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: fragment of T-cell surface glycoprotein CD3 zeta chain

<400> SEQUENCE: 9

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

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

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

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

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

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

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

<210> SEQ ID NO 10
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: GS linker

<400> SEQUENCE: 10

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

<210> SEQ ID NO 11
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine cmHsp70.1 heavy chain variable region

<400> SEQUENCE: 11

Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln

-continued

```
1               5                   10                  15

Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Arg Asn
            20                  25                  30

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

Gly Met Ile Trp Gly Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys Ser Gln Val Phe Leu
65                  70                  75                  80

Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Met Tyr Phe Cys Ala
                85                  90                  95

Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> SEQ ID NO 12
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized cmHsp70.1 heavy chain variable region

<400> SEQUENCE: 12

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Ser Leu Ser Arg Asn
            20                  25                  30

Ser Val His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
        35                  40                  45

Gly Met Ile Trp Gly Gly Gly Ser Thr Asp Tyr Asn Ser Ala Leu Lys
    50                  55                  60

Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65                  70                  75                  80

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

Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp Gly Gln Gly Thr Thr
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR1 of cmHsp70.1 heavy chain variable region

<400> SEQUENCE: 13

Gly Phe Ser Leu Ser Arg Asn Ser Val His
1               5                   10


<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: CDR2 of cmHsp70.1 heavy chain variable region

<400> SEQUENCE: 14

Trp Leu Gly Met Ile Trp Gly Gly Gly Ser Thr Asp Tyr Asn Ser Ala
1 5 10 15

Leu Lys Ser

<210> SEQ ID NO 15
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR3 of cmHsp70.1 heavy chain variable region

<400> SEQUENCE: 15

Asn Gly Gly Tyr Asp Val Phe His Tyr
1 5

<210> SEQ ID NO 16
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Murine cmHsp70.1 light chain variable region

<400> SEQUENCE: 16

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

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly
35 40 45

Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe
50 55 60

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

Gln Thr Glu Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn
85 90 95

His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 110

<210> SEQ ID NO 17
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Humanized cmHsp70.1 light chain variable region

<400> SEQUENCE: 17

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

Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser
20 25 30

Asn Tyr Ala Asn Trp Val Gln Gln Lys Pro Gly Gln Ala Pro Arg Gly
35 40 45

Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Trp Thr Pro Ala Arg Phe
50 55 60

Ser Gly Ser Leu Leu Gly Gly Lys Ala Ala Leu Thr Leu Ser Gly Val

-continued

```
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Glu Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn
                85                  90                  95

His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
            100                 105                 110


<210> SEQ ID NO 18
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR1 of cmHsp70.1 light chain variable region

<400> SEQUENCE: 18

Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val
1               5                   10                  15


<210> SEQ ID NO 19
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR2 of cmHsp70.1 light chain variable region

<400> SEQUENCE: 19

Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro
1               5                   10


<210> SEQ ID NO 20
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR3 of cmHsp70.1 light chain variable region

<400> SEQUENCE: 20

Ala Leu Trp Tyr Ser Asn His Leu Val
1               5


<210> SEQ ID NO 21
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V1 mouse cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 21

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
```

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```
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Gly Leu Ala Val Ser Thr Ile Ser Ser
            260                 265                 270

Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly
        275                 280                 285

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
    290                 295                 300

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
305                 310                 315                 320

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
                325                 330                 335

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
            340                 345                 350

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
        355                 360                 365

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
    370                 375                 380

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
385                 390                 395                 400

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
                405                 410                 415

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
            420                 425                 430

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

Leu His Met Gln Ala Leu Pro Pro Arg
    450                 455


<210> SEQ ID NO 22
<211> LENGTH: 460
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V2 mouse cmHsp70.1 scCAR polypeptide
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<400> SEQUENCE: 22

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                  10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Gly Leu Ala Val Ser Thr Ile Ser Ser
            260                 265                 270

Phe Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr
        275                 280                 285

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

Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
305                 310                 315                 320

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

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
            340                 345                 350

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
        355                 360                 365

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

Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys
385                 390                 395                 400
```

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```
Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala
                405                 410                 415

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

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

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


<210> SEQ ID NO 23
<211> LENGTH: 486
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V3 mouse cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 23

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285
```

-continued

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

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305                 310                 315                 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
                325                 330                 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
            340                 345                 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
        355                 360                 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
    370                 375                 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385                 390                 395                 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
                405                 410                 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
            420                 425                 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
        435                 440                 445

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
    450                 455                 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465                 470                 475                 480

Gln Ala Leu Pro Pro Arg
                485


<210> SEQ ID NO 24
<211> LENGTH: 489
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V4 mouse cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 24

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140
```

-continued

```
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285

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

Phe Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala
305                 310                 315                 320

Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys
                325                 330                 335

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
            340                 345                 350

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
        355                 360                 365

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
    370                 375                 380

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
385                 390                 395                 400

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
                405                 410                 415

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
            420                 425                 430

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
        435                 440                 445

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
    450                 455                 460

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
465                 470                 475                 480

Leu His Met Gln Ala Leu Pro Pro Arg
                485
```

<210> SEQ ID NO 25
<211> LENGTH: 672
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V5 mouse cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 25

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```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                  10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Glu Pro Lys Ser Pro Asp Lys Thr His
            260                 265                 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
        275                 280                 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
    290                 295                 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305                 310                 315                 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                325                 330                 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
            340                 345                 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
        355                 360                 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
    370                 375                 380

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
385                 390                 395                 400
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Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                405                 410                 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
            420                 425                 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
        435                 440                 445

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    450                 455                 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465                 470                 475                 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr
                485                 490                 495

Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
            500                 505                 510

Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
        515                 520                 525

Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
    530                 535                 540

Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
545                 550                 555                 560

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
                565                 570                 575

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            580                 585                 590

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        595                 600                 605

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    610                 615                 620

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
625                 630                 635                 640

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                645                 650                 655

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            660                 665                 670


<210> SEQ ID NO 26
<211> LENGTH: 675
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V6 mouse cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 26

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Lys Leu Gln Glu Ser Gly Pro Gly Leu
            20                  25                  30

Val Ala Pro Ser Gln Ser Leu Ser Phe Thr Cys Thr Val Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Pro Pro Gly Lys
    50                  55                  60

Gly Leu Glu Trp Leu Gly Met Ile Trp Gly Gly Gly Ser Thr Asp Tyr
65                  70                  75                  80
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Asn Ser Ala Leu Lys Ser Arg Leu Asn Ile Ser Lys Asp Ser Ser Lys
                85                  90                  95

Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala
            100                 105                 110

Met Tyr Phe Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp
    210                 215                 220

Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile
225                 230                 235                 240

Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Glu Pro Lys Ser Pro Asp Lys Thr His
            260                 265                 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
        275                 280                 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
    290                 295                 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305                 310                 315                 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                325                 330                 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
            340                 345                 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
        355                 360                 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
    370                 375                 380

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

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                405                 410                 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
            420                 425                 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
        435                 440                 445

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    450                 455                 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465                 470                 475                 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile
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                485                 490                 495

Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe
            500                 505                 510

Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu
        515                 520                 525

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
    530                 535                 540

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
545                 550                 555                 560

Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
                565                 570                 575

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
            580                 585                 590

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
        595                 600                 605

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
    610                 615                 620

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
625                 630                 635                 640

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
                645                 650                 655

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
            660                 665                 670

Pro Pro Arg
        675


<210> SEQ ID NO 27
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V1 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 27

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
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145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Gly Leu Ala Val Ser Thr Ile Ser Ser
            260                 265                 270

Phe Phe Pro Pro Gly Tyr Gln Ile Tyr Ile Trp Ala Pro Leu Ala Gly
        275                 280                 285

Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys
    290                 295                 300

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
305                 310                 315                 320

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
                325                 330                 335

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
            340                 345                 350

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
        355                 360                 365

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
    370                 375                 380

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
385                 390                 395                 400

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
                405                 410                 415

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
            420                 425                 430

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

Leu His Met Gln Ala Leu Pro Pro Arg
    450                 455


<210> SEQ ID NO 28
<211> LENGTH: 460
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V2 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 28

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
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        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Gly Leu Ala Val Ser Thr Ile Ser Ser
            260                 265                 270

Phe Phe Pro Pro Gly Tyr Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr
        275                 280                 285

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

Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro
305                 310                 315                 320

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

Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe
            340                 345                 350

Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu
        355                 360                 365

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

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

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

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

Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr
        435                 440                 445
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Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
    450                 455                 460


<210> SEQ ID NO 29
<211> LENGTH: 486
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V3 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 29

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285

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

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305                 310                 315                 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
                325                 330                 335
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Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
            340                 345                 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
        355                 360                 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
    370                 375                 380

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385                 390                 395                 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
                405                 410                 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
            420                 425                 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
        435                 440                 445

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
    450                 455                 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465                 470                 475                 480

Gln Ala Leu Pro Pro Arg
                485
```

<210> SEQ ID NO 30
<211> LENGTH: 489
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V4 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 30

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190
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```
Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285

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

Phe Ala Cys Asp Ile Ile Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala
305                 310                 315                 320

Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe Ser Val Val Lys
                325                 330                 335

Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg
            340                 345                 350

Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro
        355                 360                 365

Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser
    370                 375                 380

Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu
385                 390                 395                 400

Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg
                405                 410                 415

Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln
            420                 425                 430

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
        435                 440                 445

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
    450                 455                 460

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
465                 470                 475                 480

Leu His Met Gln Ala Leu Pro Pro Arg
                485


<210> SEQ ID NO 31
<211> LENGTH: 672
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V5 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 31

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
        35                  40                  45
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```
Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Glu Pro Lys Ser Pro Asp Lys Thr His
            260                 265                 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
        275                 280                 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
    290                 295                 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305                 310                 315                 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                325                 330                 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
            340                 345                 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
        355                 360                 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
    370                 375                 380

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

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                405                 410                 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
            420                 425                 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
        435                 440                 445
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```
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    450                 455                 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465                 470                 475                 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Tyr
                485                 490                 495

Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu
            500                 505                 510

Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile
        515                 520                 525

Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp
    530                 535                 540

Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
545                 550                 555                 560

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
                565                 570                 575

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            580                 585                 590

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        595                 600                 605

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    610                 615                 620

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
625                 630                 635                 640

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                645                 650                 655

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
            660                 665                 670


<210> SEQ ID NO 32
<211> LENGTH: 675
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: V6 humanized cmHsp70.1 scCAR polypeptide

<400> SEQUENCE: 32

Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
            20                  25                  30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
        35                  40                  45

Ser Leu Ser Arg Asn Ser Val His Trp Val Arg Gln Ala Pro Gly Lys
    50                  55                  60

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

Asn Ser Ala Leu Lys Ser Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
                85                  90                  95

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala
            100                 105                 110

Val Tyr Tyr Cys Ala Arg Asn Gly Gly Tyr Asp Val Phe His Tyr Trp
        115                 120                 125
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```
Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
    130                 135                 140

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu
145                 150                 155                 160

Pro Ser Leu Thr Val Ser Pro Gly Gly Thr Val Thr Leu Thr Cys Arg
                165                 170                 175

Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln
            180                 185                 190

Gln Lys Pro Gly Gln Ala Pro Arg Gly Leu Ile Gly Gly Thr Asn Asn
        195                 200                 205

Arg Ala Pro Trp Thr Pro Ala Arg Phe Ser Gly Ser Leu Leu Gly Gly
    210                 215                 220

Lys Ala Ala Leu Thr Leu Ser Gly Val Gln Pro Glu Asp Glu Ala Glu
225                 230                 235                 240

Tyr Tyr Cys Ala Leu Trp Tyr Ser Asn His Leu Val Phe Gly Gly Gly
                245                 250                 255

Thr Lys Leu Thr Val Leu Gly Glu Pro Lys Ser Pro Asp Lys Thr His
            260                 265                 270

Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe
        275                 280                 285

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro
    290                 295                 300

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
305                 310                 315                 320

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
                325                 330                 335

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
            340                 345                 350

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
        355                 360                 365

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
    370                 375                 380

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

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
                405                 410                 415

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
            420                 425                 430

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
        435                 440                 445

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
    450                 455                 460

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
465                 470                 475                 480

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ile Ile
                485                 490                 495

Ser Phe Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe
            500                 505                 510

Phe Leu Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu
        515                 520                 525

Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln
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    530                 535                 540

Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly
545                 550                 555                 560

Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr
                565                 570                 575

Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg
            580                 585                 590

Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met
        595                 600                 605

Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
    610                 615                 620

Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
625                 630                 635                 640

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
                645                 650                 655

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
            660                 665                 670

Pro Pro Arg
        675


<210> SEQ ID NO 33
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD20 mimotope

<400> SEQUENCE: 33

Cys Pro Tyr Ser Asn Pro Ser Leu Cys Ser
1               5                   10


<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Epitope for palivizumab

<400> SEQUENCE: 34

Asn Ser Glu Leu Leu Ser Leu Ile Asn Asp Met Pro Ile Thr Asn Asp
1               5                   10                  15

Gln Lys Lys Leu Met Ser Asn Asn
            20


<210> SEQ ID NO 35
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Mimotope 1 for cetuximab

<400> SEQUENCE: 35

Cys Gln Phe Asp Leu Ser Thr Arg Arg Leu Lys Cys
1               5                   10


<210> SEQ ID NO 36
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
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-continued

<220> FEATURE:
<223> OTHER INFORMATION: Mimotope 2 for cetuximab

<400> SEQUENCE: 36

Cys Gln Tyr Asn Leu Ser Ser Arg Ala Leu Lys Cys
1 5 10

<210> SEQ ID NO 37
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Mimotope 3 for cetuximab

<400> SEQUENCE: 37

Cys Val Trp Gln Arg Trp Gln Lys Ser Tyr Val Cys
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Mimotope 4 for cetuximab

<400> SEQUENCE: 38

Cys Met Trp Asp Arg Phe Ser Arg Trp Tyr Lys Cys
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Epitope 1 for nivolumab

<400> SEQUENCE: 39

Ser Phe Val Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp
1 5 10 15

Lys Leu Ala Ala Phe Pro Glu Asp Arg
20 25

<210> SEQ ID NO 40
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Epitope 2 for nivolumab

<400> SEQUENCE: 40

Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu Ala Pro Lys Ala Gln
1 5 10 15

Ile Lys Glu

<210> SEQ ID NO 41
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Epitope 1 for CD34

<400> SEQUENCE: 41

Thr Phe Ser Asn Val Ser Thr Asn Val Ser Tyr Gln Glu Thr

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1 5 10

<210> SEQ ID NO 42
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Epitope 2 for CD34

<400> SEQUENCE: 42

Asn Thr Asn Ser Ser Val Gln Ser Gln Thr Ser Val Ile Ser
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Target TALEN TRAC_T01

<400> SEQUENCE: 43

Thr Thr Gly Thr Cys Cys Cys Ala Cys Ala Gly Ala Thr Ala Thr Cys
1 5 10 15

Cys Ala Gly Ala Ala Cys Cys Cys Thr Gly Ala Cys Cys Cys Thr Gly
20 25 30

Cys Cys Gly Thr Gly Thr Ala Cys Cys Ala Gly Cys Thr Gly Ala Gly
35 40 45

Ala

<210> SEQ ID NO 44
<211> LENGTH: 530
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAL binding domain TRAC_T01-L

<400> SEQUENCE: 44

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Lys
1 5 10 15

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
20 25 30

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly
35 40 45

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
50 55 60

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

Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
85 90 95

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
100 105 110

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
115 120 125

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala
130 135 140

Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg
145 150 155 160

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Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
                165                 170                 175

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
            180                 185                 190

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
        195                 200                 205

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

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

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

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

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

Gln Ala Leu Glu Thr Val Gln Ala Leu Leu Pro Val Leu Cys Gln Ala
    290                 295                 300

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly
305                 310                 315                 320

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

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

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

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

Ser Asn Gly Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
385                 390                 395                 400

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

Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala
            420                 425                 430

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

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

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu
465                 470                 475                 480

Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu
                485                 490                 495

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

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
        515                 520                 525

Leu Glu
    530


<210> SEQ ID NO 45
<211> LENGTH: 530
<212> TYPE: PRT
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<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: TAL binding domain TRAC_T01-R

<400> SEQUENCE: 45

Leu Thr Pro Glu Gln Val Val Ala Ile Ala Ser His Asp Gly Gly Lys
1  5  10  15

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
            20  25  30

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly
        35  40  45

Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys
    50  55  60

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

Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val
                85  90  95

Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val Val Ala Ile Ala
            100  105  110

Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala Leu Leu
        115  120  125

Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln Gln Val Val Ala
    130  135  140

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

Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Glu Gln Val
                165  170  175

Val Ala Ile Ala Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val
            180  185  190

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
        195  200  205

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

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

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys Gln Ala
                245  250  255

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

Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Asn Gly Gly Lys
        275  280  285

Gln Ala Leu Glu Thr Val Gln Arg Leu Leu Pro Val Leu Cys Gln Ala
    290  295  300

His Gly Leu Thr Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly
305  310  315  320

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

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

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

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

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    370                 375                 380

Ser His Asp Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Arg Leu Leu
385                 390                 395                 400

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

Ile Ala Ser Asn Ile Gly Gly Lys Gln Ala Leu Glu Thr Val Gln Ala
            420                 425                 430

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

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

Gln Arg Leu Leu Pro Val Leu Cys Gln Ala His Gly Leu Thr Pro Gln
465                 470                 475                 480

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

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

Pro Gln Gln Val Val Ala Ile Ala Ser Asn Gly Gly Gly Arg Pro Ala
        515                 520                 525

Leu Glu
    530


<210> SEQ ID NO 46
<211> LENGTH: 2814
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide encoding  TRAC_T01-L TALEN

<400> SEQUENCE: 46

atgggcgatc ctaaaaagaa acgtaaggtc atcgattacc catacgatgt tccagattac     60

gctatcgata tcgccgatct acgcacgctc ggctacagcc agcagcaaca ggagaagatc    120

aaaccgaagg ttcgttcgac agtggcgcag caccacgagg cactggtcgg ccacgggttt    180

acacacgcgc acatcgttgc gttaagccaa cacccggcag cgttagggac cgtcgctgtc    240

aagtatcagg acatgatcgc agcgttgcca gaggcgacac acgaagcgat cgttggcgtc    300

ggcaaacagt ggtccggcgc acgcgctctg gaggccttgc tcacggtggc gggagagttg    360

agaggtccac cgttacagtt ggacacaggc caacttctca agattgcaaa acgtggcggc    420

gtgaccgcag tggaggcagt gcatgcatgg cgcaatgcac tgacgggtgc cccgctcaac    480

ttgacccccc agcaggtggt ggccatcgcc agcaatggcg gtggcaagca ggcgctggag    540

acggtccagc ggctgttgcc ggtgctgtgc caggcccacg gcttgacccc ccagcaggtg    600

gtggccatcg ccagcaataa tggtggcaag caggcgctgg agacggtcca gcggctgttg    660

ccggtgctgt gccaggccca cggcttgacc ccccagcagg tggtggccat cgccagcaat    720

ggcggtggca agcaggcgct ggagacggtc cagcggctgt tgccggtgct gtgccaggcc    780

cacggcttga ccccggagca ggtggtggcc atcgccagcc acgatggcgg caagcaggcg    840

ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gaccccggag    900

caggtggtgg ccatcgccag ccacgatggc ggcaagcagg cgctggagac ggtccagcgg    960

ctgttgccgg tgctgtgcca ggcccacggc ttgaccccgg agcaggtggt ggccatcgcc   1020
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agccacgatg gcggcaagca ggcgctggag acggtccagc ggctgttgcc ggtgctgtgc 1080

caggcccacg gcttgacccc ggagcaggtg gtggccatcg ccagcaatat tggtggcaag 1140

caggcgctgg agacggtgca ggcgctgttg ccggtgctgt gccaggccca cggcttgacc 1200

ccggagcagg tggtggccat cgccagccac gatggcggca agcaggcgct ggagacggtc 1260

cagcggctgt tgccggtgct gtgccaggcc cacggcttga ccccggagca ggtggtggcc 1320

atcgccagca atattggtgg caagcaggcg ctggagacgg tgcaggcgct gttgccggtg 1380

ctgtgccagg cccacggctt gacccccag caggtggtgg ccatcgccag caataatggt 1440

ggcaagcagg cgctggagac ggtccagcgg ctgttgccgg tgctgtgcca ggcccacggc 1500

ttgaccccgg agcaggtggt ggccatcgcc agcaatattg gtggcaagca ggcgctggag 1560

acggtgcagg cgctgttgcc ggtgctgtgc caggcccacg gcttgacccc ccagcaggtg 1620

gtggccatcg ccagcaatgg cggtggcaag caggcgctgg agacggtcca gcggctgttg 1680

ccggtgctgt gccaggccca cggcttgacc ccggagcagg tggtggccat cgccagcaat 1740

attggtggca agcaggcgct ggagacggtg caggcgctgt tgccggtgct gtgccaggcc 1800

cacggcttga ccccccagca ggtggtggcc atcgccagca atggcggtgg caagcaggcg 1860

ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gaccccggag 1920

caggtggtgg ccatcgccag ccacgatggc ggcaagcagg cgctggagac ggtccagcgg 1980

ctgttgccgg tgctgtgcca ggcccacggc ttgacccctc agcaggtggt ggccatcgcc 2040

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ccggcgttgg ccgcgttgac caacgaccac ctcgtcgcct tggcctgcct cggcgggcgt 2160

cctgcgctgg atgcagtgaa aaagggattg ggggatccta tcagccgttc ccagctggtg 2220

aagtccgagc tggaggagaa gaaatccgag ttgaggcaca agctgaagta cgtgccccac 2280

gagtacatcg agctgatcga gatcgcccgg aacagcaccc aggaccgtat cctggagatg 2340

aaggtgatgg agttcttcat gaaggtgtac ggctacaggg gcaagcacct gggcggctcc 2400

aggaagcccg acggcgccat ctacaccgtg ggctccccca tcgactacgg cgtgatcgtg 2460

gacaccaagg cctactccgg cggctacaac ctgcccatcg gccaggccga cgaaatgcag 2520

aggtacgtgg aggagaacca gaccaggaac aagcacatca accccaacga gtggtggaag 2580

gtgtaccct ccagcgtgac cgagttcaag ttcctgttcg tgtccggcca cttcaagggc 2640

aactacaagg cccagctgac caggctgaac cacatcacca actgcaacgg cgccgtgctg 2700

tccgtggagg agctcctgat cggcggcgag atgatcaagg ccggcaccct gaccctggag 2760

gaggtgagga ggaagttcaa caacggcgag atcaacttcg cggccgactg ataa 2814

<210> SEQ ID NO 47
<211> LENGTH: 2832
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: polynucleotide encoding TRAC_T01-R TALEN

<400> SEQUENCE: 47

atgggcgatc ctaaaaagaa acgtaaggtc atcgataagg agaccgccgc tgccaagttc 60

gagagacagc acatggacag catcgatatc gccgatctac gcacgctcgg ctacagccag 120

cagcaacagg agaagatcaa accgaaggtt cgttcgacag tggcgcagca ccacgaggca 180

-continued

ctggtcggcc acgggtttac acacgcgcac atcgttgcgt taagccaaca cccggcagcg 240

ttagggaccg tcgctgtcaa gtatcaggac atgatcgcag cgttgccaga ggcgacacac 300

gaagcgatcg ttggcgtcgg caaacagtgg tccggcgcac gcgctctgga ggccttgctc 360

acggtggcgg gagagttgag aggtccaccg ttacagttgg acacaggcca acttctcaag 420

attgcaaaac gtggcggcgt gaccgcagtg gaggcagtgc atgcatggcg caatgcactg 480

acgggtgccc cgctcaactt gaccccggag caggtggtgg ccatcgccag ccacgatggc 540

ggcaagcagg cgctggagac ggtccagcgg ctgttgccgg tgctgtgcca ggcccacggc 600

ttgacccccc agcaggtggt ggccatcgcc agcaatggcg gtggcaagca ggcgctggag 660

acggtccagc ggctgttgcc ggtgctgtgc caggcccacg gcttgacccc ggagcaggtg 720

gtggccatcg ccagccacga tggcggcaag caggcgctgg agacggtcca gcggctgttg 780

ccggtgctgt gccaggccca cggcttgacc ccggagcagg tggtggccat cgccagcaat 840

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cacggcttga ccccccagca ggtggtggcc atcgccagca ataatggtgg caagcaggcg 960

ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gaccccggag 1020

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ctgttgccgg tgctgtgcca ggcccacggc ttgacccccc agcaggtggt ggccatcgcc 1140

agcaatggcg gtggcaagca ggcgctggag acggtccagc ggctgttgcc ggtgctgtgc 1200

caggcccacg gcttgacccc ccagcaggtg gtggccatcg ccagcaataa tggtggcaag 1260

caggcgctgg agacggtcca gcggctgttg ccggtgctgt gccaggccca cggcttgacc 1320

ccccagcagg tggtggccat cgccagcaat aatggtggca agcaggcgct ggagacggtc 1380

cagcggctgt tgccggtgct gtgccaggcc cacggcttga ccccccagca ggtggtggcc 1440

atcgccagca atggcggtgg caagcaggcg ctggagacgg tccagcggct gttgccggtg 1500

ctgtgccagg cccacggctt gaccccggag caggtggtgg ccatcgccag caatattggt 1560

ggcaagcagg cgctggagac ggtgcaggcg ctgttgccgg tgctgtgcca ggcccacggc 1620

ttgaccccgg agcaggtggt ggccatcgcc agccacgatg gcggcaagca ggcgctggag 1680

acggtccagc ggctgttgcc ggtgctgtgc caggcccacg gcttgacccc ggagcaggtg 1740

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ccggtgctgt gccaggccca cggcttgacc ccggagcagg tggtggccat cgccagccac 1860

gatggcggca agcaggcgct ggagacggtc cagcggctgt tgccggtgct gtgccaggcc 1920

cacggcttga ccccccagca ggtggtggcc atcgccagca ataatggtgg caagcaggcg 1980

ctggagacgg tccagcggct gttgccggtg ctgtgccagg cccacggctt gacccctcag 2040

caggtggtgg ccatcgccag caatggcggc ggcaggccgg cgctggagag cattgttgcc 2100

cagttatctc gccctgatcc ggcgttggcc gcgttgacca acgaccacct cgtcgccttg 2160

gcctgcctcg gcgggcgtcc tgcgctggat gcagtgaaaa agggattggg ggatcctatc 2220

agccgttccc agctggtgaa gtccgagctg gaggagaaga aatccgagtt gaggcacaag 2280

ctgaagtacg tgccccacga gtacatcgag ctgatcgaga tcgcccggaa cagcacccag 2340

gaccgtatcc tggagatgaa ggtgatggag ttcttcatga aggtgtacgg ctacaggggc 2400

aagcacctgg gcggctccag gaagcccgac ggcgccatct acaccgtggg ctcccccatc 2460

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gactacggcg tgatcgtgga caccaaggcc tactccggcg gctacaacct gcccatcggc 2520

caggccgacg aaatgcagag gtacgtggag gagaaccaga ccaggaacaa gcacatcaac 2580

cccaacgagt ggtggaaggt gtacccctcc agcgtgaccg agttcaagtt cctgttcgtg 2640

tccggccact tcaagggcaa ctacaaggcc cagctgacca ggctgaacca catcaccaac 2700

tgcaacggcg ccgtgctgtc cgtggaggag ctcctgatcg gcggcgagat gatcaaggcc 2760

ggcaccctga ccctggagga ggtgaggagg aagttcaaca acggcgagat caacttcgcg 2820

gccgactgat aa 2832

<210> SEQ ID NO 48
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: GS linker

<400> SEQUENCE: 48

Ser Gly Gly Ser
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<210> SEQ ID NO 49
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<210> SEQ ID NO 58
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<210> SEQ ID NO 59
<211> LENGTH: 8
<212> TYPE: PRT
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<400> SEQUENCE: 59

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<210> SEQ ID NO 61
<211> LENGTH: 11
<212> TYPE: PRT
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1 5 10

**1**. A heat shock protein 70 (hHSP70) specific chimeric antigen receptor (CAR) comprising at least:

an extra cellular ligand binding-domain specific for hHSP70,

a transmembrane domain, and

a cytoplasmic signaling domain,

**2**. The hHSP70 specific CAR comprising at least:

an extra cellular ligand binding-domain specific for hHSP70,

a transmembrane domain, and

a cytoplasmic signaling domain,

provided that said anti-HSP70 CAR does not bind to a "mut HSP70-2" antigen.

**3**. The hHSP70 specific CAR according to claim **1**, wherein said CAR binds to a human membrane HSP70 antigen (mHSP70-1 antigen).

**4**. The hHSP70 specific CAR according to claim **1**, wherein said CAR binds to human mHSP70-1 antigen.

**5**. The hHSP70 specific CAR according to claim **1**, further comprising a co-stimulatory domain.

**6**. The hHSP70 specific CAR according to claim **1**, further comprising a CD28 and/or a 4-1BB co-stimulatory domain.

**7**. The hHSP70 specific CAR according to claim **1**, wherein said transmembrane domain comprises a CD8α transmembrane domain.

**8**. The hHSP70 specific CAR according to claim **1**, further comprising a hinge.

**9**. The hHSP70 specific CAR according to claim **1**, wherein said cytoplasmic signaling domain comprises a T-cell activating domain.

**10**. The hHSP70 specific CAR according to claim **1**, wherein said CAR is expressed as a single polypeptide.

**11**. The hHSP70 specific CAR according to claim **1**, wherein said extra cellular ligand binding-domain comprises domains from a monoclonal anti-HSP70 antibody.

**12**. The hHSP70 specific CAR according to claim **8**, wherein said extra cellular ligand binding-domain comprises a complementary determining region (CDR) from a $V_H$ domain and from a $V_L$ domain of at least one monoclonal anti-HSP70 antibody

**13**. The hHSP70 specific CAR according to claim **12**, wherein said CDRs are SEQ ID NO. 13-15 or 18-20.

**14**. The hHSP70 specific CAR according to claim **1** having a polypeptide structure of V1, V3 or V5, said structure comprising an extra cellular ligand binding-domain comprising a $V_H$ and a $V_L$ from a monoclonal anti-HSP70 antibody, a hinge transmembrane domain, a cyto-

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

**15**. The hHSP70 specific CAR according to claim **1**, wherein said CAR has a structure of V1, the structure comprising a FcγRIIIα hinge and CD8α transmembrane domain.

**16**. The hHSP70 specific scCAR according to claim **1**, wherein said CAR has a structure of V3, the structure comprising a CD8α hinge and a CD8α transmembrane domain.

**17**. The hHSP70 specific scCAR according claim **1**, wherein said CAR has a structure of V5, the structure comprising an IgG1 hinge and a CD8α transmembrane domain.

**18**. The hHSP70 specific scCAR according to claim **14**, wherein said $V_H$ and $V_L$ have at least 80% sequence identity with a polypeptide sequence of SEQ ID NO. 11-12 or SEQ ID NO. 16-17.

**19**. The hHSP70 specific CAR according to claim **1**, wherein the cytoplasmic signaling domain comprises a co-stimulatory domain from 4-1BB having at least 80% sequence identity with SEQ ID NO. 8.

**20**. The hHSP70 specific CAR according to claim **1**, wherein the cytoplasmic signaling domain comprises a CD3 zeta signaling domain having at least 80% sequence identity with SEQ ID NO. 9.

**21**. The hHSP70 specific CAR according to claim **1**, wherein said CAR further comprises a FcγRIIIα hinge having has at least 80% sequence identity with SEQ ID NO. 3.

**22**. The hHSP70 specific CAR according to claim **1**, wherein said CAR further comprises a CD8α hinge having at least 80% sequence identity with SEQ ID NO. 4.

**23**. The hHSP70 specific CAR according to claim **1**, wherein said CAR further comprises an IgG1 hinge having has at least 80% sequence identity with SEQ ID NO. 5.

**24**. The hHSP70 specific CAR according to claim **1**, wherein said transmembrane domain is a CD8α transmembrane domain having at least 80% sequence identity with SEQ ID NO. 6.

**25**. The hHSP70 specific CAR according to claim **1**, further comprising another extracellular ligand binding domain that is not specific for HSP70.

**26**. The hHSP70 specific CAR according to claim **1**, wherein the CAR has a structure of V3 comprising a polypeptide sequence having at least 80% sequence identity with SEQ ID NO. 23 or 29.

**27**. The hHSP70 specific CAR according to claim **1**, wherein the CAR has a of structure of V1 comprising a polypeptide sequence having at least 80% sequence identity with SEQ ID NO. 21 or 27.

**28**. The hHSP70 specific CAR according to claim **1**, wherein the CAR has a structure of V5 comprising a polypeptide sequence having at least 80% sequence identity with SEQ ID NO. 25 or 31.

**29**. The hHSP70 specific CAR according to claim **1**, further comprising a signal peptide.

**30**. The hHSP70 specific CAR according to claim **29**, wherein said signal peptide has at least 80% sequence identity with SEQ ID NO. 1 or SEQ ID NO. 2.

**31**. The hHSP70 specific CAR according to claim **1**, wherein the extracellular binding domain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mAb-specific epitopes.

**32**. The hHSP70 specific CAR according to claim **1**, wherein the extracellular binding domain comprises 1, 2, 3 or, 4 mAb-specific epitopes.

**33**. The hHSP70 specific CAR according to claim **1**, wherein the extracellular binding domain comprises 2, 3 or, 4 mAb-specific epitopes

**34**. The hHSP70 specific CAR according to claim **1**, wherein the extracellular binding domain comprises the following sequence

V1-L1-V2-(L)x-Epitope1-(L)x-,
V1-L1-V2-(L)x-Epitope1-(L)x-Epitope2-(L)x-;
V1-L1-V2-(L)x-Epitope1-(L)x-Epitope2-(L)x-Epitope3-(L)x-,
(L)x-Epitope1-(L)x-V1-L1-V2;
(L)x-Epitope1-(L)x-Epitope2-(L)x-V1-L1-V2;
Epitope1-(L)x-Epitope2-(L)x-Epitope3-(L)x-V1-L1-V2;
(L)x-Epitope1-(L)x-V1-L1-V2-(L)x-Epitope2-(L)x;
(L)x-Epitope1-(L)x-V1-L1-V2-(L)x-Epitope2-(L)x-Epitope3-(L)x-;
(L)x-Epitope1-(L)x-V1-L1-V2-(L)x-Epitope2-(L)x-Epitope3-(L)x-Epitope4-(L)x-;
(L)x-Epitope1-(L)x-Epitope2-(L)x-V1-L1-V2-(L)x-Epitope3-(L)x-;
(L)x-Epitope1-(L)x-Epitope2-(L)x-V1-L1-V2-(L)x-Epitope3-(L)x-Epitope4-(L)x-;
V1-(L)x-Epitope1-(L)x-V2;
V1-(L)x-Epitope1-(L)x-V2-(L)x-Epitope2-(L)x;
V1-(L)x-Epitope1-(L)x-V2-(L)x-Epitope2-(L)x-Epitope3-(L)x,
V1-(L)x-Epitope1-(L)x-V2-(L)x-Epitope2-(L)x-Epitope3-(L)x-Epitope4-(L)x;
(L)x-Epitope1-(L)x-V1-(L)x-Epitope2-(L)x-V2; or,
(L)x-Epitope1-(L)x-V1-(L)x-Epitope2-(L)x-V2-(L)x-Epitope3-(L)x;

wherein,

V1 is $V_L$ and V2 is $V_H$ or V1 is $V_H$ and V2 is $V_L$;

L1 is a linker suitable to link a $V_H$ chain to the $V_L$ chain;

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

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

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

**35**. The hHSP70 specific CAR to claim **34**, wherein the extracellular binding domain comprises the following sequence

V1-L1-V2-L-Epitope1; V1-L1-V2-L-Epitope1-L; V1-L1-V2-L-Epitope1-L-Epitope2; V1-L1-V2-L-Epitope1-L-Epitope2-L; V1-L1-V2-L-Epitope1-L-Epitope2-L-Epitope3; V1-L1-$V_2$-L-Epitope1-L-Epitope2-L-Epitope3-L; V1-L1-V2-Epitope1; V1-L1-V2-Epitope1-L; V1-L1-V2-Epitope1-L-Epitope2; V1-L1-$V_2$-Epitope1-L-Epitope2-L; V1-L1-V2-Epitope1-L-Epitope2-L-Epitope3; V1-L1-V2-Epitope1-L-Epitope2-L-Epitope3-L; Epitope1-V1-L1-V2; Epitope1-L-V1-L1-V2; L-Epitope1-V1-L1-V2; L-Epitope1-L-V1-L1-V2; Epitope1-L-Epitope2-V1-L1-$V_2$; Epitope1-L-Epitope2-L-V1-L1-V2; L-Epitope1-L-Epitope2-V1-L1-V2; L-Epitope1-L-Epitope2-L-V1-L1-V2; Epitope1-L-Epitope2-L-Epitope3-V1-L1-V2; Epitope1-L-Epitope2-L-Epitope3-L-V1-L1-V2; L-Epitope1-L-Epitope2-L-

Epitope3-V1-L1-V2; L-Epitope1-L-Epitope2-L-Epitope3-L-V1-L1-V2; V1-L-Epitope1-L-$V_2$; L-Epitope1-L-V1-L-Epitope2-L-V2; V1-L-Epitope1-L-V2-L-Epitope2-L; V1-L-Epitope1-L-V2-L-Epitope2-L-Epitope3; V1-L-Epitope1-L-V2-L-Epitope2-Epitope3; V1-L-Epitope1-L-V2-L-Epitope2-L-Epitope3-Epitope4; L-Epitope1-L-V1-L-Epitope2-L-V2-L-Epitope3-L; Epitope1-L-V1-L-Epitope2-L-V2-L-Epitope3-L; L-Epitope1-L-V1-L-Epitope2-L-V2-L-Epitope3; L-Epitope1-L-V1-L1-V2-L-Epitope2-L; L-Epitope1-L-V1-L1-V2-L-Epitope2-L-Epitope3; L-Epitope1-L-V1-L1-V2-L-Epitope2-Epitope3, or Epitope1-L-V1-L1-V2-L-Epitope2-L-Epitope3-Epitope4

wherein

V1 is $V_L$ and V2 is $V_H$ or V1 is $V_H$ and V2 is $V_L$;

L1 is any linker suitable to link a $V_H$ chain to the $V_L$ chain;

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

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

**36**. The hHSP70 specific CAR according to claim **34**, wherein L1 is a linker comprising Glycine and/or Serine.

**37**. The hHSP70 specific CAR according to claim **34**, wherein L1 is a linker comprising the amino acid sequence (Gly-Gly-Gly-Ser)n or (Gly-Gly-Gly-Gly-Ser)n, where n is 1, 2, 3, 4 or 5.

**38**. The hHSP70 specific CAR according to claim **34**, wherein L1 is a linker comprising the amino acid sequence (Gly4Ser)4 or (Gly4Ser)3.

**39**. The hHSP70 specific CAR according to claim **38**, wherein L is a linker having an amino acid sequence of SGG, GGS, SGGS (SEQ ID NO. 48), SSGGS (SEQ ID NO. 49), GGGG (SEQ ID NO. 50), SGGGG (SEQ ID NO. 51), GGGGS (SEQ ID NO. 52), SGGGGS (SEQ ID NO. 53), GGGGGS (SEQ ID NO. 54), SGGGGGS (SEQ ID NO. 55), SGGGGG (SEQ ID NO. 56), GSGGGGS (SEQ ID NO. 57), GGGGGGGS (SEQ ID NO. 58), SGGGGGGG (SEQ ID NO. 59), SGGGGGGGS (SEQ ID NO. 60), or SGGGGSGGGGS (SEQ ID NO. 61).

**40**. The hHSP70 specific CAR according to claim **39**, wherein L is a SGGGG (SEQ ID NO. 51), GGGGS (SEQ ID NO. 52), or SGGGGS (SEQ ID NO. 53).

**41**. The hHSP70 specific CAR according to claim **31** wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes specifically recognized by ibritumomab, tiuxetan, muromonab-CD3, tositumomab, abciximab, basiliximab, brentuximab vedotin, cetuximab, infliximab, rituximab, alemtuzumab, bevacizumab, certolizumab pegol, daclizumab, eculizumab, efalizumab, gemtuzumab, natalizumab, omalizumab, palivizumab, ranibizumab, tocilizumab, trastuzumab, vedolizumab, adalimumab, belimumab, canakinumab, denosumab, golimumab, ipilimumab, ofatumumab, panitumumab, QBEND-10, alemtuzumab or ustekinumab.

**42**. The hHSP70 specific CAR according to claim **31** wherein Epitope 1, Epitope 2, Epitope 3 and Epitope 4 are independently selected from mAb-specific epitopes having an amino acid sequence of anyone of SEQ ID NO 33 to SEQ ID NO 42.

**43**. The hHSP70 specific CAR according to claim **31** wherein Epitope 1 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 33.

**44**. The hHSP70 specific CAR according to claim **31** wherein Epitope 2 is a mAb-specific epitope having an amino acid sequence of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**45**. The hHSP70 specific CAR according to claim **31** wherein Epitope 3 is a mAb-specific epitope having an amino acid sequence of anyone of SEQ ID NO 33 or SEQ ID NO 35 to 38.

**46**. The hHSP70 specific CAR according to claim **31** wherein Epitope 4 is an mAb-specific epitope having an amino acid sequence of SEQ ID NO 41 or 42.

**47**. A polynucleotide encoding a CAR according to claim **1**.

**48**. An expression vector comprising the nucleic acid of claim **47**.

**49**. An engineered lymphoid immune cell expressing the CAR according to claim **1**, wherein the CAR is cell surface membrane expressed.

**50**. The engineered lymphoid immune cell according to claim **49**, wherein the cell is derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

**51**. (canceled)

**52**. The engineered cell according to claim **49**, formulated as a medicament.

**53**. A method of treating a pre-malignant or malignant cancer, comprising administering to a patient a cell comprising the hHSP70 specific CAR according to claim **1**, wherein the pre-malignant or malignant cancer is characterized by hHSP70-expressing cells.

**54**. The engineered cell according to claim **49**, wherein the pre-malignant or malignant cancer is characterized by an overabundance of hHSP70-expressing cells.

**55**. The engineered cell according to claim **49**, wherein the pre-malignant or malignant cancer is a hematological cancer condition.

**56**. The engineered cell according to claim **49**, wherein the hematological cancer condition is leukemia.

**57**. The engineered cell according to claim **56**, wherein the leukemia is acute myelogenous leukemia (AML).

**58**. The engineered cell according to claim **49**, wherein the cell has a suppressed expression of TCR in said cell.

**59**. The engineered cell according to claim **49**, wherein the cell has a repressed or suppressed expression of at least one MHC protein in said cell.

**60**. The engineered cell according to claim **49**, wherein the cell is mutated to confer resistance to at least one immune suppressive or chemotherapy drug.

**61**. A method of treating a disease associated with Hsp70.1 overexpressing cells comprising administering to a subject in need thereof an immune cell according to claim **49** and an antibody directed against soluble Hsp70 A.

**62**. The method according to claim **61**, wherein the antibody directed against soluble Hsp70 is first administered until the level of the soluble Hsp70 is reduced in the plasma of the patient by at least 50%, compared to that before administration of said antibodies, said administration of soluble Hsp70 specific monoclonal antibodies being followed by the administration of aid an anti-mHsp70 CAR expressing immune cells.

**63**. A method of impairing a hematologic cancer cell comprising contacting said cell with an engineered cell according to claim **49** in an amount effective to cause impairment of said cancer cell.

**64**. A composition comprising an immune cell modified to at least express an anti-mHsp70 CAR according to claim **1** in combination with a drug.

**65**. A method of engineering an immune cell to express at least one hHSP70 specific CAR comprising

expressing at the cell's surface at least one HSP70 single-chain specific CAR according to claim **1**.

**66**. The method of engineering an immune cell of claim **65**, wherein the expression of at least one hHSP70 single chain CAR comprises introducing into and expressing in

at least one polynucleotide encoding said HSP70 single-chain specific CAR.

**67**. The method of engineering an immune cell of claim **66**, further comprising

introducing at least one other CAR which is not specific for HSP70.

**68**. A method of treating a disease or condition associated with over-expression of hHSP70 in a subject in need thereof comprising administering

an immune cell surface expressing an anti-HSP70 single-chain specific CAR according to claim **1**.

**69**. The method according to claim **68**, wherein said immune cell is provided from a donor.

**70**. The method according to claim **68**, wherein said immune cell is provided from the patient himself.

**71**. A method for depleting engineered lymphoid immune cell expressing a HSP70 specific CAR and at least one epitope according to claim **31** in a patient, wherein an antibody specific to said epitope is administered to said patient in case of need.

**72**. The cell engineered cell according to claim **59**, wherein the at least one MHC protein is β2m or HLA.

* * * * *