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(71) 申请人: **科济生物医药（上海）有限公司 (CARSGEN THERAPEUTICS, LTD)** [CN/CN]; 中国上海市徐汇区银都路 466 号 3 号楼 4 楼, Shanghai 200231 (CN)。

(72) 发明人: **王华茂 (WANG, Huamao)**; 中国上海市徐汇区银都路 466 号 3 号楼 4 楼, Shanghai 200231 (CN)。 **宋波 (SONG, Bo)**; 中国上海市徐汇区银都路 466 号 3 号楼 4 楼, Shanghai 200231 (CN)。 **王鹏 (WANG, Peng)**; 中国上海市徐汇区银都路 466 号 3 号楼 4 楼, Shanghai 200231 (CN)。

(74) 代理人: **上海一平知识产权代理有限公司 (XU & PARTNERS,LLC.)**; 中国上海市普陀区真北路 958 号天地科技广场 1 号楼 106 室, Shanghai 200333 (CN)。

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(54) **Title**: FULLY HUMAN ANTI-MESOTHELIN ANTIBODIES AND IMMUNE EFFECTOR CELLS TARGETING MESOTHELIN

(54) **发明名称** : 抗间皮素全人抗体以及靶向间皮素的免疫效应细胞

(57) **Abstract**: The present invention provides fully human anti-mesothelin antibodies and immune effector cells targeting mesothelin.

(57) **摘要**: 本发明提供抗间皮素全人抗体以及靶向间皮素的免疫效应细胞。

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FULLY HUMAN ANTI-MESOTHELIN ANTIBODIES AND IMMUNE EFFECTOR CELLS TARGETING MESOTHELIN

Technical field

The present invention relates to the field of immunotherapy or diagnosis of tumor, and in particular, to fully human anti-mesothelin antibodies and immune effector cells targeting mesothelin.

Background

The role of immune effector cells in the tumor immune response is gaining more and more attention. Adoptive immunotherapy based on immune effector cells has achieved some effects in some tumors, and this immunotherapy method can overcome the defects of antibody treatment, however the therapeutic effects in most tumors are still unsatisfactory [Grupp SA, et al. Adoptive cellular therapy. Curr Top Microbiol Immunol., 2011; 344: 149-72.]. In recent years, it was discovered that the recognition specificity of cytotoxic lymphocytes (CTLs) to target cells depends on T cell receptors (TCRs), scFv of antibodies against tumor cell associated antigens and intracellular signal-activating motifs of T lymphocyte receptor CD3 ζ or Fc ε RI γ were fused to a chimeric antigen receptor (CAR), and T lymphocyte was genetically modified by the chimeric antigen receptors on its surface by means of, for example, lentivirus infection. Such CAR T lymphocytes are capable of selectively targeting T lymphocytes to tumor cells and specifically killing the tumor in a non-limiting manner by Major Histocompatibility Complex (MHC). CAR T lymphocyte is a new immunotherapy strategy in the field of tumor immunotherapy. CAR modified NK cells or NKT cells also exhibit antitumor activities in preclinical studies.

When designing CAR-modified immune effector cells, especially T cells, the targeted antigen genes are in fact a crucial choice. Given the complexity of gene expressions in vivo and various uncontrollable factors, selection for suitable genes for a CAR is very difficult. Moreover, for many tumor-specific antigens, it is difficult to find a specific molecule directed against them and suitable for constructing CAR-modified immune effector cells.

Mesothelin is a cell surface glycoprotein, molecular weight of which is 40-kDa. It is highly expressed in a variety of tumors, such as pancreatic cancer, ovarian cancer, and thymus mesothelioma. In normal tissues, it is expressed only on the normal mesothelial cells of the pleura, pericardium and peritoneum. Mesothelin is synthesized as a 71 kDa precursor protein, the mature portion of which is expressed on the cell surface. The precursor protein is proteolytically cleaved by furin into a 31 kDa shedding part (termed megakaryocyte chimeric factor, or MPF) and a 40 kDa mesothelin fraction). The latter component may remain bound to the cell surface via GPI

linkage and may also shed off via the proteolytic enzyme mechanism.

Antibodies against mesothelin or other targeted therapies have been reported. CAR-T has also been reported in clinical studies (Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, Zhao Y, Kalos M, June CH. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res. 2013; 1 (1): 26-31; Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, Chew A, Zhao Y, Levine BL, Albelda SM, Kalos M, June CH. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res. 2014 Feb; 2 (2): 112-20). However, it has also been found that CAR-T constructed with mouse anti-human mesothelin antibody clinically shows side effects, such as anti-mouse antibody and allergy, indicating that mesothelin may be a potential therapeutic target, but the properties of the antibody itself may affect its efficacy and side effects. Therefore, there is still a need in the art to find solutions that can overcome problems caused by antibodies that are not ideal or have toxic side effects.

Summary of the invention

It is an object of the present invention to provide fully human anti-mesothelin antibodies as well as immune effector cells targeting mesothelin.

In the first aspect of the invention, a fully human antibody that specifically binds to mesothelin is provided, which is selected from a group consisting of:

(a) an antibody comprising a heavy chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 54, CDR2 comprising the amino acid sequence of SEQ ID NO: 55, CDR3 comprising the amino acid sequence of SEQ ID NO: 56;

(b) an antibody comprising a light chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 51, CDR2 comprising the amino acid sequence of SEQ ID NO: 52, CDR3 comprising the amino acid sequence of SEQ ID NO: 53;

(c) an antibody comprising a heavy chain variable region of said antibody of (a) and a light chain variable region of said antibody of (b);

(d) an antibody comprising a heavy chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 60, CDR2 comprising the amino acid sequence of SEQ ID NO: 61, CDR3 of the amino acid sequence of ID NO: 62;

(e) an antibody comprising a light chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 57, CDR2 comprising the amino acid sequence of SEQ ID NO: 58, CDR3 of the amino acid of ID NO: 59;

(f) an antibody comprising a heavy chain variable region of said antibody of (d) and a light chain variable region of the antibody of (e);

(g) an antibody which recognizes the same antigenic determinant as that recognized by the

antibody according to any one of (a) to (f).

In a preferred embodiment, the fully human antibody comprises a heavy chain variable region and a light chain variable region, the amino acid sequence of the heavy chain variable region is shown in positions 1 to 123 of SEQ ID NO: 6; and the amino acid sequence of the light chain variable region is shown in positions 139-254 of SEQ ID NO: 6; or

the fully human antibody comprises a heavy chain variable region and a light chain variable region, the amino acid sequence of the heavy chain variable region is shown in positions 1 to 124 of SEQ ID NO: 8; and the amino acid sequence of the light chain variable region is shown in positions 140-247 of SEQ ID NO: 8.

In another preferred embodiment, the fully human antibody that specifically binds to mesothelin may be single chain antibody (scFV), monoclonal antibody, domain antibody, Fab fragment, Fd fragment, Fv fragment, F $(ab')_2$ fragment and a derivative thereof, or other forms of antibody; preferably single chain antibody.

In another aspect of the invention, a nucleic acid encoding the antibody is provided.

In another aspect of the invention, an expression vector comprising the nucleic acid is provided.

In another aspect of the invention, a host cell is provided, which comprises the expression vector or has the nucleic acid integrated into the genome.

In another aspect of the present invention, use of the antibodies described above is provided for the preparation of a targeted drug, antibody-drug conjugate, or a polyfunctional antibody that specifically targets tumor cells expressing mesothelin; or for the preparation of a reagent for diagnosing a tumor expressing mesothelin; or for the preparation of chimeric antigen receptor-modified immune cells.

In another aspect of the present invention, a chimeric antigen receptor (CAR) of the antibody is provided, and said chimeric antigen receptor comprises sequentially linked: the antibody of the present invention, a transmembrane region and intracellular signal region.

In a preferred embodiment, the intracellular signal region is selected from a group consisting of intracellular signal region sequences of CD3ζ, FcεRIγ, CD27, CD28, CD137, CD134, MyD88, CD40 or a combination thereof.

In another preferred embodiment, the transmembrane region comprises a transmembrane region of CD8 or CD28.

In another preferred embodiment, the chimeric antigen receptor comprises the following sequentially linked antibody, transmembrane region and intracellular signal region:

The antibody, CD8 and CD3ζ;

The antibody, CD8, CD137 and CD3ζ;

The antibody, the transmembrane region of CD28 molecule, the intracellular signal region of

CD28 molecule and CD3ζ; or

The antibody, the transmembrane region of CD28 molecule, the intracellular signal region of CD28 molecule, CD137 and CD3ζ.

In another preferred embodiment, the antibody is a single chain antibody or domain antibody.

In another preferred embodiment, the chimeric antigen receptor has:

SEQ ID NO: 41 or the amino acid sequence shown in positions 22-353 thereof;

SEQ ID NO: 42 or the amino acid sequence shown in positions 22-454 thereof;

SEQ ID NO: 43 or the amino acid sequence shown in positions 22-498 thereof;

SEQ ID NO: 44 or the amino acid sequence shown in positions 22-501 thereof;

SEQ ID NO: 45 or the amino acid sequence shown in positions 22-543 thereof;

SEQ ID NO: 46 or the amino acid sequence shown in positions 22-346 thereof;

SEQ ID NO: 47 or the amino acid sequence shown in positions 22-447 thereof;

SEQ ID NO: 48 or the amino acid sequence shown in positions 22-491 thereof;

SEQ ID NO: 49 or the amino acid sequence shown in positions 22-494 thereof; or

SEQ ID NO: 50 or the amino acid sequence shown in positions 22-536 thereof.

In another aspect of the invention, a nucleic acid encoding the chimeric antigen receptor is provided.

In another preferred embodiment, the nucleic acid encoding the chimeric antigen receptor has:

SEQ ID NO: 31 or the nucleotide sequence set forth in positions 473-1468 thereof;

SEQ ID NO: 32 or the nucleotide sequence set forth in positions 473-1771 thereof;

SEQ ID NO: 33 or the nucleotide sequence set forth in positions 473-1903 thereof;

SEQ ID NO: 34 or the nucleotide sequence set forth in positions 473-1912 thereof;

SEQ ID NO: 35 or the nucleotide sequence set forth in positions 473-2038 thereof;

SEQ ID NO: 36 or the nucleotide sequence set forth in positions 473-1447 thereof;

SEQ ID NO: 37 or the nucleotide sequence set forth in positions 473-1750 thereof;

SEQ ID NO: 38 or the nucleotide sequence set forth in positions 473-1882 thereof;

SEQ ID NO: 39 or the nucleotide sequence set forth in positions 473-1891 thereof;

SEQ ID NO: 40 or the nucleotide sequence set forth in positions 473 to 2017 thereof.

In another aspect of the present invention, an expression vector comprising the nucleic acid is provided.

In another preferred embodiment, the expression vector is derived from lentiviral plasmid pWPT (or pWPT-eGFP).

In another aspect of the present invention, a virus comprising said vector is provided.

In another aspect of the invention, use of the chimeric antigen receptor, or the nucleic acid,

or the expression vector, or the virus is provided for the preparation of genetically modified immune cells targeting tumor cells expressing mesothelin.

In a preferred embodiment, the mesothelin-expressing tumor includes, but is not limited to pancreatic cancer, ovarian cancer and thymus mesothelioma.

In another aspect of the present invention, a genetically modified immune cell is provided, which is transduced with the nucleic acid, or the expression vector or the virus; or expresses the chimeric antigen receptor on its surface-expressed.

In a preferred embodiment, the immune cell further carries an encoding sequence of an exogenous cytokine; and preferably, the cytokine includes: IL-12, IL-15 or IL-21.

In another preferred embodiment, the immune cell also expresses another chimeric antigen receptor which does not contain CD3ζ but contains the intracellular signaling domain of CD28, the intracellular signaling domain of CD137, or a combination of both.

In another preferred embodiment, the immune cell further expresses a chemokine receptor; and preferably, the chemokine receptor includes: CCR2.

In another preferred embodiment, the immune cell further expresses siRNA which can reduce expression of PD-1 or a protein which blocks PD-L1.

In another preferred embodiment, the immune cell further expresses a safety switch; and preferably, the safety switch includes iCaspase-9, Truancated EGFR or RQR8.

In another preferred embodiment, the immune cells include T lymphocytes, NK cells or NKT cells.

In another aspect of the invention, use of the genetically modified immune cells is provided for the preparation of a tumor-inhibiting drug, and the tumor is a tumor expressing mesothelin.

In another aspect of the present invention, a multi-functional immunoconjugate is provided, comprising: any one of the above described antibodies; and a functional molecule linked thereto (including covalently linked, conjugated, attached, adsorbed); the functional molecule is selected from a group consisting of a molecule that targets a tumor surface marker, a tumor-suppressing molecule, a molecule that targets a surface marker of an immune cell, or a detectable label.

In a preferred embodiment, in the multifunctional immunoconjugate, the molecule that targets the tumor surface marker is an antibody or ligand that binds to a tumor surface marker; or the tumor-suppressing molecule is an anti-tumor cytokine or an anti-tumor toxin; and preferably, the cytokines include but are not limited to: IL-12, IL-15, IFN-beta, TNF-alpha.

In another preferred embodiment, in the multi-functional immunoconjugate, the detectable label includes a fluorescent label or a chromogenic label.

In another preferred embodiment, in the multifunctional immunoconjugate, the antibody that binds to a tumor surface marker refers to an antibody that recognizes an antigen other than mesothelin, and the other antigen includes EGFR EGFRvIII, mesothelin, HER2, EphA2, Her3,

EpCAM, MUC1, MUC16, CEA, Claudin 18.2, folate receptor, Claudin 6, CD3, WT1, NY-ESO- 1, MAGE 3, ASGPR1 or CDH16.

In another preferred embodiment, in the multifunctional immunoconjugate, the molecule that targets the surface marker of the immune cell is an antibody that binds to T cell surface marker and forms a T-cell-engaging bifunctional antibody with the above described antibody (bispecific T cell engager, BiTE).

In another preferred embodiment, in the multifunctional immunoconjugate, the antibody that binds to T cell surface marker is an anti-CD3 antibody.

In another preferred embodiment, the anti-CD3 antibody is a single chain antibody (scFV), a monoclonal antibody, a Fab fragment, an Fd fragment, an Fv fragment, an $F(ab')_2$ fragment and a derivative thereof, antibody; preferably single chain antibody.

In another preferred embodiment, the anti-CD3 antibody is humanized, fully human, chimeric or murine antibody.

In another preferred embodiment, the multifunctional immunoconjugate is a fusion polypeptide, and further comprises a linker peptide (linker) between the above described antibody of the invention and the functional molecule linked thereto.

In another preferred embodiment, the linker peptide has the sequence (GlyGlyGlyGlySer)n, wherein n is an integer from 1 to 5; more preferably, n = 3.

In another preferred embodiment, the multi-functional immunoconjugate is administered in a form of polypeptide or in the manner of gene administration.

In another aspect of the invention, a nucleic acid encoding the multi-functional immunoconjugate is provided.

In another aspect of the present invention, use of the multi-functional immunoconjugate is provided, for the preparation of an antineoplastic agent or an agent for diagnosis of tumors that express mesothelin; or for the preparation of chimeric antigen receptor modified immune cells; and preferably, the immune cells include T lymphocyte, NK cell or NKT lymphocyte.

In another aspect of the invention, a pharmaceutical composition (including medicament or diagnostic reagent) is provided, comprising:

the antibody or a nucleic acid encoding the antibody; or

the immunoconjugate or a nucleic acid encoding the conjugate; or

the chimeric antigen receptor or a nucleic acid encoding the chimeric antigen receptor; or

the genetically modified immune cell.

In another aspect of the invention, an antibody is provided, which is capable of competing for binding to mesothelin with the antibody of the invention.

In another aspect of the invention, an antibody is provided, which is capable of binding

to mesothelin epitope as shown in SEQ ID NO: 66. In a preferred embodiment, an antibody that binds to mesothelin epitope as shown in SEQ ID NO: 72 is also provided.

Other aspects of the invention will be apparent to a skilled person in the art from the disclosure herein.

Description of drawings

Figure 1. Binding of antibodies P1A6E and P3F2 to hu-mesothelin and control BSA in a single-phage ELISA assay. The values of antibodies P1A6E and P3F2 against human mesothelin and negative control BSA demonstrated that the two selected antibodies could specifically bind to human mesothelin.

Figure 2. Binding of two different single chain antibodies P1A6E and P3F2 to human mesothelin and BSA detected by ELISA.

Figure 3. Electrophoresis of purified SDS-PAGE of anti-human mesothelin antibodies.

Figure 4. SDS-PAGE electrophoresis of the monoclonal antibodies P1A6E and P3F2.

Figure 5. Binding curves of monoclonal antibody P1A6E to different concentrations of human mesothelin in Biacore.

Figure 6. Binding curve of the monoclonal antibody P3F2 to different concentrations of human mesothelin in Biacore.

Figure 7. Assay of specific binding of four single-chain antibodies (P1A6E, P3F2 and control antibodies SS, C10) to PANC-1-MSLN cells as shown by Fluorescence Activated Cell Sorter (FACS).

Figure 8. Assay of specific binding of four monoclonal antibodies (P1A6E, P3F2 and control antibody SS, C10) to PANC-1-MSLN cells as shown by Fluorescence Activated Cell Sorter (FACS).

Figure 9. ELISA showing binding of the antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc to regions R1, R2, R3.

Figure 10. ELISA showing binding of the antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc to regions R1A, R1B, R1C, R1AB, R1BC.

Mode for carrying out the invention

The present inventors investigated many kinds of tumor-specific genes in the early stage and found that a significant proportion of these genes were also expressed in normal cells of some tissues and were relatively difficult to be applied to immune effector cell technology of chimeric antigen receptor modification. Some tumor specific genes exhibit better tumor-specific expression characteristics, however, the CAR-modified immune effector cells based on them have no tumor cell killing activity or low activity, because the target can induce secretion of immune effect

cell-inhibiting factors, such as PD-L1 by tumor cells.

After repeated investigation and screening, the present inventors found mesothelin from many candidate molecules as a target for designing CAR. The present inventors have demonstrated that CAR-modified T cells prepared based on antibodies against mesothelin do selectively target mesothelin-positive tumor cells and are highly cytotoxic to tumor cells. The inventors believe that the corresponding CAR-modified immune effector cells, particularly T cells, should be useful for the treatment of human tumors.

Antibodies against mesothelin

Specific antibodies which have good binding properties to mesothelin and are suitable for preparing genetically modified immune effector cells, were screened and obtained in all-human natural antibody libraries by the present inventors, and key CDR regions for them to exert their binding properties were also found by the inventors.

Antibodies of the invention may be intact immunoglobulin molecules or antigen-binding fragments, including but not limited to Fab fragments, Fd fragments, Fv fragments, F $(ab')_2$ fragments, complementarity determining region (CDR) fragments, single-chain antibody (scFv), domain antibody, bivalent single chain antibody, single chain phage antibody, bispecific diabody, triple chain antibody, quadruple chain antibody.

The antigen-binding properties of an antibody can be described by three specific regions located in variable regions of the heavy and light chains, termed complementarity determining regions (CDRs), which divide the variable regions into four framework regions (FR), and the amino acid sequences of four FRs are relatively conservative, not directly involved in binding reaction. These CDRs form a loop structure, in which β-folds formed by the FRs are located close to each other in space and the antigen binding site of the antibody is constituted by CDRs on the heavy chain and CDRs on the corresponding light chain. It is possible to determine which amino acids make up FR or CDR regions by comparing the amino acid sequences of the same type of antibody. The CDR regions are sequences of immunologically interesting proteins and the CDR regions of the antibodies of the invention are brand new. The antibody may comprise two, three, four, five, or all six of the CDR regions disclosed herein.

Another aspect of the invention includes functional variants of the antibodies described herein. If the variant is capable of competing with the parental antibody for specific binding to mesothelin 1 and its ability to recognize mesothelin expressed by tumor cells is close to that of the specific antibodies provided in Examples of the present invention. The functional variants may have conservative sequence modifications, including nucleotide and amino acid substitutions, additions and deletions. These modifications can be introduced by standard techniques known in

the art, such as directed mutagenesis and random PCR-mediated mutagenesis, and can include both natural and non-natural nucleotides and amino acids. Preferably, modification of the sequence occurs on a region outside the CDR region of the antibody.

The antibodies of the present invention can be applied to prepare various targeted antitumor drugs and drugs for diagnosing tumors, and in particular to prepare immune effector cells targeting mesothelin.

Chimeric antigen receptor and genetically modified immune cell

In the present invention, a chimeric antigen receptor expressed on the surface of an immune effector cell (immune cell) is provided, wherein the chimeric antigen receptor comprises sequentially linked: extracellular binding region, transmembrane region and intracellular signal region, and the extracellular binding region comprises the antibody of the invention. By expressing the chimeric antigen receptor on the surface of immune effector cells, immune effector cells can have a highly specific cytotoxic effect on tumor cells expressing mesothelin.

As used herein, "immune cells" and "immune effector cells" are used interchangeably and include: T lymphocytes, NK cells or NKT cells, and the like.

As a preferred embodiment of the present invention, the antibody contained in the chimeric antigen receptor is a single chain antibody, which is connected to CD8 or the transmembrane region of CD28 through the hinge region of CD8, and the transmembrane region is immediately followed by the intracellular signal region.

The invention also includes nucleic acids encoding the chimeric antigen receptors. The present invention also relates to variants of the above described polynucleotides, which encode a polypeptide, or a fragment, analog and derivative of the polypeptide having the same amino acid sequence as the present invention.

The transmembrane region of the chimeric antigen receptor may be selected from the transmembrane region of a protein such as CD8 or CD28. The human CD8 protein is a heterodimer composed of two chains, αβ or γδ. In one embodiment of the invention, the transmembrane region is selected from the transmembrane region of CD8a or CD28. In addition, the CD8α hinge is a flexible region so that CD8 or CD28 and the transmembrane region as well as the hinge region are used to connect the target recognition domain scFv of the chimeric antigen receptor CAR to the intracellular signal region.

The intracellular signal region may be selected from a group consisting of intracellular signal region of CD3ζ, FcεRIγ, CD27, CD28, CD137, CD134, MyD88, CD4 protein, and combinations thereof. The CD3 molecule consists of five subunits, in which CD3ζ subunit (also known as CD3 zeta, abbreviated as Z) contains 3 ITAM motifs that are important signal transduction regions in

TCR-CD3 complex. CD3δZ is a truncated CD3ζ sequence without ITAM motif and is generally constructed in the present invention as a negative control. FcsRIγ is mainly distributed on the surface of mast cells and basophils, which contains an ITAM motif, which is similar to CD3ζ in structure, distribution and function. In addition, as mentioned above, CD28, CD137 and CD134 are co-stimulatory signaling molecules. The co-stimulatory effect of their intracellular signaling segments upon binding to the respective ligands results in the continued proliferation of immune effector cells, primarily T lymphocytes, and increase in the level of cytokines such as IL-2 and IFN-γ secreted by immune effector cells, and the survival period and anti-tumor effect of CAR immune effector cells *in vivo* are increased.

The chimeric antigen receptor of the present invention can be sequentially linked as follows:

The antibody of the invention, CD8 and CD3 ζ ;

The antibody of the invention, CD8, CD137 and CD3 ζ ;

The antibody of the invention, the transmembrane region of CD28 molecule, the intracellular signal region of CD28 molecule and CD3 ζ ; or

The antibodies of the invention, the transmembrane region of CD28 molecule, the intracellular signal region of CD28 molecule, CD137 and CD3.

And combinations thereof, wherein CD28a in the relevant chimeric antigen receptor protein represents the transmembrane region of CD28 molecule and CD28b represents the intracellular signal region of CD28 molecule. The various chimeric antigen receptors described above are collectively referred to as scFv (mesothelin)-CAR.

The present invention also provides a vector comprising the above-mentioned nucleic acid encoding a chimeric antigen receptor protein expressed on the surface of an immune effector cell. In a specific embodiment, the vector used in the present invention is a lentiviral plasmid vector pWPT-eGFP. This plasmid belongs to the third generation of self-inactivating lentiviral vector system. The system has three plasmids, packaging plasmid psPAX2 encoding protein Gag / Pol, encoding Rev protein; envelope plasmid PMD2.G encoding VSV-G protein; and empty vector pWPT-eGFP, which can be used for recombinant introduction of a nucleic acid sequence of interest, i.e., a nucleic acid encoding CAR. In the empty vector pWPT-eGFP, the expression of enhanced green fluorescent protein (eGFP) is regulated by elongation factor-1α (EF-1α) promoter. While in the recombinant expression vector pWPT-eGFP-F2A-CAR containing the nucleic acid sequence encoding CAR, co-expression of eGFP and CAR is achieved by ribosomal skipping sequence 2A (abbreviated as F2A) from food-and-mouth disease virus (FMDV). It is to be understood that other expression vectors are also useful.

The invention also includes viruses comprising the vectors described above. The viruses of

the invention include packaged infectious viruses as well as viruses to be packaged that contain the necessary components for packaging into infectious viruses. Other viruses known in the art that can be used to transduce exogenous genes into immune effector cells and their corresponding plasmid vectors are also useful in the present invention.

The present invention further includes a genetically modified T lymphocyte, which is transduced with a nucleic acid of the present invention or transduced with the above-mentioned recombinant plasmid containing the nucleic acid of the present invention or a viral system containing the plasmid. Conventional nucleic acid transduction methods in the art, including non-viral and viral transduction methods, can be used in the present invention. Non-viral transduction methods include electroporation and transposon methods. Recently, nucleofector nuclear transfection instrument developed by Amaxa can directly introduce foreign genes into nucleus to achieve highly efficient transduction of target genes. In addition, compared with conventional electroporation, the transduction efficiency of transposon system based on Sleeping Beauty system or PiggyBac transposon was significantly improved. The combination of nucleofector transfection instrument and SB Sleeping Beauty transposon system has been reported [Davies JK., et al. Combining CD19 redirection and alloanergization to generate tumor-specific human T cells for allogeneic cell therapy of B-cell malignancies. Cancer Res, 2010, 70(10): OF1-10.], and high transduction efficiency and site-directed integration of target genes can be achieved by this method. In one embodiment of the invention, the transduction method of a T lymphocyte modified by a chimeric antigen receptor gene is a transduction method based on a virus such as a retrovirus or a lentivirus. The method has the advantages of high transduction efficiency and stable expression of exogenous gene, and the time for in vitro culturing T lymphocytes to clinical level can be shorten. The transduced nucleic acid is expressed on the surface of the transgenic T lymphocytes by transcription, translation. In vitro cytotoxicity assay performed on various cultured tumor cells demonstrated that the immune effector cells of the present invention have highly specific tumor cell killing effects (also known as cytotoxicity). Therefore, the nucleic acid encoding a chimeric antigen receptor protein of the present invention, a plasmid comprising the nucleic acid, a virus comprising the plasmid, and a transgenic immune effector cells transfected with the nucleic acid, plasmid or virus described above can be effectively used in tumor immunotherapy.

The immune cells of the present invention may also carry exogenous encoding sequences for cytokines, including but not limited to IL-12, IL-15 or IL-21. These cytokines have immunomodulatory or antitumor activity, enhance the function of effector T cells and activated NK cells, or directly exert anti-tumor effects. Therefore, those skilled in the art will understand that the use of these cytokines will help the immune cells to function better.

In addition to the chimeric antigen receptor described above, the immune cells of the present

invention may also express another chimeric antigen receptor, which does not contain CD3ζ, but contains intracellular signaling domain of CD28 and intracellular signal domain of CD137, or a combination of both.

The immune cells of the present invention may also express chemokine receptors; the chemokine receptors include, but are not limited to, CCR2. A skilled person will understand that the CCR2 chemokine receptor can competitively bind CCR2 in the body and is beneficial for blocking the metastasis of the tumor.

The immune cells of the present invention may also express siRNAs that can reduce PD-1 expression or PD-L1-blocking proteins. A skilled person will understand that competitive blocking of the interaction between PD-L1 and its receptor PD-1 will facilitate the recovery of anti-tumor T-cell responses, thereby inhibiting tumor growth.

The immune cells of the present invention may also express a safety switch; preferably, the safety switch includes iCaspase-9, Truancated EGFR or RQR8.

Immunoconjugate

In the present invention, a multifunctional immunoconjugate is also provided, comprising the antibodies described herein and further comprising at least one functional molecule of other type. The functional molecule is selected from, but not limited to, a molecule that targets a tumor surface marker, a tumor-suppressing molecule, a molecule that targets a surface marker of an immune cell, or a detectable label. The antibody and the functional molecule may form a conjugate by covalent attachment, coupling, attachment, cross-linking, or the like.

As a preferred mode, the immunoconjugate may comprise an antibody of the invention and at least one molecule that targets a tumor surface marker or a tumor-suppressing molecule. The tumor-suppressing molecule may be anti-tumor cytokines or anti-tumor toxins. Preferably, the cytokines include but are not limited to IL-12, IL-15, IFN-beta, TNF-alpha. The molecules that target tumor surface markers, for example, can act synergistically with the antibodies of the invention to more precisely target tumor cells.

As a preferred mode, the immunoconjugate may comprise an antibody of the present invention and a detectable label. Such detectable labels include, but are not limited to, fluorescent labels, chromogenic labels such as enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron-emitting metals and non-radioactive paramagnetic metal ion. More than one marker can also be included. The label

used to label the antibody for the purpose of detection and / or analysis and / or diagnosis depends on the used particular detection / analysis / diagnosis technique and / or method, eg, immunohistochemical staining (tissue) samples, flow cytometry, and the like. Suitable labels for detection / analysis / diagnosis techniques and / or methods known in the art are well known to those skilled in the art.

As a preferred mode, the immunoconjugate may comprise an antibody of the invention as well as a molecule that targets a surface marker of an immune cell. The molecule that targets surface markers of immune cells can recognize immune cells and carry the antibodies of the invention to the immune cells, so that the antibodies of the invention can target the immune cells to the tumor cells and thus trigger immunocyte for specifically killing tumor.

As a means of chemically generating an immunoconjugate by conjugation, either directly or indirectly (eg, by a linker), the immunoconjugate can be produced as a fusion protein comprising an antibody of the invention and other suitable proteins. The fusion protein can be produced by a method known in the art, for example recombinantly produced by constructing and subsequently expressing the nucleic acid molecule which comprises the nucleotide sequence encoding the antibody in frame with a nucleotide sequence encoding a suitable label.

In another aspect of the invention, a nucleic acid molecule encoding at least one antibody of the invention, a functional variant, or an immunoconjugate thereof is provided. Once obtaining the relevant sequence, the recombination method can be used to obtain the relevant sequence in large quantities. This is usually done by cloning it into a vector, transferring it to a cell, and then isolating the relevant sequence from the proliferating host cells by conventional methods.

The present invention also relates to vectors comprising the appropriate DNA sequences described above as well as appropriate promoters or control sequences. These vectors can be used to transform an appropriate host cell to enable expression of the protein. The host cell may be a prokaryotic cell, such as a bacterial cell; or a lower eukaryotic cell, such as a yeast cell; or a higher eukaryotic cell, such as a mammalian cell.

Pharmaceutical composition

The antibodies, immunoconjugates comprising the antibodies, and genetically modified immune cells of the present invention can be used in the preparation of a pharmaceutical

composition or diagnostic reagent. In addition to an effective amount of the antibody, immunological conjugate, or immune cell, the composition may further comprise a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means that when the molecular entities and compositions are properly administered to animals or humans, they do not cause adverse, allergic or other untoward reactions.

Specific examples of some of the substances which may be used as pharmaceutically acceptable carriers or components thereof are sugars, such as lactose, dextrose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as carboxymethylcellulose sodium, ethylcellulose and methylcellulose; gum tragacanth; malt; gelatin; talc; solid lubricants such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and cocoa butter; polyhydric alcohols such as propylene glycol, glycerin, sorbitol, mannitol and polyethylene glycol; alginic acid; emulsifiers such as Tween®; wetting agents such as sodium lauryl sulfate; coloring agents; flavoring agents; tablets, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline solutions; and phosphate buffers and the like.

The composition of the present invention can be prepared into various dosage forms as needed, and the dosage to be administered to a patient can be determined by a physician according to factors, such as type, age, body weight, and general disease condition of a patient, mode of administration, and the like. For example, injection or other treatment may be used.

The present invention is further described below with reference to specific embodiments. It should be understood that these examples are only for illustrating the present invention and are not intended to limit the scope of the present invention. Experimental procedures in the following examples where no specific conditions are indicated are generally carried out in accordance with the conditions described in customary conditions such as those compiled by J. Sambrook et al., Molecular Cloning Experiments Guide, Third Edition, Science Press, 2002, or according to the manufacturer Suggested conditions.

Example 1. Construction of Cell Lines Stably Expressing Mesothelin

1.1 Construction of plasmid vector

The vector system used in this example belongs to the third generation of self-inactivating lentiviral vector system. The system has three plasmids, packaging plasmid psPAX2 encoding protein Gag / Pol, encoding Rev protein; envelope plasmid PMD2.G encoding VSV-G protein; and recombinant plasmid pWPT-MSLN encoding the extracellular and transmembrane region of the target gene human mesothelin based on empty vector pWPT (purchased from Addgene).

According to GenBank Accession No. NM_005823, the target gene fragment (SEQ ID NO: 1 (nucleotide), 2 (amino acid)) containing signal peptide, Flag tag, extracellular domain and transmembrane region of human mesothelin was synthesized using a gene synthesis method based on bridge-PCR. PCR amplification was performed by primer pairs pWmslnF (SEQ ID NO: 3, GCTTACGCGTCCTAGCGCTACCGGTCGCCACCATGAGGGCCTGGATC) and pWmslnR (SEQ ID NO: 4, CGAGGTCGAC CTAGGCCAGGGTGGAGGCTAGGAGCAGTGCCAGGACGG) under the following conditions: pre-denaturation: 94°C for 4 min; denaturation: 94°C for 30 s; annealing: 58°C for 30 s; extension: 68°C for 80 s; 30 cycles. The theoretical size of the obtained fragment was 1113bp. The amplification product was confirmed by agarose electrophoresis and consistent with the theoretical size. MluI and SalI restriction sites were introduced upstream and downstream to the open reading frame. The target gene obtained above was double-digested with MluI and SalI and ligated into the same double-digested pWPT vector to construct a successful lentiviral vector pWPT-MSLN. The constructed vector was identified by MluI and SalI digestion and sequenced correctly, which was ready for lentivirus packaging.

1.2 Plasmid transfecting 293T cells for packaging lentivirus

293T cells (ATCC: CRL-11268) cultured at passage 6 to passage 10 were seeded at a density of 6×10^6 in 10 cm dishes and cultured overnight at 37°C in 5% CO_2 for transfection. The medium was DMEM (Invitrogen) containing 10% fetal bovine serum (Sigma). And the next day, the medium was changed to serum-free DMEM about 2 hours prior to transfection.

Transfection steps were as follows:

1) 5 μg of target gene plasmid pWPT-MSLN was solved into 500 μl of MillQ water with 7.5 μg of packaging plasmid PAX2 and 2.5 μg of envelope plasmid pMD2.G, respectively, and mixed,

2) 62 μL of 2.5 M $CaCl_2$ (Sigma) was added dropwise and mixed at 1200 rpm / min vortex,

3) Finally, 500 μL of 2 × HBS (280 mM NaCl, 10 mM KCl, 1.5 mM Na_2HPO_4, 12 mM glucose, 50 mM Hepes (Sigma), pH 7.05, and sterized through 0.22 μM filter) was added dropwise and mixed by shaking at 1200 rpm/min for 10 s,

4) Immediately added to the culture dish, gently shake at 37°C, 5% CO_2, cultured for 4 ~ 6h, replaced with DMEM containing 10% fetal bovine serum.

After 48 or 72 hours of transfection, cell debris was removed by centrifugation and the virus was collected by filtration through a 0.45 μm filter (Millipore).

1.3 Recombinant lentivirus infecting PANC-1 cells

The collected virus solution was concentrated and titrated, and cells PANC-1 (purchased from the ATCC) plated in 6 cm plate were infected. Three days after infection, cells were

harvested, part of mixed clones were taken, and lysed with cell lysis liquid. And then, 40 μg of cell protein was subjected to SDS-PAGE gel electrophoresis followed by immunoblotting and staining with mouse anti-Flag-tag antibody. After washing with PBS, the protein was incubated with horseradish peroxidase-labeled goat anti-mouse antibody, washed and finally developed with ECL reagent. Western blot results showed that a band with a molecular weight of about 38 kDa was detected in PANC-1 cells infected with human mesothelin MSLN (i.e., PANC-1-MSLN), while no corresponding band was detected in uninfected empty cells. Remaining cells were expanded, frozen and stored for later experiments.

Example 2. Preparation of human mesothelin antigen

According to GenBank Accession No. NM_005823, the gene fragment of human mesothelin (positions 88-942 of SEQ ID NO: 1 (nucleotide), positions 30-314 of SEQ ID NO: 12 (amino acid)) were synthesized using a gene synthesis method based on bridge-PCR, and PCR amplification was performed. The amplified product was inserted into plasmid vector pCMV-V5 (the vector has 6 × His tag fused and expressed downstream to the multiple cloning site, purchased from Shanghai Rui Jin Biotechnology Co., Ltd.) by NheI/BglII, and transformed into host strain TOP10. Positive clones were picked out, identified by PCR and confirmed by sequencing to obtain recombinant expression plasmid V5-MSLN.

The above expression plasmids were transfected into well-growing HEK-293F cells and cultured continuously at 37°C, 5% CO_2, 12.5 rpm on a shaker for 7 days and centrifuged at 4000rpm for 10min, the pelletes were removed, the supernatant was collected and filtered through a 0.45 μm membrane filter, the processes sample was purified with HisTrap (from GE) affinity chromatography column to finally obtain purified human mesothelin protein, and the identification results are shown in FIG. 1.

Example 3. Screening of single chain antibody against human mesothelin

3.1 Screening of human mesothelin-specific binding antibodies based on phage display

Using phage display technology, human mesothelin specific antibody was screened from the all-human natural antibodies library. For this purpose, glycerol bacteria (purchased from Shanghai Rui Jin Biotechnology Co., Ltd.) from the natural library of phage-displayed all-human single-chain antibody were inoculated in 400 ml of 2 × YT/ampicillin medium so that the cell density reached OD_{600} = 0.1, and incubated at 37°C and 200 rpm until cell density reached OD_{600} = 0.5. Cells were infected with 10^{12} pfu of M13KO7 helper phage (purchased from Invitrogen) and incubated at 30°C and 50 rpm for 30 minutes. After 50 mg/L kanamycin was added and shaking-culture was performed at 37°C and 200 rpm for 30 minutes, the pellet was separated by centrifugation (15 minutes, 1600 × g, 4°C) and

resuspended in 400 ml of 2 × YT/Penicillin/kanamycin medium and shaken for 16 hours at 37°C and 200 rpm. Finally, the pellet was separated by centrifugation (5000 rpm, 4°C for 20 minutes) and discarded. The supernatant was filtered through a 0.45 μm filter and 1/4 volume of 20% (w/v) PEG 8000, 2.5 M NaCl solution was added and incubated in an ice bath for 1 hour to precipitate bacteriophage pellets. The pellet was then percipitated by centrifugation (20 min, 8000 × g, 4°C) and the supernatant discarded. The phage were resuspended in 25 ml of prechilled PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2PO_4) and centrifuged (5 minutes, 20000 × g, 4°C). 1/4 volume of 20% (w/v) PEG8000, 2.5 M NaCl solution was added to the supernatant and incubated in an ice bath for 30 minutes to precipitate phage particles again. The pellets were centrifuged (30 min, 20000 × g, 4°C) and the phage pellets were resuspended in 2 ml of prechilled PBS again, kept on ice for 30 min and centrifuged (30 min, 17000 xg, 4°C). Supernatants were mixed with 4% (w/v) BSA in PBS at 1: 1, placed on a rotary mixer and incubated for 30 minutes at room temperature before being directly used for screening.

Using the above phage antibody library, four rounds of directional screening were performed on biotinylated human mesothelin recombinant protein with the following scheme: The phage antibody library was incubated with biotin-labeled antigen mesothelin at room temperature for 2 hours and then incubated with streptavidin magnetic beads MyOne Cl (from Invitrogen) blocked with 2% (w/v) BSA (bovine serum albumin) at room temperature for 30 minutes. The beads were then washed with PBST (containing 0.1% Tween-20) buffer to remove phages which were not specifically bound or with weak binding capacities. Strongly-binding phages were then eluted from magnetic beads with glycine-HCl (pH 2.2), neutralized with Tris neutralizing solution (pH 9.1), and used to infect E. coli ER2738 in the mid-logarithmic growth phase and for the next round of screening. In the four rounds of screening, the beads were used in an amount of 50 μl, 20 μl, 10 μl and 10 μl, and the concentrations of biotin-labeled human mesothelin were 100 nM, 10 nM, 5 nM and 1 nM, respectively, and the time for PBST-washing was 10, 10, 15 and 20, respectively.

3.2 Identification of human mesothelin-specific binding antibodies

96 clones were randomly selected in the clones obtained from the fourth round of screening and their binding capability to human mesothelin was analyzed by single phage ELISA (enzyme-linked immunosorbent assay). For this purpose, each single colony was inoculated in 300 μl of 2 × YT/ampicillin medium (containing 2% glucose) in a 96-well deep-well plate and cultured with shaking at 37°C and 250 rpm for 16 hours. 20 μl of culture was inoculated into 500 μl of 2 × YT/ampicillin medium (containing 0.1% glucose) and shaken at 37°C and 250 rpm for 1.5 hours. To prepare the helper phage solution, 75 μl of M13KO7 (titer of 3×10^{12} pfu/ml) was taken and mixed into 15 ml of 2 × YT medium and

added into a culture plate at 50 μl/well, and incubated at 37°C and 150 rpm for 30 minutes. And then prepared kanamycin solution (180 μl of 50 mg/ml kanamycin was taken and added into 15 ml of 2 × YT medium) was added at 50 μl/well and incubated with shaking for 16 hours at 37°C and 250 rpm. Finally, cells were precipitated by centrifugation (30 mins, 5000 × g, 4°C) and the supernatant was transferred to a new 96-well deep-well plate.

For single phage ELISA, 100 ng/well of antigen human mesothelin and negative control protein BSA (100 μl/well) were used in a 96-well MediSorp ELISA plate (purchased from Nunc) and coated overnight at 4°C. Each well was blocked with PBST containing 2% BSA (w/v). The wells were then washed with PBST for three times and PBST was discarded. Then, each phage solution prepared above was added into each well of the plate at 100 μl/well. After incubated at 37°C for 2 hours, the plate was washed for three times with PBST. To detect bound phage, anti-M13 antibody peroxide dismutase conjugate (purchased from GE Healthcare) was diluted at 1: 5000 in PBST and 100 μl was taken and added into each well. After incubated at 37°C for 1 hour, the wells were rinsed for three times with PBST and then rinsed for three times with PBS. Finally, 50 μl of TMB substrate was pipetted into the wells and developed for 10 minutes at room temperature, followed by addition of 50 μl of 2M H_2SO_4 per well to quench the color reaction. Extinction values were measured at 450 nm with an enzyme-linked immunosorbent (Bio-Rad). Two different single chain antibodies P1A6E (SEQ ID NO: 5 (nucleotide), 6 (amino acid)) and P3F2 (SEQ ID NO: 7 (nucleotide), 8 (amino acid)) were observed with sequencing analysis, which exhibited significantly stronger binding signal to human mesothelin (hu-mesothelin) in ELISA assay, while not binding to BSA (Figure 2).

SEQ ID NO: 5 (nucleotide)

caggtacagctggaacagtcaggtctaggactggtgaagccctcgcagaccctctctctcacctgtgccatctccggggacactgtctctag
cgacagtgctgcttggaactggatcaggcagtccccatcgagaggccttgagtggctgggaaggacatactacaggtccaagtggtttaatgattatg
cagtatctgtgaaaggtcgaataaccatcaactcagacacatccaagaaccagttctccctgcagttgaactctgtgactcccgaggacacggctgtgt
attattgtgcaagaagtaatagttactactactacgctatggacgtctggggccaaggcaccctggtcaccgtctcgagtggtggaggcggttcaggc
ggaggtggttctggcggtggcggatcgcaggctgtgctgactcagccgtcttccctctctgcatctcctggagcatcagccagtctcacctgcaccttg
cgcagtggcatcaatgttggtatctacaggatatactggtaccaacagaggccagggagtcctccccagattctcctgacttacaaatcagactcagat
aagtaccagggctctggagtccccagtcgcttctctggatccaaagatgcttcggccaatgcagggattttactcatctctgggctccagtctgaagatg
aggctgactattactgcatgatttggcacagcggcggttgggtgttcggcggagggaccaaggtcaccgtcctaggt

SEQ ID NO: 6 (amino acid)

QVQLEQSGLGLVKPSQTLSLTCAISGDTVSSDSAAWNWIRQSPSRGLEWLGRTYYRSKWFN
DYAVSVKGRITINSDTSKNQFSLQLNSVTPEDTAVYYCARSNSYYYYAMDVWGQGTLVTVSSGG
GGSGGGGSGGGGSQAVLTQPSSLSASPGASASLTCTLRSGINVGIYRIYWYQQRPGSPPQILLTYKS
DSDKYQGSGVPSRFSGSKDASANAGILLISGLQSEDEADYYCMIWHSGGWVFGGGTKVTVLG

Wherein the amino acid sequence of the heavy chain variable region is shown in positions 1 to 123 of SEQ ID NO: 6 and the amino acid sequence of the light chain variable region is shown in positions 139 to 254 of SEQ ID NO: 6.

Wherein the amino acid sequence of light chain CDR1 is TLRSGINVGIYRIY (SEQ ID NO:

51), the amino acid sequence of light chain CDR2 is YKSDSDKYQGS (SEQ ID NO: 52), the amino acid sequence of light chain CDR3 is MIWHSGGWV (SEQ ID NO: 53); the amino acid sequence of heavy chain CDR1 is GDTVSSDSAAWN (SEQ ID NO: 54), the amino acid sequence of heavy chain CDR2 is RTYYRSKWFNDYAVSVKG (SEQ ID NO: 55), and the amino acid sequence of heavy chain CDR3 is SNSYYYYAMDV (SEQ ID NO: 56).

SEQ ID NO: 7 (nucleotide)

cagatgcagctagtgcagtctggggctgaggtgaagaagcctggggcctcagtgaaggtttcctgcaaggcatctggatacaccttcacca
gctactatatgcactgggtgcgacaggcccctggacaagggcttgagtggatgggaataatcaaccctagtggtggtagcacaagctacgcacagaa
gttccagggcagagtcaccatgaccagggacacgtccacgagcacagtctacatggagctgagcagcctgagatctgaggacacggccgtgtatta
ctgtgcgagtagtcggagtgggactacggtggtaaatcatgatgcttttgatatctgggggaaagggaccacggtcaccgtctcgagtggtggaggc
ggttcaggcggaggtggttctggcggtggcggatcggacatccagttgacccagtctccatcctccctgtctgcgtctgtaggagacagagtcaccat
cacttgccgggcaagccaggtcattagccgtgctttagcctggtatcaacaaacaccagggaaacctcctaaactcctgatctatgatgcctccaatttg
cagagtggggtcccatcaaggttcagcggcagtggatctgggacagatttcactctcaccatcagccgcctgcagcctgaagattttgcaacttattact
gtcaacagtttaatagttaccctctcactttcggcggagggaccaagctggagatcaaacgt

SEQ ID NO: 8 (amino acid)

QMQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGSTS
YAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCASSRSGTTVVNHDAFDIWGKGTTVTVS
SGGGGSGGGGSGGGGSDIQLTQSPSSLSASVGDRVTITCRASQVISRALAWYQQTPGKPPKLLIYD
ASNLQSGVPSRFSGSGSGTDFTLTISRLQPEDFATYYCQQFNSYPLTFGGGTKLEIKR

Wherein, the amino acid sequence of the heavy chain variable region is shown in positions 1 to 124 of SEQ ID NO: 8; the amino acid sequence of the light chain variable region is shown in positions 140-247 of SEQ ID NO: 8.

Wherein the amino acid sequence of light chain CDR1 is RASQVISRALA (SEQ ID NO: 57), the amino acid sequence of light chain CDR2 is DASNLQS (SEQ ID NO: 58), the amino acid sequence of light chain CDR3 is QQFNSYPLT (SEQ ID NO: 59); the amino acid sequence of heavy chain CDR1 is GYTFTSYYMH (SEQ ID NO: 60), the amino acid sequence of heavy chain CDR2 is IINPSGGSTSYAQKFQG (SEQ ID NO: 61) and the amino acid sequence of heavy chain CDR3 is SRSGTTVVNHDAFDI (SEQ ID NO: 62).

Example 4. Preparation of single chain antibody and monoclonal antibody

4.1 Preparation of single chain antibody against human mesothelin

scFv-P1A6E fragment was amplified from the resulting clones using primer pair V5-P1A6E-F (SEQ ID NO: 9) and V5-P1A6E-R (SEQ ID NO: 10); scFv-P3F2 fragment was amplified using primer pair V5-P3F2-F (SEQ ID NO: 11) and V5-P3F2-R (SEQ ID NO: 12), digested by NheI / BamHI restriction enzyme, connected to NheI/BamHI double-digested vector plasmid pCMV-V5-Fc (in the vector, Fc fragment of human antibody IgG1 was fused downstream to multiple cloning sites, hereinafter referred to as V5-Fc, purchased from Shanghai Rui Jin Biotech Co., Ltd.) with T4 DNA ligase, and transformed into host strain TOP10. Clones were picked up, and positive clones were identified by PCR and confirmed by sequencing to obtain eukaryotic expression plasmids, V5-scFv-P1A6E-Fc and V5-scFv-P3F2-Fc, respectively.

SEQ ID NO: 9：ACAGTGCTAGCACAGGTACAGCTGGAACAG；
SEQ ID NO: 10：TTGTCGGATCCACCTAGGACGGTGACC；
SEQ ID NO: 11：ACAGTGCTAGCACAGATGCAGCTAGTGC；
SEQ ID NO: 12：TTGTCGGATCCACGTTTGATCTCCAGC。

The above expression plasmids were transfected into well-growing HEK-293F cells respectively, cultured at 37°C, 5% CO_2, 125rpm on a shaker continuously for 7 days, centrifuged at 4000rpm for 10min. Pelletes were removed, and the supernatant was collected and filtered with 0.45 μm membrane. The processed sample was affinity-purified with protein A (from GE) affinity column to finally obtain the purified antibody-Fc fusion proteins scFv-P1A6E-Fc and scFv-P3F2-Fc. The identification results are shown in FIG. 3.

4.2 Preparation of monoclonal antibody against human mesothelin

In this example, the monoclonal antibody was expressed using a two-plasmid system. The gene of antibody heavy chain variable region shall be constructed into pIH plasmid containing human IgG1 CH gene, and the gene of antibody light chain variable region be constructed into PIK plasmid containing human IgG CL gene (plasmid purchased from Shanghai Rui Jin Biotechnology Co., Ltd.).

VH-P1A6E fragment was amplified from the template plasmid V5-scFv-P1A6E-Fc using primer pair P1A6E-HF (SEQ ID NO: 13, gcctttcctggtttcctgtctcaggtacagctgg aacagtc) and P1A6E-HR (SEQ ID NO: 14, GATGGGCCCTTGGTGGAGGCACTCGAGACGGTGACCAG). HF1 fragment was amplified from the template plasmid pIH using primer pair HF1F (SEQ ID NO: 15, ggctaactagagaacccactgc) and HF1R (SEQ ID NO: 16, AGACAGGAAACCAGGAAAGGC); and HF3 fragment was amplified from the template plasmid pIH primers HF3F (SEQ ID NO: 17, gcctccaccaagggcccatc) and HF3R (SEQ ID NO: 18, gacaatcttagcgcagaagtc). The three fragments were mixed at equimolar ratio, and then splicing-PCR was performed. Fragments were recovered by restriction endonuclease NheI / NotI double digestion and connected into NheI/NotI double-digested vector plasmid pIH with T4 DNA ligase and transformed into host strain TOP10. Clones were picked out and the positive clones were identified by PCR and confirmed by sequencing to obtain pIH-P1A6E eukaryotic expression plasmid. pIH-P3F2 eukaryotic expression plasmid was also obtained in the same manner.

To obtain pIK-P1A6E eukaryotic expression plasmid, VL1-P1A6E fragment was obtained from the template plasmid V5-scFv-P1A6E-Fc using the primer pair P1A6E-LF (SEQ ID NO: 19, ctttggtttccaggtgcaagatgtcaggctgtgctgactcag) and P1A6E-LR (SEQ ID NO: 20, GAAGACAGATGGTGCAGCCACCGTACCTAGGACGGTGACCTTG); LF1 fragment was amplified from the template plasmid pIK using the primer pair LF1F (SEQ ID NO: 21, ggctaactagagaacccactgc) and LF1R (SEQ ID NO: 22, ACATCTTGCACCTGGAAACCAAAG); LF3 fragment was amplified from the template plasmid pIK using the primer pair LF3F (SEQ ID NO: 23, acggtggctgcaccatctgtcttc) and LF3R (SEQ ID NO: 24,

GACAATCTTAGCGCAGAAGTC). The three fragments were mixed in equimolar ratio for splicing PCR. After the fragments were recovered, the fragments were digested with EcoRV / NotI restriction endonucleases and ligated in EcoRI/NotI double-digested vector plasmid pIK with T4 DNA ligase, and transformed into host strain TOP10. Clones were picked out and positive clones were identified by PCR and confirmed by sequencing. pIK-P3F2 eukaryotic expression plasmid was also obtained in the same manner.

Expression plasmids pIH-P1A6E and pIK-P1A6E were equimolarly mixed, pIH-P3F2 and pIK-P3F2 were equimolarly mixed and transfected into well-growing HEK-293F cells respectively. The cells were cultured at 37°C, 5% CO_2, 125rpm on a shaker continuously for 7 days, centrifuged at 4000rpm for 10min. Pelletes were removed, and the supernatant was collected and filtered with 0.45 μm membrane. The processed sample was affinity-purified with protein A (from GE) affinity column to finally obtain the purified antibody P1A6E and P3F2. The identification results are shown in FIG. 4.

Example 5. Affinity of antibody against human mesothelin

To quantitatively analyze the binding of an antibody to human mesothelin, the affinity and kinetic parameters of single-chain antibody and monoclonal antibody of P1A6E and P3F2 were measured by capture method using Biacore T200 system (from GE). An anti-human IgG (Fc) antibody (purchased from GE) was coupled to carboxymethyl dextran surface of sensor chip CM5 through primary amino with NHS / EDC coupling according to the manufacturer's instructions. Measurements were performed in 1 × HBS-EP + working buffer at 25°C, 30 μl/min, and regeneration condition was 3 M MgCl2, 10 μl/min for 30 seconds. In each round of the testing cycle, the antibody to be tested is firstly captured onto the chip. Analyte (human mesothelin) of a certain concentration flowed over the chip surface. Due to the produced SPR signal, the interaction between human mesothelin and the captured antibody can be detected. The detected signal is defined as resonance unit (RU), which was plotted *vs* time (second) to obtain the corresponding binding curve and dissociation curve. In different test cycles, concentrations of human mesothelin were 10 nM, 20 nM, 40 nM, 80 nM and 160 nM, respectively. The resulting curves were evaluated using Biacore T200 evaluation software and the affinity KD values were calculated. Figure 5 and Figure 6 show kinetic curves of the monoclonal antibodies P1A6E and P3F2, in a Biacore Affinity Assay, respectively. The binding data for single-chain antibody and monoclonal antibody of P1A6E and P3F2 to human mesothelin are summarized in Table 1.

Table 1. Affinity parameters of single chain antibodies and monoclonal antibodies of P1A6E and P3F2 to human mesothelin

Sample of antibody	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
P1A6E monoclonal antibody	2.88E+05	1.16E-03	4.04E-09

P3F2 monoclonal antibody	1.10E+05	7.89E-04	7.17E-09
P1A6E single chain antibody	8.25E+04	1.56E-03	1.89E-08
P3F2 single chain antibod	5.77E+04	9.35E-04	1.62E-08

Example 6. Cell-binding properties of antibody to human mesothelin (single-chain antibody and monoclonal antibody)

Each of antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc was analyzed for binding ability to mesothelin at cell surface by Fluorescence Activated Cell Sorter (FACS) (Guava 8HT, supplied by Millipore).

Specific methods are as follows:

1) inoculating PANC-1-MSLN and PANC-1 in logarithmic growth phase into a 6 cm dish respectively at inoculation cell density of about 90%, and incubating overnight at 37°C in an incubator.

2) digesting cells with 10 mM EDTA, collecting cells through centrifugation at 200 g × 5 mins, and resuspending cells in 1% phosphate buffered saline (NBS PBS) containing calf serum at 1×10^6 to 1×10^7/mL into a flow-specific tube in an amount of 100 μl per tube.

3) centrifuging at 200g × 5min, and discarding the supernatant.

4) antibodies P1A6E and P3F2 to be tested were added into the two experimental groups, respectively, adding antibodies ss and C10 (purchased from Shanghai Rui Jin Biotechnology Co., Ltd.) were added into two positive control groups added as positive controls, and another control group is PBS blank control without antibody. The final concentration of each antibody was 20 μg/ml. 100 μl was added to each tube, and incubated in an ice bath for 45 minutes.

5). Adding 2 ml of 1% NBS PBS to each tube and centrifuging at 200 g × 5 min for two times.

6) Discarding the supernatant and adding goat anti-human antibody-FITC (Shanghai Karrie Biotech Co., Ltd.) at a dilution of 1: 100 with 100ul being added to each tube, incubating in an ice bath for 45 minutes.

7). Adding 2 ml of 1% NBS PBS into each tube, centrifuging at 200 g × 5 min for two times.

8) Discarding the supernatant, resuspending in 300 ul of 1% NBS PBS and detecting by flow cytometry.

9) Analyzing the data using flow cytometry data analysis software Flowjo7.6.

Flow cytometry results showed that four antibodies, P1A6E and P3F2, as well as control antibodies SS and C10, either in single-chain antibody format (Figure 7) or in monoclonal full antibody format (Figure 8, fluorescence peak of PANC-1-MSLN cells was significantly different from that of blank control (PBS) (FIG. 7B, FIG. 8B), while no significant difference from PANC-1 cells (FIG. 7A, FIG.

8A)), can specifically recognize PANC-1-MSLN cells stably expressing human mesothelin, but do not bind to human mesothelin-negative PANC-1 cells, indicating that the four antibodies could specifically recognize human mesothelin.The fluorescence peaks of antibodies P1A6E and P3F2 were significantly stronger than those of control antibodies SS and C10, indicating that the binding efficiencies of P1A6E and P3F2 to PANC-1-MSLN cells are higher than those of SS and C10.

Example 7. Preparation of CAR T containing antibody to human mesothelin

To construct a chimeric antigen receptor, the connection order of the parts of the chimeric antigen receptor exemplified in the present invention is shown in Table 2.

Table 2

Chimeric antigen receptor	Extracellular binding region - transmembrane region - intracellular signal region 1 - intracellular signal region 2 and the like	Description
P1A6E-δZ	scFv(MSLN)-CD8-CD3δzeta	Negative control
P1A6E-Z	scFv(MSLN)-CD8-CD3 zeta	1st generation
P1A6E-BBZ	scFv(MSLN)-CD8-CD137-CD3 zeta	2nd generation
P1A6E-28Z	scFv(MSLN)-CD28a-CD28b-CD3 zeta	2nd generation
P1A6E-28BBZ	scFv(MSLN)-CD28a-CD28b-CD137-CD3 zeta	3rd generation
P3F2-δZ	scFv(MSLN)-CD8-CD3δzeta	Negative control
P3F2-Z	scFv(MSLN)-CD8-CD3 zeta	1st generation
P3F2-BBZ	scFv(MSLN)-CD8-CD137-CD3 zeta	2nd generation
P3F2-28Z	scFv(MSLN)-CD28a-CD28b-CD3 zeta	2nd generation
P3F2-28BBZ	scFv(MSLN)-CD28a-CD28b-CD137-CD3 zeta	3rd generation

Note: CD28a represents the transmembrane region of CD28 molecule and CD28b represents the intracellular signaling region of CD28 molecule.

The lentiviral plasmid vector system used in the present example belongs to lentiviral 4-plasmid system of the third generation, which has 4 plasmids, namely, envelope plasmid pCMV-VSV-G encoding VSV-G protein (from addgene), packaging plasmid pRSV-REV encoding Rev protein (from addgene); pMDLg/pRRE encoding Gal and Pol (from addgene) and the recombinant expression vector encoding the gene of interest CAR based on empty vector pRRLSIN-cPPT.PGK-GFP.WPRE (from addgene). For the promoter in all vectors of CAR gene, elongation factor-1α (EF-1α) of the vector disclosed in 201310164725.X was used. Specific construction method is as follows:

(1) Obtaining Promoter Fragment: a fragment with the promoter EF-1α was amplified by PCR using the vector pWPT-eGFP-F2A-CAR, primers pwpxlF (SEQ ID NO: 25, 5'-gcaggggaaagaatagtaga ca-3') and pWPT-MluIR (SEQ ID NO: 26, 5'-aggccagcggcaggagcaaggcggtcactggta aggccatggtggcgaccggtagc-3').

(2) Obtaining fragment of target CAR: P1A6E part and P3F2 part of the target CAR fragment was amplified using the above aobtained V5-scFv- P1A6E-Fc and V5-scFv- P3F2-Fc as templates and using primers P1A6E-F (SEQ ID NO: 27,

5'-ctcctgccgctggccttgctgctccacgccgccaggccgcaggtacagc tggaaca-3') and primer P1A6E-R (SEQ ID NO: 28, 5'-gcggcgctggcgtcgtggtacctaggacggtgacc-3'), primer P3F2-F (SEQ ID NO: 29, 5'ctcctgccgctggccttgctgctccacgccgccaggccgcagatgcagctagt gca-3') and P3F2-R (SEQ ID NO: 30, 5'gcggcgctggcgtcgtggtacgtttgatctccag-3').

(3) The first, second, third generation of consensus sequence and negative control sequence of CAR were obtained by PCR: fragments CD8-CD3δ zeta(δZ), CD8-CD3 zeta (Z), CD28a-CD28b-CD3 zeta (28Z) and CD28a-CD28b-CD137-CD3 zeta (28BBZ) sequences were obtained by using pWPT-eGFP-F2A-GPC3-δZ, pWPT-eGFP-F2A-GPC3-Z, pWPT-eGFP-F2A-GPC3-28Z and pWPT-eGFP-F2A-GPC3-28BBZ in 201310164725.X as templates and primer HF (SEQ ID NO: 63, 5'accacgacgccagcgccgcgaccac) and primer pwpxlR (SEQ ID NO: 64, 5'-tagcgtaaaaggagcaacatag), respectively.

(4) fragments of consensus sequence CD8-CD137-CD3 zeta (BBZ) were synthesized using a gene synthesis method based on bridge-PCR with reference to BBZ sequence in US 8,911,993 B2 (COMPOSITIONS FOR TREATMENT OF CANCER).

(5) After the above obtained promoter fragment, target CAR fragments and fragments of consensus sequence CD8-CD3δ zeta(δZ), CD8-CD3 zeta(Z), CD8-CD137-CD3 zeta(BBZ), CD28a-CD28b-CD3 zeta(28Z) and CD28a-CD28b-CD137-CD3 zeta(28BBZ) were respectively routinely bridged, primers pwpxlF and pwpxlR were used for amplification to obtain fragments containing the EF-1α and target gene CAR and respectively referred to as:

P1A6E-δZ (SEQ ID NO: 31);
P1A6E-Z (SEQ ID NO: 32);
P1A6E-BBZ (SEQ ID NO: 33);
P1A6E-28Z (SEQ ID NO: 34);
P1A6E-28BBZ (SEQ ID NO: 35).
P3F2-δZ (SEQ ID NO: 36);
P3F2-Z (SEQ ID NO: 37);
P3F2-BBZ (SEQ ID NO: 38);
P3F2-28Z (SEQ ID NO: 39);
P3F2-28BBZ (SEQ ID NO: 40).

(6) The CAR fragment with the promoter and the target gene CAR obtained in the above step was double-digested with ClaI and SalI and ligated into the same digested vector pRRLSIN.cPPT.PGK-GFP.WPRE to construct a lentiviral vector expressing each chimeric antigen receptor. The successfully constructed vector was identified by Mlu and Sal digestion and confirmed by sequencing for lentivirus packaging.

The resulting vectors containing each target CAR are as follows:

pRRLSIN-EF1α-P1A6E-δZ;
pRRLSIN-EF1α-P1A6E-Z;
pRRLSIN-EF1α-P1A6E-BBZ;
pRRLSIN-EF1α-P1A6E-28Z;
pRRLSIN-EF1α-P1A6E-28BBZ;
pRRLSIN-EF1α- P3F2-δZ;

pRRLSIN-EF1α- P3F2-Z;
pRRLSIN-EF1α- P3F2-BBZ;
pRRLSIN-EF1α- P3F2-28Z;
pRRLSIN-EF1α- P3F2-28BB.

Through the above construction, 10 CAR polypeptide sequences can be obtained respectively, which are referred to as:

P1A6E-δZ (SEQ ID NO: 41);
P1A6E-Z (SEQ ID NO: 42);
P1A6E-BBZ (SEQ ID NO: 43);
P1A6E-28Z (SEQ ID NO: 44);
P1A6E-28BBZ (SEQ ID NO: 45).
P3F2-δZ (SEQ ID NO: 46);
P3F2-Z (SEQ ID NO: 47);
P3F2-BBZ (SEQ ID NO: 48);
P3F2-28Z (SEQ ID NO: 49);
P3F2-28BBZ (SEQ ID NO: 50).

Transfection of 293T by Plasmid for packaging lentivirus

HEK-293T cells (ATCC: CRL-11268) cultured at passage 6 to passage 10 were seeded at a density of 6×10^6 in 10 cm dishes and cultured overnight at 37°C in 5% CO_2 for transfection. The medium was DMEM containing 10% fetal bovine serum.

Transfection steps are as follows:

Preparation of liquid A: dissolving 10 μg of desired gene plasmids pRRLSIN-cPPT.EF-1α-CAR (selected from pRRLSIN-EF1α-P1A6E-δZ, pRRLSIN-EF1α-P1A6E-Z, pRRLSIN-EF1α-P1A6E-BBZ, pRRLSIN-EF1α-P1A6E-28Z, pRRLSIN-EF1α-P1A6E-28BBZ, pRRLSIN-EF1α-P3F2-Z, pRRLSIN-EF1α-P3F2-BBZ, pRRLSIN-EF1α-P3F2-28Z, pRRLSIN-EF1α-P3F2-28BBZ) with 7.5 μg of packaging plasmid pMDLg RRE and pRSV-REV and 3 μg of envelope plasmid pCMV-VSV-G into 800 μL of serum-free DMEM medium and mixing well.

Preparation of liquid B: dissolving 60 μg PEI (polyethylenimine 1 μg/μl, purchased from Polysciences) in 800μL serum-free DMEM medium, mixing gently and incubating at room temperature for 5min.

Formation of transfection complex: adding liquid A into liquid B and gently mixing, vortexing or gently mixing immediately after addition, incubating at room temperature for 20min.

Adding 1.6 ml of the transfection complex into HEK-293T cells dropwise, and after 4-5 h, changing to DMEM with 2% FBS for transfected 293T cells.

After 72 h of transfection, the virus was collected by filtration using a 0.45 μm filter and centrifuged at 28,000 rpm using a Beckman Optima L-100XP ultracentrifuge for 2 hours at 4°C. The supernatant was discarded and the resulting pellet was centrifuged at 1/10 ~ 1/50 stock solution of AIM-V medium (purchased from Invitrogen) and resuspend at 100 μL/tube in -80°C for virus titration or infection of T lymphocytes.

Example 8. Infection of CTL cells by Recombinant lentivirus

Human peripheral blood mononuclear cells were obtained from healthy human peripheral blood by density gradient centrifugation (supplied by Shanghai Blood Center), and CTLs were obtained from peripheral blood mononuclear cells by negative sorting method using CTL magnetic beads (purchased from Stem Cell Technologies). Sorted CTL cells were subjected to flow cytometry to detect the purity of CTL cells. The positive rate of CTL cells $\geqslant$ 95% was appropriate for the next step. Cells were added in Quantum 007 lymphocyte medium (purchased from PAA) at a density of about 1×10^6 / mL. Magnetic beads coated with anti-CD3 and CD28 antibodies (Invitrogen) were added in a 1: 1 ratio of cells to magnetic beads, and recombinant human IL-2 (purchased from Shanghai Huaxin Biotechnology Co., Ltd.) at a final concentration of 300U/mL was added for stimulation and culture for 24 h. And then CTL cells were infected with the above recombinant lentivirus at MOI $\approx$ 5. The infected cells were passaged every other day at a density of 5×10^5/mL and recombinant human IL-2 at a final concentration of 300 U / mL was supplemented in the lymphocyte culture medium.

Infected CTL cells were detected by flow cytometry on day 8 of culture for the expression of different chimeric antigen receptors. Firstly, the infected CAR T cells were incubated with biotinylated human mesothelin recombinant protein for 1 h at 37°C, washed in D-PBS twice and then incubated with PE-labeled streptavidin for 40 min at 37°C. After washed with D-PBS for 3 times, the ratio of positive cells was determined by flow cytometry. Uninfected T lymphocytes was used as a negative control, the positive rates of virus-infected T cells expressing different chimeric antigen receptors are shown in Table 3. The positive rate results show that a certain positive rate of CAR^+T cells can be obtained by lentivirus infection.

Table 3

CTL cells transfected by following CARs	Positive rate of CTL cells transfection
P1A6E -δZ (negative control)	75%
P1A6E -Z	58%
P1A6E-BBZ	85%
P1A6E-28Z	73%
P1A6E-28BBZ	69%
P3F2 -δZ (negative control)	71%
P3F2 -Z	68%
P3F2-BBZ	83%
P3F2-28Z	86%
P3F2-28BBZ	77%

CTL cells were infected with viruses that had different chimeric antigen receptors packaged, respectively, and then subcultured at a cell density of 5×10^5 / ml quaque die alterna, counted, and supplemented with IL-2 (final concentration of 300 U / ml). On the 11th day of culture, about 20 ~

40 times of amplification was obtained, indicating that the CTL cells expressing different chimeric antigen receptors can be expanded in a certain amount *in vitro*, which ensures subsequent *in vitro* toxicity tests and *in vivo* experiments.

Example 9. *in vitro* toxicity test of T lymphocytes expressing chimeric antigen receptors

In vitro toxicity experiments used the following materials:

Mesothelin-negative pancreatic cancer cell line (PANC-1) and PANC-1 (PANC-1-MSLN) cell line transfected with mesothelin gene as shown in Table 4 were used as target cells and effector cells were CTL cultured for 12 days *in vitro*, which were verified in Example 4 and detected chimeric antigen receptor-expression positive by FACS. Effective target ratios were 3: 1, 1: 1 and 1: 3, respectively. The number of target cells was 10000/well, and effector cells corresponded to different effective target ratio. Each group had 5 replicate wells, average of 5 wells was calculated, and detection time was 18h.

Each experimental group and each control group are listed as follows:

Each experimental group: each target cell + CTL expressing different chimeric antigen receptors;

Control group 1: target cells with maxium LDH release;

Control group 2: target cells with spontaneous LDH release;

Control group 3: effector cells with spontaneous LDH release.

Detection method: CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega) is used, which is a colorimetric based assay that can replace 51Cr release assay. CytoTox 96® Assay measures lactate dehydrogenase (LDH) quantitatively. LDH is a stable cytosolic enzyme that is released upon lysis of cells and is released in the same way as radioactive 51Cr is released. The supernatant with released LDH medium can be detected by a 30-minute coupled enzyme reaction in which LDH converts a tetrazolium salt (INT) to a red formazan. The amount of red product produced is proportional to the number of lysed cells. Details can be found in instructions of CytoTox 96 non-radioactive cytotoxicity detection kit.

Cytotoxicity is calculated as:

Cytotoxicity % = [(experiment group – control group 2 – control group 3)/(control group 1 – control group 2)]×100

Specifically, as shown in Table 4, the CARs of anti-mesothelin single chain antibody (P1A6E，P3F2) of the present invention exhibited significant killing activity on mesothelin-positive pancreatic cancer cells, and the second and third generations of anti-mesothelin CAR T cells were slightly more potent than the antitumor activity of the first generation. There was no siginificant killing effects in mock group. In addition, all CAR T

cells showed no cytotoxic activity on mesothelin-negative PANC-1 pancreatic cancer cells. These results indicate that anti-mesothelin CAR T cells of the invention (including 1st, 2nd, and 3rd generation of CAR T) can selectively target mesothelin-positive pancreatic cancer cells and kill them effectively. In addition, the first, second and third generation of anti-mesothelin CAR T of the present invention exhibited a effector target ratio gradient dependency, that is, the higher the effector target ratio, the stronger the cytotoxic effects.

Table 4. *In vitro* anti-tumor activity of CAR T cells having single chain antibody fused and expressed

CYTOTOXICITY (%)	P1A6E -28BBZ			P1A6E -BBZ			P1A6E -28Z			P1A6E -Z			P1A6E -δZ (mock)		
	Different effector target ratio			Different effector target ratio			Different effector target ratio			Different effector target ratio			Different effector target ratio		
	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3
PANC-1-MSLN	95.3	63.8	32.5	86.5	56.7	25.3	89.4	58.1	23.9	63	33.1	13.7	2.5	1.8	3.6
PANC-1	3.5	4.3	2.2	1.8	2.3	3.4	2.3	3.9	2.8	1.7	2.7	3.5	2.1	2.4	2.8

CYTOTOXICITY (%)	P3F2-28BBZ			P3F2 -BBZ			P3F2 -28Z			P3F2 -Z			P3F2-δZ(mock)		
	Different effector target ratio			Different effector target ratio			Different effector target ratio			Different effector target ratio			Different effector target ratio		
	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3	3:1	1:1	1:3
PANC-1-MSLN	85.6	62.9	30.7	89.3	60.2	31.9	92.	59.3	28.7	58.4	29.9	15.7	3.1	1.5	2.8
PANC-1	2.8	3.1	2.5	4.8	1.8	3.6	4.1	1.7	2.8	3	3.9	2.2	1.8	3.3	3.5

Example 10. Epitope analysis of antibody to human mesothelin

Human mesothelin gene fragment was amplified by PCR from SEQ ID NO: 1 and ligated into eukaryotic expression vector pCMV-V5-muFc containing mouse Fc fragment by NheI/BamHI double-digestion. HEK-293F cells were transiently transfected according to Example 4 and the culture supernatant of cells was processed and affinity-purified through protein G (from GE) affinity column to finally obtain purified human mesothelin fragment-muFc fusion protein, and the binding of antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc was identified through ELISA. The mature human mesothelin is divided into three regions, region R1 (E296-T390, SEQ ID NO: 66), region R2 (S391-Q486, SEQ ID NO: 67), region R3 (N487-G581, SEQ ID NO: 68) according to Genbank Accession No. NP_001170826.1 (SEQ ID NO: 65). ELISA results showed that both of antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc only bind to region 1 (E296-T390). Region 1 was further divided into 5 small fragments, and fused and expressed with muFc, respectively. Region R1A (296E-337D, SEQ ID NO: 69), Region R1B (328D-369I, SEQ ID NO: 70), Region R1C (360Y- 405T, SEQ ID NO: 71), Region R1AB (296E- 359L, ID NO: 72), R1BC (328D-405T, SEQ ID NO: 73). The results from ELISA are shown in FIG. 9 and FIG. 10, in which the

antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc significantly bound to region R1AB while weakly bound to region R1A and not bound to region R1B. Therefore, the binding sites for the antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc should be located around the sites where R1A and R1B overlap. This region contains 10 amino acids "DAALLATQMD", based on which 10 amino acids or 5 amino acids were extended to both ends to form two peptides R1J10: "YKKWELEACVDAALLATQMDRVNAIPFTYE (SEQ ID NO: 74)" and R1J5: "LEACVDAALLATQMDRVNAI (SEQ ID NO: 75)" and fused and expressed with muFc, respectively. ELISA results showed that antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc did not bind to R1J10 or R1J5. Based on the above results, the epitopes of the antibodies scFv-P1A6E-Fc and scFv-P3F2-Fc should be a conformational epitope located in region R1AB (SEQ ID NO: 72).

All references mentioned in the present invention are incorporated herein by reference, as if each reference was individually incorporated by reference. In addition, it should be understood that after reading the above teachings of the present invention, those skilled in the art can make various modifications or changes to the present invention, and these equivalent forms also fall within the scope of the appended claims of the present application.

Claims

1. A fully human antibody that specifically binds to mesothelin, wherein the fully human antibody is selected from a group consisting of:

(a) an antibody comprising a heavy chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 54, CDR2 comprising the amino acid sequence of SEQ ID NO: 55, CDR3 comprising the amino acid sequence of SEQ ID NO: 56;

(b) an antibody comprising a light chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 51, CDR2 comprising the amino acid sequence of SEQ ID NO: 52, CDR3 comprising the amino acid sequence of SEQ ID NO: 53;

(c) an antibody comprising a heavy chain variable region of said antibody of (a) and a light chain variable region of said antibody of (b);

(d) an antibody comprising a heavy chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 60, CDR2 comprising the amino acid sequence of SEQ ID NO: 61, CDR3 of the amino acid sequence of ID NO: 62;

(e) an antibody comprising a light chain variable region having CDR1 comprising the amino acid sequence of SEQ ID NO: 57, CDR2 comprising the amino acid sequence of SEQ ID NO: 58, CDR3 of the amino acid of ID NO: 59;

(f) an antibody comprising a heavy chain variable region of said antibody of (d) and a light chain variable region of the antibody of (e);

(g) an antibody which recognizes the same antigenic determinant as that recognized by the antibody according to any one of (a) to (f).

2. The fully human antibody of chaim 1, wherein the fully human antibody comprises a heavy chain variable region and a light chain variable region, the amino acid sequence of the heavy chain variable region is shown in positions 1 to 123 of SEQ ID NO: 6; and the amino acid sequence of the light chain variable region is shown in positions 139-254 of SEQ ID NO: 6; or

the fully human antibody comprises a heavy chain variable region and a light chain variable region, the amino acid sequence of the heavy chain variable region is shown in positions 1 to 124 of SEQ ID NO: 8; and the amino acid sequence of the light chain variable region is shown in positions 140-247 of SEQ ID NO: 8.

3. A nucleic acid encoding the antibody of claim 1 or 2.

4. An expression vector comprising the nucleic acid of claim 3.

5. A host cell, comprising the expression vector of claim 4 or having the nucleic acid of claim 3 integrated into the genome.

6. Use of the antibody of claim 1 or 2 for the preparation of a targeted drug, antibody-drug

conjugate, or a polyfunctional antibody that specifically targets tumor cells expressing mesothelin; or

for the preparation of a reagent for diagnosing a tumor expressing mesothelin; or

for the preparation of chimeric antigen receptor-modified immune cells.

7. A chimeric antigen receptor, wherein said chimeric antigen receptor comprises sequentially linked: the antibody of claim 1 or 2, a transmembrane region and intracellular signal region.

8. The chimeric antigen receptor of claim 7, wherein the intracellular signal region is selected from a group consisting of intracellular signal region sequences of CD3ζ, FcεRIγ, CD27, CD28, CD137, CD134, MyD88, CD40 or a combination thereof.

9. The chimeric antigen receptor of claim 7, wherein the transmembrane region comprises a transmembrane region of CD8 or CD28.

10. The chimeric antigen receptor of claim 7, comprising the following sequentially linked antibody, transmembrane region and intracellular signal region:

The antibody of claim 1 or 2, CD8 and CD3ζ;

The antibody of claim 1 or 2, CD8, CD137 and CD3ζ;

The antibody of claim 1 or 2, the transmembrane region of CD28 molecule, the intracellular signal region of CD28 molecule and CD3ζ; or

The antibody of claim 1 or 2, the transmembrane region of CD28 molecule, the intracellular signal region of CD28 molecule, CD137 and CD3ζ.

11. The chimeric antigen receptor of claim 7, wherein the antibody is a single chain antibody or domain antibody.

12. The chimeric antigen receptor of claim 7, wherein the chimeric antigen receptor has:

SEQ ID NO: 41 or the amino acid sequence shown in positions 22-353 thereof;

SEQ ID NO: 42 or the amino acid sequence shown in positions 22-454 thereof;

SEQ ID NO: 43 or the amino acid sequence shown in positions 22-498 thereof;

SEQ ID NO: 44 or the amino acid sequence shown in positions 22-501 thereof;

SEQ ID NO: 45 or the amino acid sequence shown in positions 22-543 thereof;

SEQ ID NO: 46 or the amino acid sequence shown in positions 22-346 thereof;

SEQ ID NO: 47 or the amino acid sequence shown in positions 22-447 thereof;

SEQ ID NO: 48 or the amino acid sequence shown in positions 22-491 thereof;

SEQ ID NO: 49 or the amino acid sequence shown in positions 22-494 thereof; or

SEQ ID NO: 50 or the amino acid sequence shown in positions 22-536 thereof.

13. A nucleic acid encoding the chimeric antigen receptor of any one of claims 7-12.

In another preferred embodiment, the nucleic acid encoding the chimeric antigen receptor has:

SEQ ID NO: 31 or the nucleotide sequence set forth in positions 473-1468 thereof;

SEQ ID NO: 32 or the nucleotide sequence set forth in positions 473-1771 thereof;

SEQ ID NO: 33 or the nucleotide sequence set forth in positions 473-1903 thereof;

SEQ ID NO: 34 or the nucleotide sequence set forth in positions 473-1912 thereof;

SEQ ID NO: 35 or the nucleotide sequence set forth in positions 473-2038 thereof;

SEQ ID NO: 36 or the nucleotide sequence set forth in positions 473-1447 thereof;

SEQ ID NO: 37 or the nucleotide sequence set forth in positions 473-1750 thereof;

SEQ ID NO: 38 or the nucleotide sequence set forth in positions 473-1882 thereof;

SEQ ID NO: 39 or the nucleotide sequence set forth in positions 473-1891 thereof;

SEQ ID NO: 40 or the nucleotide sequence set forth in positions 473 to 2017 thereof.

14. An expression vector comprising the nucleic acid of claim 13.

15. A virus, wherein the virus comprises the vector of claim 14.

16. Use of the chimeric antigen receptor of any one of claims 7-12, or the nucleic acid of claim 13, or the expression vector of claim 14, or the virus of claim 15 for the preparation of genetically modified immune cells targeting tumor cells expressing mesothelin.

17. The chimeric antigen receptor of claim 16, wherein the mesothelin-expressing tumor includes: pancreatic cancer, ovarian cancer and thymus mesothelioma.

18. A genetically modified immune cell, wherein it is transduced with the nucleic acid of claim 13, or the expression vector of claim 14 or the virus of claim 15; or

expresses the chimeric antigen receptor of any one of claims 7-12 on its surface-expressed.

19. The immune cell of claim 18, wherein it further carries an encoding sequence of an exogenous cytokine; and preferably, the cytokine includes: IL-12, IL-15 or IL-21.

20. The immune cell of claim 18, wherein the immune cell also expresses another chimeric antigen receptor which does not contain CD3ζ but contains the intracellular signaling domain of CD28, the intracellular signaling domain of CD137, or a combination of both.

21. The immune cell of claim 18, wherein the immune cell further expresses a chemokine receptor; and preferably, the chemokine receptor includes: CCR2.

22. The immune cell of claim 18, wherein the immune cell further expresses siRNA which can reduce expression of PD-1 or a protein which blocks PD-L1.

23. The immune cell of claim 18, wherein the immune cell further expresses a safety switch; and preferably, the safety switch includes: iCaspase-9, Truancated EGFR or RQR8.

24. The immune cell of claim 18, wherein the immune cell includes: T lymphocyte, NK cell or NKT cell.

25. Use of the genetically modified immune cell of any one of claims 18-24 for the preparation of a tumor-inhibiting drug, and the tumor is a tumor expressing mesothelin.

26. A multi-functional immunoconjugate, wherein the multi-functional immunoconjugate

comprises:

The antibody of claim 1 or 2; and

a functional molecule linked thereto; and the functional molecule is selected from a molecule that targets a tumor surface marker, a tumor-suppressing molecule, a molecule that targets a surface marker of an immune cell, or a detectable label.

27. The multi-functional immunoconjugate of claim 26, wherein the molecule that targets the tumor surface marker is an antibody or ligand that binds to a tumor surface marker; or

the tumor-suppressing molecule is an anti-tumor cytokine or an anti-tumor toxin; and preferably, the cytokine includes: IL-12, IL-15, IFN-beta, TNF-alpha.

28. The multi-functional immunoconjugate of claim 26, wherein the detectable label includes a fluorescent label or a chromogenic label.

29. The multi-functional immunoconjugate of claim 27, wherein the antibody that binds to a tumor surface marker refers to an antibody that recognizes an antigen other than mesothelin, and the other antigen includes EGFR EGFRvIII, mesothelin, HER2, EphA2, Her3, EpCAM, MUC1, MUC16, CEA, Claudin 18.2, folate receptor, Claudin 6, CD3, WT1, NY-ESO- 1, MAGE 3, ASGPR1 or CDH16.

30. The multi-functional immunoconjugate of claim 26, wherein the molecule that targets the surface marker of the immune cell is an antibody that binds to T cell surface marker and forms a T-cell-engaging bifunctional antibody with the antibody of claim 1 or 2.

31. The multi-functional immunoconjugate of claim 30, wherein the antibody that binds to T cell surface marker is an anti-CD3 antibody.

32. The multi-functional immunoconjugate of claim 31, wherein it is a fusion polypeptide, and further comprises a linker peptide between the antibody of claim 1 or 2 and the functional molecule linked thereto.

33. A nucleic acid encoding the multi-functional immunoconjugate of any one of claims 26-32.

34. Use of the multi-functional immunoconjugate of any one of claims 26-32 for the preparation of an antineoplastic agent or an agent for diagnosis of tumors that express mesothelin; or

for the preparation of chimeric antigen receptor modified immune cells; and preferably, the immune cells include T lymphocyte, NK cell or NKT lymphocyte.

35. A pharmaceutical composition, comprising:

the antibody of claim 1 or 2 or a nucleic acid encoding the antibody; or

the immunoconjugate of any one of claims 26-32 or a nucleic acid encoding the conjugate; or

the chimeric antigen receptor of any one of claims 7-12 or a nucleic acid encoding the

chimeric antigen receptor; or

the genetically modified immune cell of any one of claims 18-24.

36. An antibody capable of competing for binding to mesothelin with the antibody of claim 1 or 2.

37. An antibody capable of binding to mesothelin epitope as shown in SEQ ID NO: 66; and preferrably, the antibody can bind to mesothelin epitope as shown in SEQ ID NO: 72.

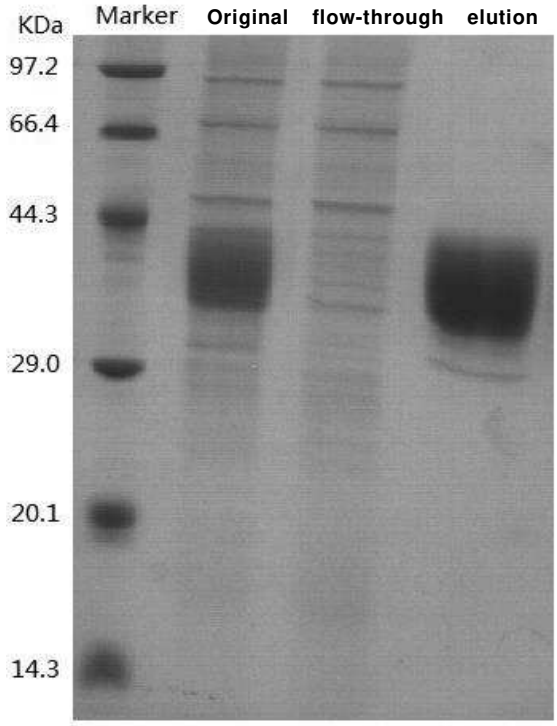


Fig. 1

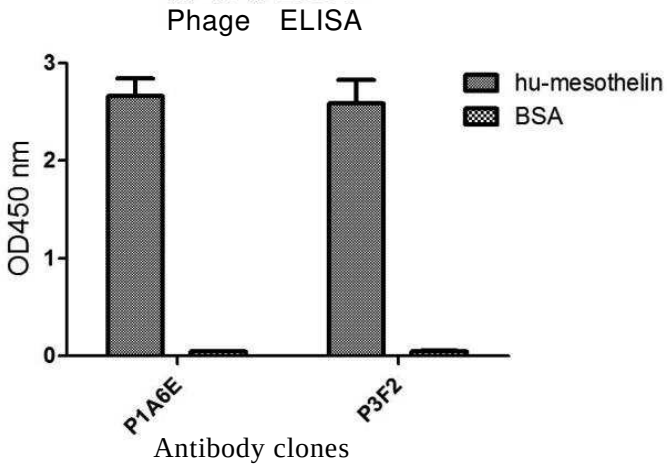


Fig. 2

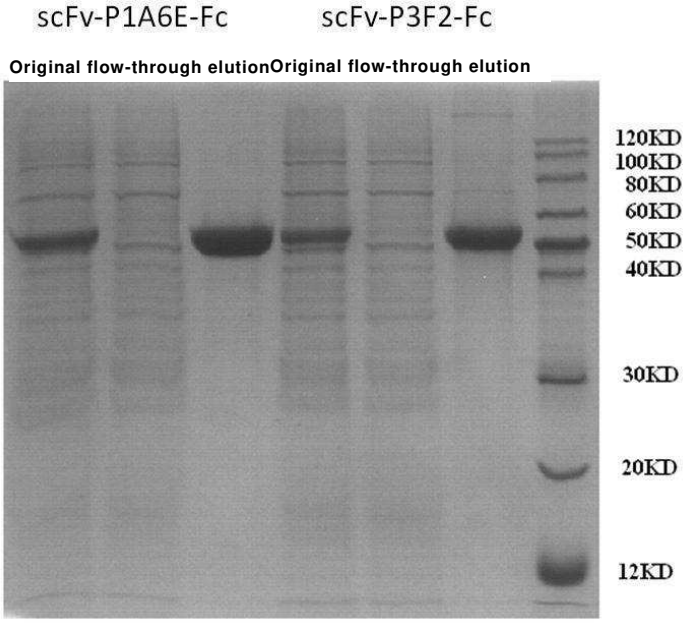
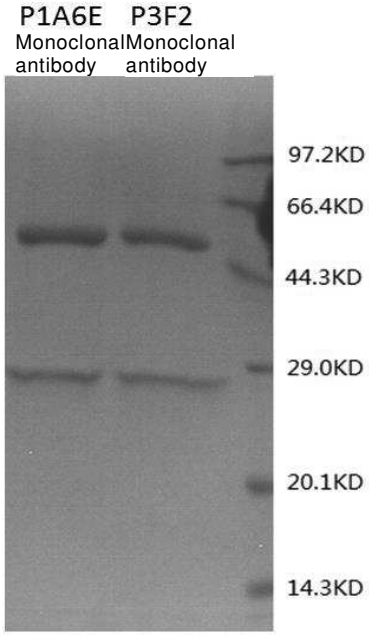


Fig. 3



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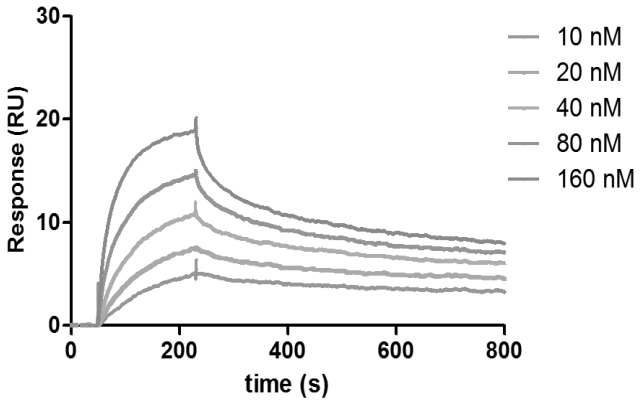

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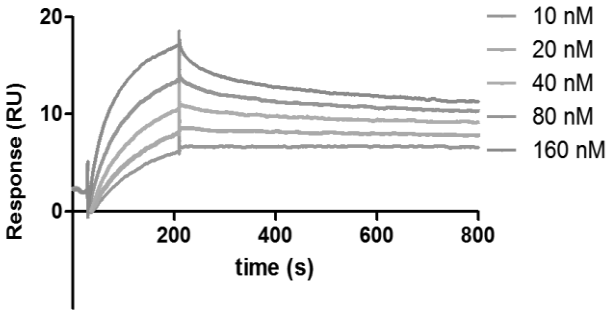

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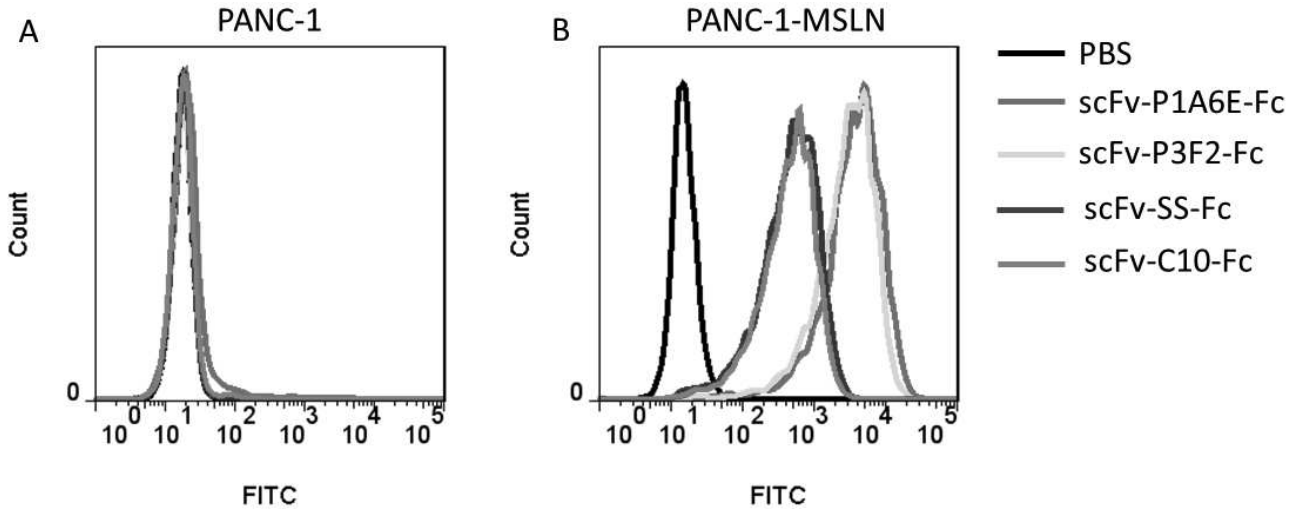

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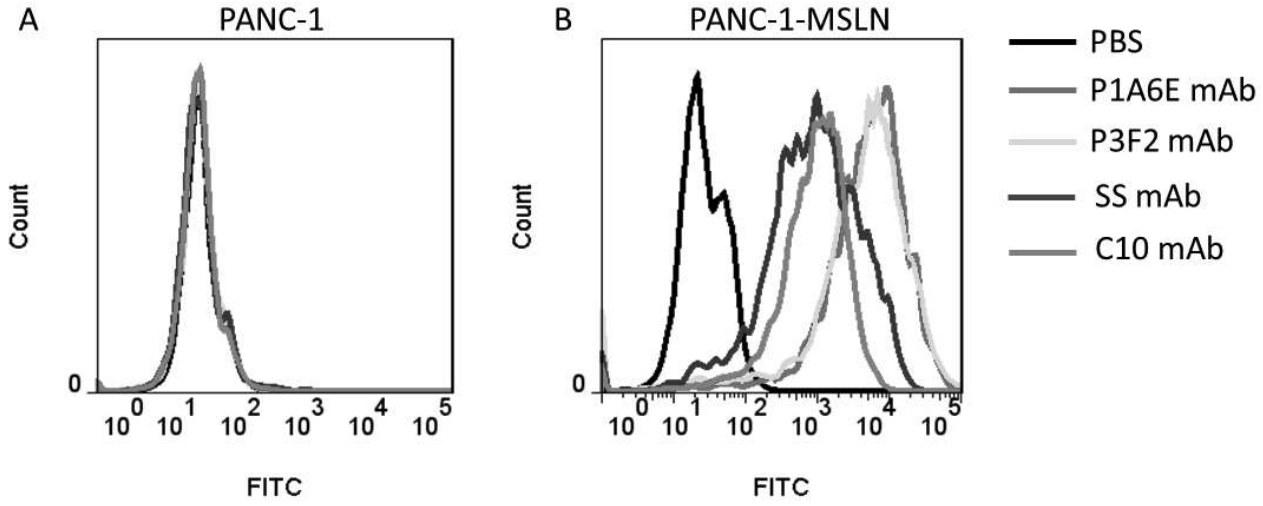

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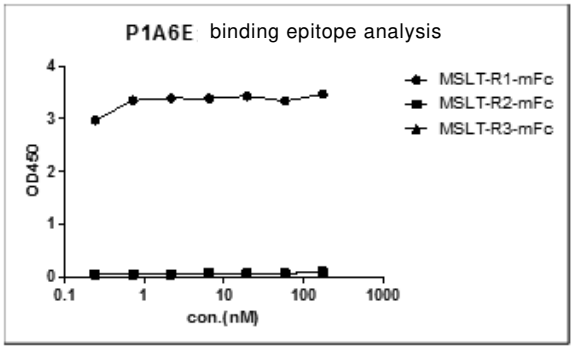
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con.(nM)
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MSLT-R2-mFc
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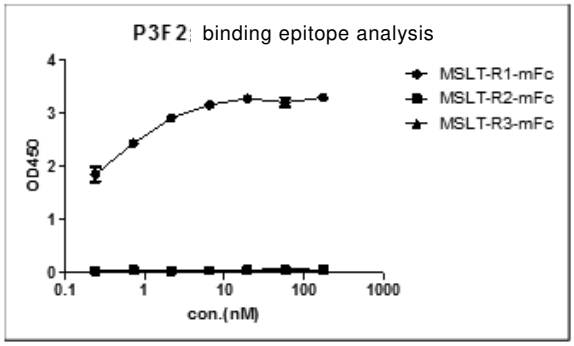
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con.(nM)
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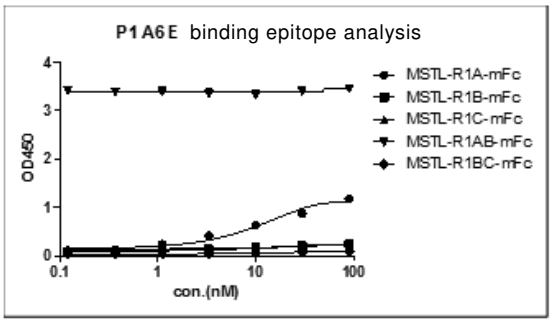
MSLT-R3-mFc

Fig. 9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OD450
con.(nM)
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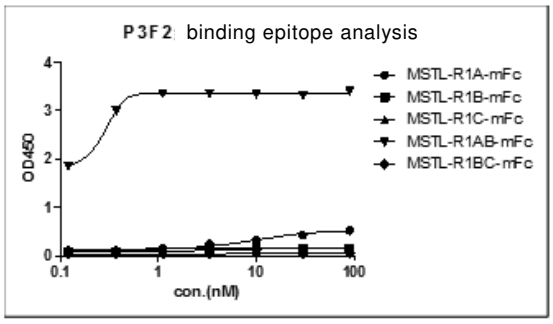
MSTL-R1B-mFc
MSTL-R1C-mFc
MSTL-R1AB-mFc
MSTL-R1BC-mFc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con.(nM)
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MSTL-R1BC-mFc

Fig. 10

Sequence listing

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<130> P2016-1262

<150> CN201510519214.4
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Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val Asp Ala Ala
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Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr
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Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu Tyr Pro Gln
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Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu Phe Leu Lys
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Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr Ser Leu Glu Thr
115 120 125

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145 150 155 160

Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys
165 170 175

Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro Ser Ser Ile Trp
180 185 190

Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp
195 200 205

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210 215 220

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

225 230 235 240

Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser Met Asp Leu Ala Thr
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Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro Leu Thr Val Ala Glu
260 265 270

Val Gln Lys Leu Leu Gly Pro His Val Glu Gly Leu Lys Ala Glu Glu
275 280 285

Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp
290 295 300

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35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Phe Asn Asp Tyr Ala
50 55 60

Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser Asp Thr Ser Lys Asn
65 70 75 80

Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr Tyr Ala Met Asp Val
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Leu Thr Gln
130 135 140

Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser Ala Ser Leu Thr Cys
145 150 155 160

Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr Arg Ile Tyr Trp Tyr
165 170 175

Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu Leu Thr Tyr Lys Ser
180 185 190

Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro Ser Arg Phe Ser Gly
195 200 205

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

Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro Glu Asp Phe Ala Thr
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<223> P1A6E-Z polynucleotide

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<220>
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taagatggcg gaggcctaca gtgagattgg gatgaaaggc gagcgccgga ggggcaaggg 1800

gcacgatggc ctttaccagg gtctcagtac agccaccaag gacacctacg acgcccttca 1860

catgcaggcc ctgccccctc gctaggtcga cctcgaggga attccgataa tcaacctctg 1920

gattacaaaa tttgtgaaag attgactggt attcttaact atgttgctcc ttttacgcta 1980

<210> 39
<211> 1989
<212> DNA
<213> Artificial sequence

<220>
<221> misc_feature
<223> P3F2-28Z polynucleotide

<400> 39
gcaggggaaa gaatagtaga cataatagca acagacatac aaactaaaga attacaaaaa 60

caaattacaa aaattcaaaa ttttccgatc acgagactag cctcgagaag cttgatcgat 120

ggctccggtg cccgtcagtg ggcagagcgc acatcgccca cagtccccga gaagttgggg 180

ggaggggtcg gcaattgaac cggtgcctag agaaggtggc gcggggtaaa ctgggaaagt 240

gatgtcgtgt actggctccg cctttttccc gagggtgggg gagaaccgta tataagtgca 300

gtagtcgccg tgaacgttct ttttcgcaac gggtttgccg ccagaacaca ggtgtcgtga 360

cgcggatcca ggcctaagct tacgcgtcct agcgctaccg gtcgccacca tggccttacc 420

agtgaccgcc ttgctcctgc cgctggcctt gctgctccac gccgccaggc cgcagatgca 480

gctagtgcag tctggggctg aggtgaagaa gcctggggcc tcagtgaagg tttcctgcaa 540

ggcatctgga tacaccttca ccagctacta tatgcactgg gtgcgacagg cccctggaca 600

agggcttgag tggatgggaa taatcaaccc tagtggtggt agcacaagct acgcacagaa 660

gttccagggc agagtcacca tgaccaggga cacgtccacg agcacagtct acatggagct 720

gagcagcctg agatctgagg acacggccgt gtattactgt gcgagtagtc ggagtgggac 780

tacggtggta aatcatgatg cttttgatat ctgggggaaa gggaccacgg tcaccgtctc 840

gagtggtgga ggcggttcag gcggaggtgg ttctggcggt ggcggatcgg acatccagtt 900

gacccagtct ccatcctccc tgtctgcgtc tgtaggagac agagtcacca tcacttgccg 960

ggcaagccag gtcattagcc gtgctttagc ctggtatcaa caaacaccag ggaaacctcc 1020

taaactcctg atctatgatg cctccaattt gcagagtggg gtcccatcaa ggttcagcgg 1080

cagtggatct gggacagatt tcactctcac catcagccgc ctgcagcctg aagattttgc 1140

aacttattac tgtcaacagt ttaatagtta ccctctcact ttcggcggag ggaccaagct 1200

ggagatcaaa cgtaccacga cgccagcgcc gcgaccacca acaccggcgc ccaccatcgc 1260

gtcgcagccc ctgtccctgc gcccagaggc gtgccggcca gcggcggggg gcgcagtgca 1320

cacgaggggg ctggacttcg cctgtgattt ttgggtgctg gtggtggttg gtggagtcct 1380

ggcttgctat agcttgctag taacagtggc ctttattatt ttctgggtga ggagtaagag 1440

gagcaggctc ctgcacagtg actacatgaa catgactccc cgccgccccg ggccaacccg 1500

caagcattac cagccctatg ccccaccacg cgacttcgca gcctatcgct ccagagtgaa 1560

gttcagcagg agcgcagacg cccccgcgta ccagcagggc cagaaccagc tctataacga 1620

gctcaatcta ggacgaagag aggagtacga tgttttggac aagagacgtg gccgggaccc 1680

tgagatgggg ggaaagccgc agagaaggaa gaaccctcag gaaggcctgt acaatgaact 1740

gcagaaagat aagatggcgg aggcctacag tgagattggg atgaaaggcg agcgccggag 1800

gggcaagggg cacgatggcc tttaccaggg tctcagtaca gccaccaagg acacctacga 1860

cgcccttcac atgcaggccc tgcccccctcg ctaggtcgac ctcgagggaa ttccgataat 1920

caacctctgg attacaaaat ttgtgaaaga ttgactggta ttcttaacta tgttgctcct 1980

tttacgcta 1989

<210> 40
<211> 2115
<212> DNA
<213> Artificial sequence

<220>
<221> misc_feature
<223> P3F2-28BBZ polynucleotide

<400> 40
gcaggggaaa gaatagtaga cataatagca acagacatac aaactaaaga attacaaaaa 60

caaattacaa aaattcaaaa ttttccgatc acgagactag cctcgagaag cttgatcgat 120

ggctccggtg cccgtcagtg ggcagagcgc acatcgccca cagtccccga gaagttgggg 180

ggaggggtcg gcaattgaac cggtgcctag agaaggtggc gcggggtaaa ctgggaaagt 240

gatgtcgtgt actggctccg ccttttccc gagggtgggg gagaaccgta tataagtgca 300

gtagtcgccg tgaacgttct ttttcgcaac gggtttgccg ccagaacaca ggtgtcgtga 360

cgcggatcca ggcctaagct tacgcgtcct agcgctaccg gtcgccacca tggccttacc 420

agtgaccgcc ttgctcctgc cgctggcctt gctgctccac gccgccaggc cgcagatgca 480

gctagtgcag tctggggctg aggtgaagaa gcctggggcc tcagtgaagg tttcctgcaa 540

ggcatctgga tacaccttca ccagctacta tatgcactgg gtgcgacagg cccctggaca 600

agggcttgag tggatgggaa taatcaaccc tagtggtggt agcacaagct acgcacagaa 660

gttccagggc agagtcacca tgaccaggga cacgtccacg agcacagtct acatggagct 720

gagcagcctg agatctgagg acacggccgt gtattactgt gcgagtagtc ggagtgggac 780

tacggtggta aatcatgatg cttttgatat ctgggggaaa gggaccacgg tcaccgtctc 840

gagtggtgga ggcggttcag gcggaggtgg ttctggcggt ggcggatcgg acatccagtt 900

gacccagtct ccatcctccc tgtctgcgtc tgtaggagac agagtcacca tcacttgccg 960

ggcaagccag gtcattagcc gtgctttagc ctggtatcaa caaacaccag ggaaacctcc 1020

taaactcctg atctatgatg cctccaattt gcagagtggg gtcccatcaa ggttcagcgg 1080

cagtggatct gggacagatt tcactctcac catcagccgc ctgcagcctg aagattttgc 1140

aacttattac tgtcaacagt ttaatagtta ccctctcact ttcggcggag ggaccaagct 1200

ggagatcaaa cgtaccacga cgccagcgcc gcgaccacca acaccggcgc ccaccatcgc 1260

gtcgcagccc ctgtccctgc gcccagaggc gtgccggcca gcggcggggg gcgcagtgca 1320

cacgaggggg ctggacttcg cctgtgattt ttgggtgctg gtggtggttg gtggagtcct 1380

ggcttgctat agcttgctag taacagtggc ctttattatt ttctgggtga ggagtaagag 1440

gagcaggctc ctgcacagtg actacatgaa catgactccc cgccgccccg ggccaacccg 1500

caagcattac cagccctatg ccccaccacg cgacttcgca gcctatcgct ccaaacgggg 1560

cagaaagaaa ctcctgtata tattcaaaca accatttatg agaccagtac aaactactca 1620

agaggaagat ggctgtagct gccgatttcc agaagaagaa gaaggaggat gtgaactgag 1680

agtgaagttc agcaggagcg cagacgcccc cgcgtaccag cagggccaga accagctcta 1740

taacgagctc aatctaggac gaagagagga gtacgatgtt ttggacaaga gacgtggccg 1800

ggaccctgag atgggggggaa agccgcagag aaggaagaac cctcaggaag gcctgtacaa 1860

tgaactgcag aaagataaga tggcggaggc ctacagtgag attgggatga aaggcgagcg 1920

ccggaggggc aaggggcacg atggccttta ccagggtctc agtacagcca ccaaggacac 1980

ctacgacgcc cttcacatgc aggccctgcc ccctcgctag gtcgacctcg agggaattcc 2040

gataatcaac ctctggatta caaaatttgt gaaagattga ctggtattct taactatgtt 2100

gctcctttta cgcta 2115

<210> 41
<211> 353
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P1A6E-¦ÄZ amino acid sequence

<400> 41

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

His Ala Ala Arg Pro Gln Val Gln Leu Glu Gln Ser Gly Leu Gly Leu
20 25 30

Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp
35 40 45

Thr Val Ser Ser Asp Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro
50 55 60

Ser Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp
65 70 75 80

Phe Asn Asp Tyr Ala Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser
85 90 95

Asp Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro
100 105 110

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr
115 120 125

Tyr Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Ala Val Leu Thr Gln Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser

165 170 175

Ala Ser Leu Thr Cys Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr
180 185 190

Arg Ile Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu
195 200 205

Leu Thr Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro
210 215 220

Ser Arg Phe Ser Gly Ser Lys Asp Ala Ser Ala Asn Ala Gly Ile Leu
225 230 235 240

Leu Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Met
245 250 255

Ile Trp His Ser Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Val Thr
260 265 270

Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
275 280 285

Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
290 295 300

Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
305 310 315 320

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
325 330 335

Ser Leu Val Ile Thr Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
340 345 350

Ala

<210> 42
<211> 454
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P1A6E-Z amino acid sequence

<400> 42

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

His Ala Ala Arg Pro Gln Val Gln Leu Glu Gln Ser Gly Leu Gly Leu
20 25 30

Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp
35 40 45

Thr Val Ser Ser Asp Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro
50 55 60

Ser Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp
65 70 75 80

Phe Asn Asp Tyr Ala Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser
85 90 95

Asp Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro
100 105 110

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr
115 120 125

Tyr Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Ala Val Leu Thr Gln Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser
165 170 175

Ala Ser Leu Thr Cys Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr
180 185 190

Arg Ile Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu
195 200 205

Leu Thr Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro
210 215 220

Ser Arg Phe Ser Gly Ser Lys Asp Ala Ser Ala Asn Ala Gly Ile Leu
225 230 235 240

Leu Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Met
245 250 255

Ile Trp His Ser Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Val Thr
260 265 270

Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
275 280 285

Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
290 295 300

Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
305 310 315 320

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
325 330 335

Ser Leu Val Ile Thr Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro
340 345 350

Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly
355 360 365

Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro
370 375 380

Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu
385 390 395 400

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
405 410 415

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
420 425 430

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

Gln Ala Leu Pro Pro Arg
450

<210> 43
<211> 498
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P1A6E-BBZ amino acid sequence

<400> 43

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

His Ala Ala Arg Pro Gln Val Gln Leu Glu Gln Ser Gly Leu Gly Leu
20 25 30

Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp
35 40 45

Thr Val Ser Ser Asp Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro
50 55 60

Ser Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp
65 70 75 80

Phe Asn Asp Tyr Ala Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser
85 90 95

Asp Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro
100 105 110

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr
115 120 125

Tyr Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Ala Val Leu Thr Gln Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser
165 170 175

Ala Ser Leu Thr Cys Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr
180 185 190

Arg Ile Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu
195 200 205

Leu Thr Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro

210 215 220

Ser Arg Phe Ser Gly Ser Lys Asp Ala Ser Ala Asn Ala Gly Ile Leu
225 230 235 240

Leu Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Met
245 250 255

Ile Trp His Ser Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Val Thr
260 265 270

Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
275 280 285

Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
290 295 300

Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
305 310 315 320

Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly Val Leu Leu Leu
325 330 335

Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg Lys Lys Leu Leu
340 345 350

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
355 360 365

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
370 375 380

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Lys
385 390 395 400

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

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
420 425 430

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
435 440 445

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
450 455 460

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
465 470 475 480

Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
485 490 495

Pro Arg

<210> 44
<211> 501
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P1A6E-28Z amino acid sequence

<400> 44

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

His Ala Ala Arg Pro Gln Val Gln Leu Glu Gln Ser Gly Leu Gly Leu
20 25 30

Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp
35 40 45

Thr Val Ser Ser Asp Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro
50 55 60

Ser Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp
65 70 75 80

Phe Asn Asp Tyr Ala Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser
85 90 95

Asp Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro
100 105 110

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr
115 120 125

Tyr Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Ala Val Leu Thr Gln Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser
165 170 175

Ala Ser Leu Thr Cys Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr
180 185 190

Arg Ile Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu
195 200 205

Leu Thr Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro
210 215 220

Ser Arg Phe Ser Gly Ser Lys Asp Ala Ser Ala Asn Ala Gly Ile Leu
225 230 235 240

Leu Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Met
245 250 255

Ile Trp His Ser Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Val Thr
260 265 270

Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
275 280 285

Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
290 295 300

Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
305 310 315 320

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
325 330 335

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
340 345 350

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
355 360 365

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
370 375 380

Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
385 390 395 400

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

Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
420 425 430

Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr
435 440 445

Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly
450 455 460

Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
465 470 475 480

Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln
485 490 495

Ala Leu Pro Pro Arg
500

<210> 45
<211> 543
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P1A6E-28BBZ amino acid sequence

<400> 45

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

His Ala Ala Arg Pro Gln Val Gln Leu Glu Gln Ser Gly Leu Gly Leu
20 25 30

Val Lys Pro Ser Gln Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp
35 40 45

Thr Val Ser Ser Asp Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro
50 55 60

Ser Arg Gly Leu Glu Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp

65 70 75 80

Phe Asn Asp Tyr Ala Val Ser Val Lys Gly Arg Ile Thr Ile Asn Ser
85 90 95

Asp Thr Ser Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro
100 105 110

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asn Ser Tyr Tyr Tyr
115 120 125

Tyr Ala Met Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135 140

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln
145 150 155 160

Ala Val Leu Thr Gln Pro Ser Ser Leu Ser Ala Ser Pro Gly Ala Ser
165 170 175

Ala Ser Leu Thr Cys Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr
180 185 190

Arg Ile Tyr Trp Tyr Gln Gln Arg Pro Gly Ser Pro Pro Gln Ile Leu
195 200 205

Leu Thr Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser Gly Val Pro
210 215 220

Ser Arg Phe Ser Gly Ser Lys Asp Ala Ser Ala Asn Ala Gly Ile Leu
225 230 235 240

Leu Ile Ser Gly Leu Gln Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Met
245 250 255

Ile Trp His Ser Gly Gly Trp Val Phe Gly Gly Gly Thr Lys Val Thr
260 265 270

Val Leu Gly Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro
275 280 285

Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro
290 295 300

Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
305 310 315 320

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
325 330 335

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
340 345 350

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
355 360 365

Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro Arg Asp Phe Ala
370 375 380

Ala Tyr Arg Ser Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys
385 390 395 400

Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys
405 410 415

Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val
420 425 430

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
435 440 445

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
450 455 460

Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln
465 470 475 480

Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp
485 490 495

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
500 505 510

Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr
515 520 525

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
530 535 540

<210> 46
<211> 346
<212> PRT

<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P3F2-¦ÄZ amino acid sequence

<400> 46

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

His Ala Ala Arg Pro Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val
20 25 30

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
50 55 60

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
65 70 75 80

Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
85 90 95

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Ser Ser Arg Ser Gly Thr Thr Val Val Asn
115 120 125

His Asp Ala Phe Asp Ile Trp Gly Lys Gly Thr Thr Val Thr Val Ser
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Arg Ala
180 185 190

Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro
225 230 235 240

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro
260 265 270

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
275 280 285

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
290 295 300

Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
305 310 315 320

Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Arg Val
325 330 335

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
340 345

<210> 47
<211> 447
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P3F2-Z amino acid sequence

<400> 47

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

His Ala Ala Arg Pro Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val
20 25 30

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln

50 55 60

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
65 70 75 80

Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
85 90 95

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Ser Ser Arg Ser Gly Thr Thr Val Val Asn
115 120 125

His Asp Ala Phe Asp Ile Trp Gly Lys Gly Thr Thr Val Thr Val Ser
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Arg Ala
180 185 190

Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro
225 230 235 240

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro
260 265 270

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
275 280 285

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
290 295 300

Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
305 310 315 320

Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Arg Val
325 330 335

Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn
340 345 350

Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val
355 360 365

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

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

Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg
405 410 415

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

Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
435 440 445

<210> 48
<211> 491
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P3F2-BBZ amino acid sequence

<400> 48

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

His Ala Ala Arg Pro Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val
20 25 30

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
50 55 60

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
65 70 75 80

Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
85 90 95

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Ser Ser Arg Ser Gly Thr Thr Val Val Asn
115 120 125

His Asp Ala Phe Asp Ile Trp Gly Lys Gly Thr Thr Val Thr Val Ser
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Arg Ala
180 185 190

Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro
225 230 235 240

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro
260 265 270

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
275 280 285

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
290 295 300

Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu
305 310 315 320

Ala Gly Thr Cys Gly Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr
325 330 335

Cys Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe
340 345 350

Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg
355 360 365

Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser
370 375 380

Arg Ser Ala Asp Ala Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr
385 390 395 400

Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys
405 410 415

Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn
420 425 430

Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu
435 440 445

Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly
450 455 460

His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr
465 470 475 480

Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 49
<211> 494
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P3F2-28Z amino acid sequence

<400> 49

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

His Ala Ala Arg Pro Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val
20 25 30

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
50 55 60

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
65 70 75 80

Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
85 90 95

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Ser Ser Arg Ser Gly Thr Thr Val Val Asn
115 120 125

His Asp Ala Phe Asp Ile Trp Gly Lys Gly Thr Thr Val Thr Val Ser
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Arg Ala
180 185 190

Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro
225 230 235 240

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro
260 265 270

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
275 280 285

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
290 295 300

Thr Arg Gly Leu Asp Phe Ala Cys Asp Phe Trp Val Leu Val Val Val
305 310 315 320

Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile
325 330 335

Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr
340 345 350

Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln
355 360 365

Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys
370 375 380

Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln
385 390 395 400

Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu
405 410 415

Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg
420 425 430

Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys
435 440 445

Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg
450 455 460

Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys
465 470 475 480

Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
485 490

<210> 50
<211> 536
<212> PRT
<213> Artificial sequence

<220>
<221> MISC_FEATURE
<223> P3F2-28BBZ amino acid sequence

<400> 50

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

His Ala Ala Arg Pro Gln Met Gln Leu Val Gln Ser Gly Ala Glu Val
20 25 30

Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr
35 40 45

Thr Phe Thr Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln
50 55 60

Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser
65 70 75 80

Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
85 90 95

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Ser Ser Arg Ser Gly Thr Thr Val Val Asn
115 120 125

His Asp Ala Phe Asp Ile Trp Gly Lys Gly Thr Thr Val Thr Val Ser
130 135 140

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
145 150 155 160

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Val Ile Ser Arg Ala
180 185 190

Leu Ala Trp Tyr Gln Gln Thr Pro Gly Lys Pro Pro Lys Leu Leu Ile
195 200 205

Tyr Asp Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
210 215 220

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Gln Pro
225 230 235 240

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
245 250 255

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro
260 265 270

Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu
275 280 285

Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His
290 295 300

Thr Arg Gly Leu Asp Phe Ala Cys Asp Phe Trp Val Leu Val Val Val
305 310 315 320

Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile
325 330 335

Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr
340 345 350

Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln
355 360 365

Pro Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly
370 375 380

Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val
385 390 395 400

Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu
405 410 415

Glu Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp

420 425 430

Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn
435 440 445

Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg
450 455 460

Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu
465 470 475 480

Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser
485 490 495

Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly
500 505 510

Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu
515 520 525

His Met Gln Ala Leu Pro Pro Arg
530 535

<210> 51
<211> 14
<212> PRT
<213> Homo Sapiens

<400> 51

Thr Leu Arg Ser Gly Ile Asn Val Gly Ile Tyr Arg Ile Tyr
1 5 10

<210> 52
<211> 11
<212> PRT
<213> Homo Sapiens

<400> 52

Tyr Lys Ser Asp Ser Asp Lys Tyr Gln Gly Ser
1 5 10

<210> 53
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 53

Met Ile Trp His Ser Gly Gly Trp Val

1 5

<210> 54
<211> 12
<212> PRT
<213> Homo Sapiens

<400> 54

Gly Asp Thr Val Ser Ser Asp Ser Ala Ala Trp Asn
1 5 10

<210> 55
<211> 18
<212> PRT
<213> Homo Sapiens

<400> 55

Arg Thr Tyr Tyr Arg Ser Lys Trp Phe Asn Asp Tyr Ala Val Ser Val
1 5 10 15

Lys Gly

<210> 56
<211> 11
<212> PRT
<213> Homo Sapiens

<400> 56

Ser Asn Ser Tyr Tyr Tyr Tyr Ala Met Asp Val
1 5 10

<210> 57
<211> 11
<212> PRT
<213> Homo Sapiens

<400> 57

Arg Ala Ser Gln Val Ile Ser Arg Ala Leu Ala
1 5 10

<210> 58
<211> 7
<212> PRT
<213> Homo Sapiens

<400> 58

Asp Ala Ser Asn Leu Gln Ser
1 5

<210> 59
<211> 9
<212> PRT
<213> Homo Sapiens

<400> 59

Gln Gln Phe Asn Ser Tyr Pro Leu Thr
1 5

<210> 60
<211> 10
<212> PRT
<213> Homo Sapiens

<400> 60

Gly Tyr Thr Phe Thr Ser Tyr Tyr Met His
1 5 10

<210> 61
<211> 17
<212> PRT
<213> Homo Sapiens

<400> 61

Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 62
<211> 15
<212> PRT
<213> Homo Sapiens

<400> 62

Ser Arg Ser Gly Thr Thr Val Val Asn His Asp Ala Phe Asp Ile
1 5 10 15

<210> 63
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<221> misc_feature
<223> primer

<400> 63
accacgacgc cagcgccgcg accac 25

<210> 64
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<221> misc_feature
<223> primer

<400> 64
tagcgtaaaa ggagcaacat ag 22

<210> 65
<211> 622
<212> PRT
<213> Homo Sapiens

<400> 65

Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1 5 10 15

Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
20 25 30

Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
35 40 45

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

Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65 70 75 80

Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
85 90 95

Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
100 105 110

Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
115 120 125

Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
130 135 140

Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145 150 155 160

Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
165 170 175

Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
180 185 190

Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
195 200 205

Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
210 215 220

Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225 230 235 240

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

Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
260 265 270

Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
275 280 285

Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
290 295 300

Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305 310 315 320

Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
325 330 335

Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
340 345 350

Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
355 360 365

Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
370 375 380

Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385 390 395 400

Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
405 410 415

Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
420 425 430

Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
435 440 445

Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
450 455 460

Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465 470 475 480

Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
485 490 495

Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
500 505 510

Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
515 520 525

Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
530 535 540

Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545 550 555 560

Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
565 570 575

Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
580 585 590

Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
595 600 605

Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
610 615 620

<210> 66
<211> 95
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 66

Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
1 5 10 15

Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
20 25 30

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
35 40 45

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
50 55 60

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
65 70 75 80

Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr
85 90 95

<210> 67
<211> 95
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 67

Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu Val Asn Lys Gly His Glu
1 5 10 15

Met Ser Pro Gln Val Ala Thr Leu Ile Asp Arg Phe Val Lys Gly Arg
20 25 30

Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr Leu Thr Ala Phe Tyr Pro
35 40 45

Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu Leu Ser Ser Val Pro Pro
50 55 60

Ser Ser Ile Trp Ala Val Arg Pro Gln Asp Leu Asp Thr Cys Asp Pro
65 70 75 80

Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala Arg Leu Ala Phe Gln

85 90 95

<210> 68
<211> 95
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 68

Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile Gln Ser Phe Leu Gly
1 5 10 15

Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser Gln Gln Asn Val Ser
20 25 30

Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr Asp Ala Val Leu Pro
35 40 45

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

Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg Asp Trp Ile Leu Arg
65 70 75 80

Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu Gly Leu Gln Gly
85 90 95

<210> 69
<211> 42
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 69

Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
1 5 10 15

Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
20 25 30

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp
35 40

<210> 70
<211> 42

<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 70

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
1 5 10 15

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
20 25 30

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile
35 40

<210> 71
<211> 31
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 71

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
1 5 10 15

Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr
20 25 30

<210> 72
<211> 64
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 72

Glu Val Glu Lys Thr Ala Cys Pro Ser Gly Lys Lys Ala Arg Glu Ile
1 5 10 15

Asp Glu Ser Leu Ile Phe Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val
20 25 30

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
35 40 45

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu

50 55 60

<210> 73
<211> 63
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 73

Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg Val Asn Ala Ile Pro
1 5 10 15

Phe Thr Tyr Glu Gln Leu Asp Val Leu Lys His Lys Leu Asp Glu Leu
20 25 30

Tyr Pro Gln Gly Tyr Pro Glu Ser Val Ile Gln His Leu Gly Tyr Leu
35 40 45

Phe Leu Lys Met Ser Pro Glu Asp Ile Arg Lys Trp Asn Val Thr
50 55 60

<210> 74
<211> 30
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 74

Tyr Lys Lys Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala
1 5 10 15

Thr Gln Met Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu
20 25 30

<210> 75
<211> 20
<212> PRT
<213> Artificial sequence

<220>
<223> human mesothelin fragment

<400> 75

Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met Asp Arg
1 5 10 15

Val Asn Ala Ile
20