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DESCRIPTION

[0001] In a first aspect, the present invention relates to a recombinant polypeptide containing a domain comprising at least two antibody units whereby one antibody unit is an anti-CD30 single chain antibody unit while another antibody unit is an antibody unit being specific for an antigen present on the surface of a predetermined target cell according to claim 1 or claim 2. In particular, the present invention relates to a recombinant polypeptide containing at least the following domains starting from the N-terminus to the C-terminus: a first domain containing an anti-CD30 single chain antibody unit of HRS3 scFv of SEQ ID No. 2 and an antibody unit said antibody unit being specific for an antigen present on the surface of a predetermined target cell, namely, being specific for a tumor-associated antigen; optionally a spacer domain; a trans-membrane domain; and a cytoplasmatic signalling domain. The recombinant polypeptide can likewise be arranged in the order where the domain comprising at least two antibody units has the order: a first domain containing an antibody unit specific for a tumor-associated antigen and the second domain containing an anti-CD30 single chain antibody unit. In a further aspect, the present invention relates to a nucleic acid molecule encoding the polypeptide according to the present invention, as well as vectors and cells containing the same. Moreover, isolated T-cells, like CD8⁺ or a CD4⁺ T-cell, are provided expressing on its surface chimeric antigen receptors containing an anti-CD30 single chain antibody unit and an antibody unit whereby said antibody unit being specific for an antigen present on the surface of a predetermined target cell.

[0002] These cells are particularly useful in treating cancer, in particular CD30⁺ cancer. That is, the cells are for use in adoptive cell therapy for treating cancer in a subject in need thereof.

Prior art

[0003] Adoptive T-cell transfer has shown significant efficacy in the treatment of malignancies and can be curative in patients with various diseases including leukaemia or Epstein Barr virus associated malignancies. Usually, patient derived T-cells are engineered *ex vivo* to express a recombinant T-cell receptor or, alternatively, a Chimeric Antigen Receptor. Said Chimeric Antigen Receptor (CAR) is typically composed of an extracellular antigen binding domain derived from an antibody and an intracellular T-cell activation domain derived from the T-cell receptor endodomain. In contrast to the physiological TCR, the CAR is composed of one single polypeptide chain that combines antigen binding via the extracellular moiety with a T-cell activation machinery provided by the intracellular signalling moiety. Thus, due to the antibody derived binding domain, CAR modified T-cells recognize their target, usually, a cell surface antigen, independently of the Major Histocompatibility Complex (MHC) presentation of antigen and are not compromised by tumour cell variants with lowered or deficient antigen possessing which represents a commonly observed mechanism of tumour immune escape. CARs are in the focus of research in the recent years. In particular, their capacity to target and lyse pre-

defined cells are in the focus of immunotherapy. Recent clinical trials have underscored the potential of adaptive therapy of cancer with CAR-redirectioned T-cells. For example, neuroblastoma patients treated with GD2 Ganglioside-specific CAR T-cells showed some encouraging anti-tumour effects although the T-cells persist only for a brief period. Further studies proved the concept that CAR engineered T-cells can initiate a productive anti-tumour response in patients suffering from various malignancies. The CAR approach differs to other antibody mediated immune therapy strategies, e.g. by using immunotoxins, in so far that engineered cells are used instead of single molecules.

[0004] In recent years, efforts have been done in the optimization of the CAR design, see e.g. Bridgeman J.S., et al., *Curr Gene Ther* 2010, 10, 77-90. However, many challenges remain, in particular, the necessity of a more effected anti-tumour response and prolonging T-cell survival allowing long term T-cell persistence of said engineered T-cells in the body. In addition, the co-stimulatory signals required for a successful clinical application remains to be illusive, e.g. see Hombach A, et al., *Curr Mol Med*. 2013 Aug;13(7):1079-88. Hence, there is ongoing work on optimizing CAR for various approaches including adaptive immunotherapy.

[0005] Hombach A., et al., *Gene Therapy*, 2010, 17, 1206-1213 describe the modification of the IgG1 Fc spacer domain in the extracellular moiety of Chimeric Antigen Receptors for avoiding off-target activation and unintended initiation of an innate immune response. Therein, T-cells engineered with modified CAR, either an anti-CD30 CAR or an anti-CEA CAR, have been used. As demonstrated therein, anti-CEA CAR T-cells are not effective against CEA negative cells and anti-CD30 CAR T-cells not against CD30⁺ cells. The modification disclosed in this publication relates to the avoidance of target activation by unintended initiation of the immune response, e.g. due to cross reactivity with a spacer domain in the extracellular moiety of the CAR.

[0006] In Kofler et al., 2011, *Mol. Ther.* 19, 760-767 a CAR molecule is described having a CD28 endodomain combined with a CD3 endodomain and an antibody derived scFv ectodomain specific for CEA. It is described therein, that a deletion of the Ick binding moiety in the CD28 CAR endodomain improves redirectioned anti-tumour activity in presence of T-regulatory cells without impairment of interferon-gamma secretion, proliferation and cytotoxicity. It is speculated, that the CAR with the modified CD28 endodomain expedite the implementation of adoptive T-cell therapy in patients with a variety of cancer types that are heavily infiltrated by regulatory T-cells (Treg cells).

[0007] A summary of the present knowledge on chimeric antigen receptor redirectioned T-cells is given in the review article of Chmielewski M., et al, *frontiers in immunology*, 2013, 4, 1-7 as well as in Melenhorst JJ, Levine BL., *Cytotherapy*. 2013 Sep; 15(9):1046-53. In addition, a summary of adoptive therapy of cancer with CAR redirectioned T-cells is provided in Hombach A., et al., *Curr Mol Med*, 2013, 13(1), 1-10. Therein, the effects of CAR are summarized including co-stimulation activity as well as improvement and prolongation of the redirectioned anti-tumour T-cell response. In addition, the adverse effects of this kind of adaptive therapy are described including "cytokine storm" and "T-cell repression".

[0008] Recently, Chmielewski et al, Immunol Reviews vol. 257, 2014, p83-90 discusses the possibility of CAR T-cells engineered with an inducible cytokine to modulate the tumor stroma.

[0009] Wilkie, S. et al., J. Clin. Immunol., 2012, 32, 1059-1070 describe a dual targeting of ErbB2 and MUC1 in breast cancer using two separate CARs, each capable for full T cell activation. Kloss et al., Nat Biotechnol. 2013 Jan;31(1):71-5, report T cells engineered with two separate CARs which provide complementary signalling. The vectors described therein encode two separate CAR molecules expressed simultaneously by the same T cells.

[0010] Grada, Z. et al., Molecular Therapy-Nucleic Acids, 2013, vol. 2, page 105 relate to TanCAR, a novel bispecific CAR for cancer immunotherapy. The CAR molecules described therein are designed to identify ligands both being present on the target cell.

[0011] That is, currently used chimeric antigen receptor (CAR) modified T-cells for antigen-redirected targeting towards tumor cells show insufficient performance in the anti-tumor attack due to various reasons, in particular due to less amplification and cytolytic activity after adoptive transfer into the patient. Hence, a major obstacle of the strategy for clinical use is the insufficient performance of engineered T-cells in the long-term.

[0012] Further, beside beneficial effect of the CAR expressing T-cell in adoptive therapy, ample effects are known which presently hinder favourite development of respective therapy as mentioned above.

Brief description of the present invention

[0013] The present invention aims at improving persistence and performance of CAR T-cells.

[0014] In a first aspect, the present invention relates to a recombinant polypeptide as defined in claim 1 or claim 2 containing at least the following domains starting from the N-terminus to the C-terminus: a first domain containing an anti-CD30 single chain antibody unit, of HRS3 scFv of SEQ ID No. 2 and an antibody unit said antibody unit being specific for an antigen present on the surface of a predetermined target cell being specific for a tumor-associated antigen; optionally a spacer domain; a trans-membrane domain; and a cytoplasmatic signalling domain. In particular, the present invention relates to a recombinant peptide wherein the first domain comprises the anti-CD30 single chain antibody unit in combination with an antibody unit whereby this antibody unit is specific for a tumor-associated antigen. The anti-CD30 single chain antibody unit is the HRS3 scFv of SEQ ID No. 2 while the antibody unit is a unit binding to the tumor-associated antigen of carcinoembryonic antigen (CEA), in particular of SEQ ID No. 4. In a further aspect, the present invention relates to a nucleic acid molecule encoding the polypeptide according to the present invention as well as a vector comprising said nucleic acid sequence. Moreover, an isolated cell, cell line or host cell containing said nucleic acid sequence or said vector is provided.

[0015] In addition, the present invention is directed to an isolated T-cell, like a CD8⁺ and/or a CD4⁺ T-cell, expressing on its surface chimeric antigen receptors having in their extracellular domain two antibody units, an antibody unit being specific for an antigen present on the surface of a predetermined target cell, and an anti-CD30 single chain antibody unit as defined in the claims. These units are either present in a single polypeptide as defined herein or present in different functional chimeric antigen receptors. These cells are particularly useful for treating tumor cells expressing the antigen on its surface but may not express CD30 on its surface. That is, by combining these two units, namely, the anti-CD30 single chain antibody unit and an antibody domain being specific for a tumor-associated antigen, tumor cells expressing the tumor specific antigen on its surface but being CD30⁻ can be treated more efficiently. Hence, herein a method for treating subjects being afflicted with cancer whereby the tumor cells of said cancer express the antigen but are CD30⁻ (CD30 negative) is described.

[0016] In particular, the cells useful for adoptive cell therapy according to present invention express on its surface chimeric antigen receptors having in the extracellular domain the antibody unit being specific for an antigen present on the surface of a predetermined cell, in particular, wherein the antibody unit is an antibody unit being specific for a tumor-associated antigen, and an anti-CD30 single chain antibody unit whereby these two units may be present either in a single polypeptide representing a CAR or may be present in different functional chimeric antigen receptors.

Brief description of the drawings

[0017]

Figure 1: Schematic representation of the modular composition of the CAR molecules. #1138 represents the anti-CD30 CAR; #946 represents the anti-CEA CAR. The CAR #1457 is the bispecific CAR molecule according to the present invention composed of the anti-CD30 single chain antibody scFv HRS3, the anti-CEA scFv BW431/26, the spacer domain IgG1-Fc, the trans-membrane domain of CD28 and the cytoplasmic signalling domain of the CD28ΔLCK and CD3zeta chain.

Figure 2: Peripheral blood lymphocytes were engineered with CARs by standard procedures. After cultivation CD3⁺ cells were analysed for CAR expression utilising FITC-conjugated anti-CD3 and PE-conjugated anti-human IgG1Fc antibodies. As demonstrated, transduction was successfully conducted and T-cells express the anti-CD30/CEA CAR on the cell surface in similar amounts as T-cells transduced with the anti-CD30 and anti-CEA CAR, respectively.

Figure 3: Human T-cells were engineered with CARs as indicated with an initial CAR expression rate of anti-CEA-CAR: 22.2%; anti-CD30-CAR: 28.9%; anti-CEA/CD30-CAR: 22.8%. CAR T-cells were cultivated 300 hours in the presence of IL-2 and CAR expressing cells were monitored. CAR T-cells with a CD30 binding domain expand more efficiently than T-cell with

the anti-CEA CAR only.

Figure 4: Engineered human T-cells expressing different kinds of CARs as indicated (initial CAR expression: anti-CEA-CAR: 8.5%; anti-CD30-CAR: 9.3 9%; anti-CEA/CD30-CAR: 8.2%). CAR T-cells ($0.625-5 \times 10^4$ cells/well) were co-incubated for 24 hours with tumor cells ($2.5-5 \times 10^4$ cells/well) as indicated. Three different types of cell lines were used being either positive for CEA or CD30 or as control negative for both CEA and CD30. The cell line being negative for both CEA and CD30 was not lysed while specific lysis of LS174T cells was observed which is a CEA⁺/CD30⁻ cell line. Therein, the anti-CEA/CD30 CAR T-cells demonstrate a higher specific lysis compared to the anti-CEA CAR T-cells.

Figure 5: The release of interferon-gamma, a marker for T-cell activation, during co-cultivation of different numbers of effector cells with the tumor cells is shown. After 24 hours co-cultivation the supernatants were collected and analysed for interferon-gamma by ELISA. The assay was performed in triplicate and the SD was determined. As shown, anti-CEA/CD30 CAR T-cells produced IFN-gamma when co-incubated with the CEA⁺/CD30⁻ LS174T-cells as well as with the CEA⁻ CD30⁺ cells. No IFN-gamma was produced upon co-incubation with CEA⁻ CD30⁻ Colo320 cells. T-cells with the anti-CEA CAR or anti-CD30 CAR released IFN-gamma only upon co-incubation with CEA⁺ or CD30⁺ cells, respectively.

Figure 6: Figure 6 shows the cytolytic activity of CAR CIK T-cells (cytokine induced killer cells with NKT-cell phenotype). It is demonstrated that enhanced killing of CEA⁺ CD30⁻ target cells by using the anti-CEA/CD30 CAR CIK T-cells is achieved. The tumor cell lines are the same as described in figure 2.

Figure 7: Figure 7 demonstrates improved in vivo killing of CEA⁺ tumor cells by anti-CEA/CD30 CAR T-cells compared to the mono-specific anti-CEA CAR T-cells. Subtherapeutic doses of T-cells (2.5×10^5 cells/mouse) without CAR and T-cells engineered either with the anti-CEA/CD30 CAR or the anti-CEA CAR were co-injected with CEA⁺ CD30⁻ C15A3 tumor cells (1×10^6 cells/mouse) into immune deficient Rag^{-/-} common gamma chain^{-/-} mice. For comparison, C15A3 tumor cells were injected without T-cells (w/o). Tumor volumes and area under curve (AUC) were determined. Significance was determined by Student's T-test. Significant differences ($p < 0.05$) were indicated by asterisks.

Figure 8: Figure 8A shows schematic representation of anti-CD30 CARs and the CD25/CEA bispecific CAR of Seq. ID. No. 18 encoded by the sequence of Seq. ID. No.17. The HRS3-scFv of CAR #1457 was exchanged by the anti-CD25-scFv RFT5. The backbone of the resulting #1576 CAR is identical to CAR # 1457 shown in Figure 1.

Figure 8B shows the specificity of cytotoxicity. CAR T cells ($0.625-5 \times 10^4$ cells/well) were co-incubated for 24 hours with CEA⁺ or CEA⁻ tumor cells ($2.5-5 \times 10^4$ cells/well) as indicated. Only CAR T cells expressing the bispecific anti-CD30/CEA CAR lysed CEA⁺ target cells whereas CAR T cells with the bispecific anti-CD25/CEA CAR and CAR T cells with a monospecific anti

CEA CAR lysed CEA+ targets with lower efficiency. CEA- tumor cells were not lysed demonstrating the CEA-specificity of CAR T cells.

Figure 9: Figure 9A is a schematic representation of the molecular composition of the CAR molecules with specificity for CD30/MUC1 of Seq. ID. No. 20 encoded by the sequence of Seq. ID. No. 19 and CD30/TAG-72 of Seq. ID. No. 22 encoded by the sequence of Seq. ID. No. 21. B72.3 is used for the anti-TAG-72 CAR. Except for the anti-CEA scFv, the molecules are identical with the CAR shown in figure 1. Cells surface expression of CD30/Muc1 and CD30/TAG-72 CARs is demonstrated in Fig. 9B by flow cytometry.

Detailed description of the present invention

[0018] The inventors aim to provide new recombinant polypeptides as mentioned in claim 1 or claim 2 containing at least the following domains starting from the N-terminus to the C-terminus: a first domain containing an anti-CD30 single chain antibody unit of HRS3 scFv of SEQ ID No. 2 and an antibody unit said antibody unit being specific for an antigen present on the surface of a predetermined target cell being specific for a tumor-associated antigen; optionally a spacer domain; a trans-membrane domain; and a cytoplasmatic signalling domain. These recombinant polypeptides represent bispecific CAR peptides. In an embodiment of the present invention, these CAR polypeptides are expressed by genetically engineered T-cells containing and eventually expressing these CAR polypeptides.

[0019] That is, the present invention relates to a recombinant polypeptide having at least the following domains starting from the N-terminus to the C-terminus: a first domain containing an anti-CD30 single chain antibody unit of HRS3 scFv of SEQ ID No. 2 and an antibody unit said antibody unit being specific for an antigen present on the surface of a predetermined target cell, in particular, being specific for a tumor-associated antigen; optionally a spacer domain; a trans-membrane domain; and a cytoplasmatic signalling domain according to claim 2.

[0020] The present invention likewise relates to the following domains, a first domain an antibody unit said antibody unit being specific for an antigen present on the surface of a predetermined target cell, and a second domain containing an anti-CD30 single chain antibody unit, optionally a spacer domain; a trans-membrane domain; and a cytoplasmatic signalling domain.

[0021] As used herein, the term "comprise" or "comprising" as well as the terms "contain" or "containing" includes the embodiment of "consist" or "consisting".

[0022] The term "homolog" as used herein refers to molecules, either DNA or polypeptides, having a sequence homology of a certain amount, namely of at least 95%, like at least 96%, 97%, 98%, 99% of the nucleic acid sequence or the amino acid sequence it is referred to.

Homology refers to the magnitude of identity between two sequences. Homolog sequences have the same or similar characteristics, in particular, have the same or similar property of the sequence as identified. For example, the homolog of the HRS3 scFv sequence of Seq. ID. No. 2 has the same or similar binding specificity to the CD30 molecule as it is the case for the HRS3 scFv molecule. Further, homologs include nucleic acid molecules encoding the same peptide but may vary in its sequence due to the degeneracy of the genetic code. Further, "identify" refers to the presence of identical amino acid or nucleic acid molecules in the order as described for the sequence it refers to. That is, in case of at least 90% identity, 90% or more of the nucleic acid and amino acid molecules, respectively, are identical at the respective positions. Unless otherwise identified, the terms "homology" and "identity" are used herein interchangeably. In particular, the homolog of the HRS3 scFv sequence of Seq. ID. No.2 include anti CD30 single chain antibody units binding to the same epitope recognized by the HRS3 scFv.

[0023] In addition, the term "genetically engineered" refers to cells being manipulated by genetic engineering. That is, the cells contain a heterologous sequence which does not naturally occur in said cells. Typically, the heterologous sequence is introduced via a vector system or other means for introducing nucleic acid molecules into cells including liposomes. The heterologous nucleic acid molecule may be integrated into the genome of said cells or may be present extra-chromosomally, e.g. in the form of plasmids. The term also includes embodiments of introducing genetically engineered, isolated CAR polypeptides into the cell.

[0024] Generally, CARs are fusion proteins, consisting of an extracellular antibody type recognition domain fused to intracellular T-cell signalling proteins. Typically, the ectodomain containing the antigen recognition region comprises a signal peptide and an antigen recognition unit. According to the present invention, the ectodomain comprises an anti CD30 single chain unit in combination with an antibody unit being specific for an antigen present on the surface of a predetermined target cell, in particular, being specific for a tumor-associated antigen. The single chain unit is a single chain unit from HRS3 scFv of SEQ. ID. No. 2. That is the anti-CD30 single chain unit is a single chain unit being specific to the epitope of the CD30 molecule recognized by the anti CD30 single chain unit HRS3 scFv of Seq. ID. No.2.

[0025] The ectodomain may be spaced apart from the transmembrane domain by the presence of a spacer domain. Said optional spacer domain links the antigen-binding domain to the transmembrane domain and it is preferred that said trans-membrane domain is flexible enough to allow the antigen binding domain to orient in different directions to facilitate antigen recognition.

[0026] The transmembrane domain is typically a hydrophobic alpha helix that spans the membrane. Finally, the endodomain represents the signalling domain in the cytoplasm of the cells.

[0027] Further, the term "antibody unit being specific for an antigen present on the surface of a predetermined target cell" refers to the binding site derived from an antibody of any species

either in form of a single polypeptide chain or multiple covalently or non-covalently associated polypeptide chains. These domains are characterized by specific binding to a molecule on the surface of a target cell. Any other binding moiety than an antibody can alternatively be used as targeting domain in a CAR, e.g., the binding moiety of a receptor like a cytokine receptor.

[0028] Moreover, the term "antibody unit being specific for a tumor-associated antigen" refers to a binding domain with specificity to a target molecule present on the cell surface expressed by tumor cells and composed of a polypeptide, carbohydrate or lipid or combinations thereof that are exclusively or preferentially expressed by malignant cells.

[0029] The term "functional CAR" refers to a CAR expressed by immune cells that by specific binding to the cognate target molecule activates the same immune cell to increase protein biosynthesis, cytokine secretion, cell proliferation and target cell lysis.

[0030] Moreover, the term "CD30⁺ cells" or "CD30⁺ cancer cells" refers to cells (either non-cancer or cancer cells) expressing on their surface the CD30 molecule.

[0031] The terms "non-tumor cells" and "tumor cells" as well as "non-cancer cells" and "cancer cells" are used herein interchangeably unless otherwise defined.

[0032] The term "CIK T-cells" or "NK T-cells" which are used herein interchangeably refers to a heterogeneous group of T-cells that share properties of both T-cells and natural killer (NK) cells. CAR CIK T-cells are obtained by known cultivation techniques to obtain firstly NK T-cells or CIK T-cells and, thereafter, engineering the same cells with the CAR by known methods.

[0033] The recombinant polypeptide according to the present invention represents a bispecific CAR also identified herein as dual specific CAR. In an embodiment, the order in the first domain is from the N-terminus to the C-terminus: i) an anti-CD30 single chain antibody unit and an antibody unit, said antibody unit being specific for an antigen present on the surface of a predetermined target cell, or ii) an antibody unit whereby said antibody unit being specific for an antigen present on the surface of a predetermined target cell, and an anti-CD30 single chain antibody unit.

[0034] It is preferred that the antibody unit being specific for an antigen present on the surface of a predetermined target cell is an antibody unit being specific for a tumor-associated antigen.

[0035] In an embodiment of the present invention, the CAR present in the T-cell comprises a leader sequence being located N-terminally to the first domain containing the anti CD30 single chain unit and the antibody unit.

[0036] In addition, in another embodiment, the anti-CD30 single chain antibody unit is a HRS3 scFv peptide, in particular, of SEQ. ID. No. 2, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 1. It has been recognized herein that an anti-CD30 single chain antibody fragment of the variable region (scFv), in particular, of HRS3, allows displaying the desired activity. In an

embodiment, the anti CD30 single chain antibody fragment is a fragment recognizing and binding to the epitope of HRS scFv.

[0037] In another embodiment, the spacer domain of the CAR molecule is an IgG₁ hinge-CH2CH3 domain of SEQ. ID. No. 6 or homologs thereof having at least 95% identity therewith, preferably, the spacer domain is a mutated IgG₁ hinge-CH2CH3 domain according to SEQ. ID. No.6, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 5.

[0038] In some embodiments, a linker may be located between the spacer domain and the transmembrane domain.

[0039] Further, another embodiment relates to a T-cell with a chimeric antigen receptor wherein the transmembrane domain is derived from the CD28 molecule, e.g. the transmembrane domain of the CD28 molecule lacking the Ick domain of SEQ. ID. No. 8, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 7.

[0040] The signalling domain or endodomain or intracellular domain which are used herein interchangeably, contains a CD3 zeta or FcEpsilon-Receptor gamma chain signalling chain or a co-stimulatory domain or both the CD3zeta and a co-stimulatory domain or the FcEpsilon-Rezeptor gamma chain and a co-stimulatory domain. For example, the intracellular domain is a CD3 zeta signalling domain of SEQ. ID. No. 10, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 9, or a homolog thereof having at least 95% homology. In another embodiment, the intracellular domain is the IgE Fc-Receptor gamma signalling domain of SEQ. ID. No. 12, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 11, or a homolog thereof having at least 95% identity. The signalling domain is responsible for the activation of the T-cells, in particular the cytotoxic activity and cytokine release including Interferon gamma secretion.

[0041] The CAR molecule may be a so-called second generation CAR molecule. Second generation CAR molecules have improved signalling domains additionally containing a second signalling (costimulatory) domain, e.g. derived from CD28, CD134 (OX40) or CD137 (4-1BB). Third generation CAR molecules contain a combined co-stimulatory signalling domain, e.g., CD28 combined with CD137 or CD134.

[0042] An overview about the CAR molecules is provided e.g. in Gilham D.E. et al., Trans. and Molecular Medicine, 2012, 18(7), 377-384.

[0043] In a preferred embodiment of the present invention, the T-cell is a T-cell with a chimeric antigen receptor wherein the chimeric antigen receptor is a polypeptide of SEQ. ID. No. 14, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 13. Said CAR is also referred to herein as #1457.

[0044] The anti-CD30 anti-CEA CAR #1457 is expressed on the surface of T-cells and is composed in the extracellular part of the anti-CD30 single chain fragment of variable region (scFv) antibody HRS3, the BW431/26 scFv antibody corresponding to Seq. ID. No. 4, e.g.

encoded by the nucleic acid sequence of Seq. ID. No. 3, corresponding to the anti-CEA antibody BW431/26 and the modified human IgG1 CH2CH3 domain as spacer between scFv and the trans-membrane domain. The modification of the IgG1 domain consists of point mutations to convert the wild-type amino acid sequence PELLGGP X₁₃ MISRT (Seq. ID. No. 15) to PPVA-GP X₁₃ MIART (Seq. ID. No. 16) which reduces unintended binding of the CAR Fc domain to Fc receptors on other cells like innate immune cells which would mediate their activation. The transmembrane and intracellular membrane proximal part of CAR # 1457 is derived from human CD28 and is fused to the intracellular part of human CD3zeta. The CD28 sequence is mutated at P560 > A560, P563 > A563, P564 > A564 (Kofler et al., Mol. Ther. 19, 760 - 767 (2011)). Thereby the CD28 binding site for the Ick kinase is destroyed with the consequence that activation of the Ick signalling pathway and subsequent CAR mediated IL-2 secretion is prevented. Pre-clinical models imply that Treg cell mediated repression of CAR T-cell effector functions is reduced under these conditions.

[0045] As an example, the recombinant polypeptide is the polypeptide of SEQ ID No. 14, e.g. encoded by the nucleic acid sequence of Seq. ID. No. 13 correspond to the #1457 CAR shown in figure 1.

[0046] In another embodiment, the recombinant polypeptide is a polypeptide wherein the antibody unit binds to a tumor-associated antigen including any one of carcinoembryonic antigen (CEA), CA19-9, CA72-4 also known as TAG-72, PSCA, Muc-1, HMW-MAA, p97 melanotransferrin, fetal actelycholin receptor, ErbB2 (Her2/neu), multi-drug-resistance protein (MDR), CD19, CD20, TOSO.

[0047] For example, the CAR #1457 of Seq. ID. No. 14 correspond to a polypeptide wherein the antibody unit being specific to CEA. Further, CARs useful according to the present invention are shown in figure 9, namely, CD30/MUC-1 CAR or CD30/Tag72 CAR. These CARs are #1587, anti-CD30/MUC1, of Seq. ID. No. 20 encoded by the sequence of Seq. ID. No. 19, and #1650 anti-CD30/TAG-72 of Seq. ID. No. 22 encoded by the sequence of Seq. ID. No. 21.

[0048] As demonstrated in the examples, T-cells (either CD4 or CD8 T-cells) or CIK-cells demonstrate specific lysis of cells not expressing CD30 but expressing the antigen to which the antibody unit is specifically binding only. That is, surprisingly the percentage of specific lysis is higher with the bispecific CAR molecule compared to the monospecific molecule containing the antibody binding unit specific for the antigen present on the surface of the predetermined target cell only.

[0049] The target cell is a tumor cell.

[0050] Moreover, the predetermined target cell may be a cell involved in autoimmune defects in a subject. In that particular case suppressor cells, in particular regulatory T-cells, are modified with the described CAR in order to repress the acute inflammatory immune reaction in the targeted tissue. The CAR binds to tissue antigen with one scFv, e.g., anti-HLA B27, and to CD30 with the other scFv.

[0051] Although not expressed by the predetermined target cells, the anti-CD30 single chain antibody unit present in the CAR molecule increases specific lysis of the target cells. Furthermore, it has been surprisingly shown that in case of CD30 negative cells the percentage of specific lysis can be increased as demonstrated in the examples. This is not only true for lysis by CAR expressing T-cells but also by CAR CIK-cells. It is considered that autostimulation of the CAR T-cells allows to increase specific lysis of the target cells.

[0052] In addition, the present invention provides nucleic acid molecules comprising the nucleic acid sequence encoding the polypeptide according to the present invention. Furthermore, vectors are provided comprising the nucleic acid sequence according to the present invention encoding the polypeptide as described. The skilled person is well aware of suitable vector systems and vectors, in particular, vectors allowing transfection and transduction of eukaryotic cells, in particular, T-cells. For example, the vector is a viral vector.

[0053] Moreover, the present invention provides an isolated cell, cell line or a host cell containing the vector according to the present invention or a nucleic acid molecule according to the present invention. Preferably, said cell, cell line or host cell is an isolated T-cell, e.g., a CD4⁺ T-cell or a CD8⁺ T-cell.

[0054] Further, a kit or system containing the vector according to the present invention, the cell, cell line or host cell according to the present invention, or the polypeptide according to the present invention or a nucleic acid molecule according to the present invention or mixtures thereof for use in the production of T-cells expressing the chimeric antigen receptor is described. The kit or system may contain further components including means for introducing the vector, polypeptide or nucleic acid molecules into the cells. The skilled person is well aware of suitable means for doing so.

[0055] Moreover herein are provided T-cells, like CD8 and/or CD4 T-cells expressing on its surface chimeric antigen receptors having in the extracellular domain an antibody unit, said antibody unit being specific for a tumor associated antigen and/or being specific to a viral antigen present on the surface for an antigen present on the surface of a predetermined target cell, and an anti-CD30 single chain antibody unit expressed by said cells. The units may be present either in a single polypeptide, e.g. a polypeptide as defined herein, or, alternatively, may be present in different chimeric antigen receptors.

[0056] In another aspect, the lymphocyte may be a CAR CIK-T-cell as defined herein.

[0057] The lymphocytes (T-cells) including the CD8⁺, CD4⁺ or CIK-T-cells demonstrate higher specific lysis of the target cells, thus, reducing side effects and, in addition, overcoming the problem of insufficient performance in the anti-tumor attack due to various reasons, for example, less amplification and cytolytic activity after adopted transfer into the patient.

[0058] It is considered that the presence of the anti-CD30 single chain antibody unit allows to

amplify the genetically engineered lymphocytes and improve persistence of the same, thus, overcoming the problem of insufficient performance.

[0059] The cells are particularly useful in treating cancer, in particular CD30⁻ cancer whereby said cancer is a cancer expressing on the cancer cells the antigen to which the anti-tumor antigen antibody is directed to.

[0060] In a further aspect, the use of T-cells with a chimeric antigen receptor according to the present invention in adaptive cell therapy for treating CD30⁻ or CD30⁺ cancer in a subject in need thereof is disclosed. For instance the CD30⁺ cancer may be Hodgkin's lymphoma, anaplastic large cell lymphoma, acute lymphocytic leukaemia, cutaneous lymphoma, mycosis fungoides, lymphoproliferative diseases, systemic mastocytosis, teratocarcinoma, stem cell derived malignancies, or cancer stem cells or others. In particular the invention relates to the use of such immune cells for the treatment of CD30⁻ cancers, e.g., breast, lung, prostate, pancreas, gastrointestinal carcinomas, neuronal cancer and others. Thus, the present invention may foster adaptive immune therapy by CAR T-cells.

[0061] The present invention is further described by way of examples. Said examples illustrate the invention further without limiting the same thereto.

Examples

Preparation of CAR #1457

[0062] The expression cassette of the bispecific anti-CD30/CEA-CAR was generated as follows: The anti-CD30-scFv (HRS3) and the anti-CEA-scFv (BW431/26) were PCR amplified and flanked with a linker by overlapping sequences at the 3'terminus of the CD30-scFv and the 5'terminus of the anti-CEA-scFv while the linker coding for a 40 AA (Gly(4)-Ser(1))₅ linker. DNAs of scFvs were assembled by SOE PCR, digested with NcoI and BamHI and ligated into the NcoI/BamHI site of the retroviral vector pBullet-HRS3-scFv-Fc-CD28deltaIck-zeta thereby replacing the HRS3-scFv by the combined HRS3scFv-BW431/26scFv. The resulting vector coding for the bispecific anti-CD30/CEA-CAR was termed #1457. The modular composed #1457 CAR and its mono-specific anti-CEA (#946) and anti-CD30 (#1138) counterparts are schematically shown in figure 1.

[0063] The retroviral vector coding for the #1457 CAR was produced according to SOP-GL-VectProd using a Galv pseudotyped envelope. In summary vector particle production was done transiently on the human embryonic kidney cell line 293T after Polyfect[®] mediated DNA transfection. Vector particles were pseudotyped with Galv. No vector titer was determined.

[0064] Transduction of human blood lymphocytes was done according to SOP standard techniques. In summary human lymphocytes were transduced with a 2-day supernatant from transfected 293T-cells for 2 days. The CAR #1457 was expressed by 20-35 % of human T-cells as measured at day 2 by flow cytometry using an antibody directed to the extracellular constant IgG1 CH2 CH3 domain of the CAR.

[0065] CD4⁺ and CD8⁺ T-cells which express the CAR #1457 on the cell surface can be recorded by use of the 9G10 antibody which binds specifically the CAR HRS3 scFv domain. T-cells engineered with the #1457 CAR bind specifically to CD30 expressing cells and become activated indicated by increased secretion of cytokines including IFN- γ , by increase in proliferation and in cytotoxicity of CD30⁺ target cells. Noteworthy, only background levels of IL-2 are secreted when T-cells are stimulated by the CAR. IL-2, however, is secreted in physiological amounts when T-cells are stimulated by their physiological TCR and CD28. Activation of the T-cells #1457 is antigen-specific as defined by the specificity of the CAR since CD30⁻ cells do not trigger T cell activation. Soluble CD30, which accumulates in the serum of CD30⁺ lymphoma patients, does not block CAR mediated T-cell activation in concentrations up to 10 μ g/ml [Hombach A, et al., Cancer Res. 1998 Mar 15;58(6):1116-9]. This is due to the fact that the CAR must be cross-linked by binding the multiple copies of the targeted antigen in order to trigger T-cell activation which can only occur when CD30 is immobilized or expressed on the surface of target cells but does not occur when CD30 protein is present in solution.

Example 1: Activity of dual specific CAR modified T-cells toward CEA⁺ and/or CD30⁺ cell lines

[0066] Engineering of T-cells with CAR #1457 was performed as described above. The CAR was detected on the T-cell surface by flow cytometry utilizing an antibody against the Fc domain in the extracellular moiety of the CAR (Fig. 1, 2). The anti-CEA/CD30 CAR was expressed with similar efficiency as the mono-specific CARs with either the CD30 or the CEA binding domain. To test for dual specificity of the #1457 CAR, grafted T-cells were co-incubated either with CD30⁺ or CEA⁺ target cells and redirected cytotoxicity and IFN- γ secretion were monitored. Whereas T-cells with anti-CEA and anti-CD30 CAR were only activated by CEA⁺ or CD30⁺ target cells, respectively, the bispecific CAR activates T-cells after co-culture with both CEA⁺ CD30⁻ and CEA⁻ CD30⁺ tumor cells. T-cell activation results in specific target cell lysis and IFN- γ secretion (cf. Fig. 4, 5 and Example 3).

Conclusion:

[0067] T-cells with the #1457 CAR harbour dual specificity for binding, i.e., for CD30 and CEA. T-cells expressing the bispecific CAR were activated by engaging target cells with one cognate

CAR antigen only as well as by target cells with both antigens.

Example 2: Specific expansion of #1457 CAR modified T-cells

[0068] T-cells were engineered with the #1457 CAR or for comparison with the corresponding single CD30 or CEA specific CAR, respectively, and cultivated in presence of IL-2 (400 U/ml). The initial number of CAR+ T-cells was 20-30%. Cells were tested at different time points for CAR expression by flow cytometry. As summarized in Figure 3, the number of T-cells with the anti-CD30-CAR and the anti-CEA/CD30 CAR #1457 increased up to 90% of T-cells during cultivation whereas the number of anti-CEA CAR T-cells did not exceed 50%.

Conclusion

[0069] Data demonstrate that CAR T-cells with the CD30-binding domain expand superior compared to CAR T-cells with a CEA binding domain. The superior effect is also present when the anti-CD30 binding domain is linked with the anti-CEA binding domain.

Example 3: Increased specific lysis by CAR #1457 engineered T-cells and CAR #1457 engineered CIK T-cells toward CD30-negative target cells

(a) T-cells

[0070] Human T-cells were engineered to express the CAR #1457, the CEA-specific CAR and the CD30-specific CAR, respectively. The same number of CAR T-cells was co-incubated with tumor cells which express either CEA or CD30 or both. Data presented in Figure 4 show that the cell line being negative for both CEA and CD30 (Colo320) was not lysed while specific lysis of LS174T cells was observed which is a CEA⁺/CD30⁻ cell line. Therein, the anti-CEA/CD30 CAR T-cells demonstrate a higher specific lysis compared to the anti-CEA CAR T-cells.

[0071] The cytolytic activity is accompanied by release of IFN- γ , a marker for T-cell activation, as shown in Figure 5. Anti-CEA/CD30 CAR #1457 T-cells produced IFN- γ when co-incubated with the CEA⁺/CD30⁻ LS174T-cells as well as with the CEA⁻ CD30⁺ cells. No IFN- γ was produced upon co-incubation with CEA⁻ CD30⁻ Colo320 cells. T-cells with the anti-CEA CAR or anti-CD30 CAR released IFN- γ only upon co-incubation with CEA⁺ or CD30⁺ cells, respectively.

(b) CIK cells

[0072] CIK cells were generated according to standard procedures and were engineered with the anti-CD30/CEA CAR #1457. After 8 days post transduction #1457 CAR CIK cells were co-cultivated with CD30⁻CEA⁺, CD30⁻CEA⁻ or CD30⁺CEA⁻ target cells and target cell lysis was recorded. CIK cells expressing the corresponding monospecific anti-CD30 and anti-CEA CAR, respectively, served as control. Bispecific anti-CD30/CEA and mono-specific anti-CD30 CAR T-cells lysed CD30⁺ target cells with similar efficiency. In contrast, CAR #1457 CIK cells lysed CEA⁺ CD30⁻ target cells more efficiently than CIK cells with the anti-CEA CAR (Fig. 6). Data indicate that CAR #1457 CIK cells surprisingly show a higher cytolytic activity against tumor cells that do not express CD30 than CIK cells with a CEA-specific CAR.

Conclusion:

[0073] T-cells and CIK cells engineered with the CAR #1457 show enhanced anti-tumor reactivity against target cells, both which express and which lack CD30, compared to T-cells with the monospecific CAR. This is unexpected in particular for those target cells which lack CD30.

Example 4: Improved anti-tumor activity of # 1457 CAR T-cells in a xenograft mouse model.

[0074] The in vivo activity of anti-CD30/CEA CAR #1457 T-cells was monitored in the immune deficient Rag ^{-/-} common gamma chain ^{-/-} mouse. Anti-CEA-CAR and anti-CD30/CEA CAR #1457 T-cells were engineered as described above and subcutaneously co-injected in a sub-therapeutic dose (2.5x10⁵ CAR T-cells/animal) with CEA⁺ CD30⁻ C15A3 tumor cells (1x10⁶ cells/animal), that were transfected to express human CEA, into mice. Mice without T-cells and non-modified T-cells were used for control and tumor growth was monitored every 2nd-3rd day. Growth curves (Fig. 7A) and area under curve (Fig. 7B) were determined. Whereas anti-CEA-CAR T-cells produced slightly delay of tumor growth, anti-CD30/CEA CAR T-cells significantly delayed tumor growth indicating higher anti-CEA activity of the bispecific CAR T-cells #1457 in vivo than T cells with the anti-CEA CAR.

Conclusion:

[0075] Bispecific anti-CD30/CEA CAR T-cells are more effective in an prolonged anti-tumor response against CEA⁺ CD30⁻ tumor cells in vivo compared to T cells with the mono-specific anti-CEA CAR. The improved anti-tumor reactivity is due to the particular CAR design because human CD30 is neither expressed by the murine host nor by the grafted mouse tumor cells

and not recognized on tumor cells or on cells of the host.

Example 5: Improved anti-tumor activity of anti-CD30/CEA #1457 CAR but not anti-CD25CEA #1576 CAR.

[0076] The bispecific #1576 CAR of Seq. ID. No. 18 encoded by the sequence of Seq. ID. No. 17 with specificity for both the lymphocyte activation antigen CD25 and CEA was generated by substituting the HRS3 scFv with the anti-CD25 scFv RFT5 (Fig. 8A; cf Fig. 1). Engineering of T-cells with CAR #1576 was performed as described above by flanking the cDNA for the anti-CD25 scFv with overlapping sequences and sequences for appropriate restriction enzymes by PCR. The sequence of the HRS3 scFv was substituted and the resulting #1576 CAR expressed by transduction with retroviral supernatant according to transductionSOP. The CAR was detected on the T-cell surface by flow cytometry utilizing an antibody against the Fc domain in the extracellular moiety of the CAR. The anti-CD25/CEA CAR was expressed with similar efficiency than monospecific and bispecific anti-CD30 CARs, respectively. Specific CAR T cell activation and lysis of CEA+ target cells was tested by co-cultivation with CEA+ and CEA- tumor cells respectively. All CARs lysed specifically CEA+ target cells but the anti-CD30/CEA CAR lysed tumor cells with higher efficiency than monospecific and bispecific anti-CEA and anti-CD25/CEA CARs, respectively (Fig. 8B).

Conclusion:

[0077] T-cells with the #1457 anti-CD30/CEA CAR but not with the #1576 anti-CD25/CEA CAR were activated against CEA+ target cells with higher efficiency than anti-CEA-monospecific CAR T cells.

Example 6: Bispecific anti-CD30 antibodies with different second specificity

[0078] Bispecific anti-CD30 CAR molecules were generated as described in Example 1 except for substituting the anti-CEA scFv moiety with scFv moieties with specificity against MUC1, C595-scFv, resulting in anti-CD30/MUC1-CAR #1587, and with specificity against TAG-72, B72.3-scFv, resulting in anti-CD30/TAG-72 CAR #1650. The sequences thereof are Seq. Id. Nos. 20 and 19 for #1587 and Seq. ID. Nos. 22 and 21 for #1650.

[0079] Figure 9A is a schematic representation of these bispecific CARs. Figure 9B demonstrates expression of these CAR molecules on transfected 293 T-cells.

SEQUENCE LISTING

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<213> artificial

<220>

<223> synthetic nucleic acid

<220>

<221> CDS

<222> (1)..(204)

<400> 7

ttt tgg gtg ctg gtg gtg gtt ggt gga gtc ctg gct tgc tat agc ttg
Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

```

      10      20      30      40      50      60
cta gta aca gtg gcc ttt att att ttc tgg gtg agg agt aag agg agc      96
Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
      20      25      30

agg ctc ctg cac agt gac tac atg aac atg act ccc cgc cgc ccc ggg      144
Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
      35      40      45

ccc acc cgc aag cat tac cag gcc tat gcc gcc gca cgc gac ttc gca      192
Pro Thr Arg Lys His Tyr Gln Ala Tyr Ala Ala Ala Arg Asp Phe Ala
      50      55      60

gcc tat cgc tcc      204
Ala Tyr Arg Ser
65

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<210> 8

<211> 68

<212> PRT

<213> artificial

<220>

<223> Synthetic Construct

<400> 8

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Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1      5      10      15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser
      20      25      30

Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly
      35      40      45

Pro Thr Arg Lys His Tyr Gln Ala Tyr Ala Ala Ala Arg Asp Phe Ala
      50      55      60

Ala Tyr Arg Ser
65

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<210> 9

<211> 414

<212> DNA

<213> human

<220>

<221> CDS

<222> (1)..(414)

<400> 9

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gat ccc aaa ctc tgc tac ctg ctg gat gga atc ctc ttc atc tat ggt      48
Asp Pro Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly
1      5      10      15

gtc att ctc act gcc ttg ttc ctg aga gtg aag ttc agc agg agc gca      96
Val Ile Leu Thr Ala Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala
      20      25      30

```

gac gcc ccc gcg tac cag cag ggc cag aac cag ctc tat aac gag ctc 144
 Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
 35 40 45
 aat cta gga cga aga gag gag tac gat gtt ttg gac aag aga cgt ggc 192
 Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
 50 55 60
 cgg gac cct gag atg ggg gga aag ccg cag aga agg aag aac cct cag 240
 Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln
 65 70 75 80
 gaa ggc ctg tac aat gaa ctg cag aaa gat aag atg gcg gag gcc tac 288
 Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
 85 90 95
 agt gag att ggg atg aaa ggc gag cgc cgg agg ggc aag ggg cac gat 336
 Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
 100 105 110
 ggc ctt tac cag ggt ctc agt aca gcc acc aag gac acc tac gac gcc 384
 Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
 115 120 125
 ctt cac atg cag gcc ctg ccc cct cgc taa 414
 Leu His Met Gln Ala Leu Pro Pro Arg
 130 135

<210> 10

<211> 137

<212> PRT

<213> human

<400> 10

Asp Pro Lys Leu Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly
 1 5 10 15
 Val Ile Leu Thr Ala Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala
 20 25 30
 Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
 35 40 45
 Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
 50 55 60
 Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln
 65 70 75 80
 Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
 85 90 95
 Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
 100 105 110
 Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
 115 120 125
 Leu His Met Gln Ala Leu Pro Pro Arg
 130 135

<210> 11
 <211> 201
 <212> DNA
 <213> human

<220>
 <221> CDS
 <222> (1)..(201)

<400> 11
 gat ccc aaa ctc tgc tat atc ctg gat gcc atc ctg ttt ctg tat gga 48
 Asp Pro Lys Leu Cys Tyr Ile Leu Asp Ala Ile Leu Phe Leu Tyr Gly
 1 5 10 15
 att gtc ctc acc ctc ctc tac tgt cga ctg aag atc caa gtg cga aag 96
 Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile Gln Val Arg Lys
 20 25 30
 gca gct ata acc agc tat gag aaa tca gat ggt gtt tac acg ggc ctg 144
 Ala Ala Ile Thr Ser Tyr Glu Lys Ser Asp Gly Val Tyr Thr Gly Leu
 35 40 45
 agc acc agg aac cag gag act tac gag act ctg aag cat gag aaa cca 192
 Ser Thr Arg Asn Gln Glu Thr Tyr Glu Thr Leu Lys His Glu Lys Pro
 50 55 60
 cca cag tag 201
 Pro Gln
 65

<210> 12
 <211> 66
 <212> PRT
 <213> human

<400> 12
 Asp Pro Lys Leu Cys Tyr Ile Leu Asp Ala Ile Leu Phe Leu Tyr Gly
 1 5 10 15
 Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile Gln Val Arg Lys
 20 25 30
 Ala Ala Ile Thr Ser Tyr Glu Lys Ser Asp Gly Val Tyr Thr Gly Leu
 35 40 45
 Ser Thr Arg Asn Gln Glu Thr Tyr Glu Thr Leu Lys His Glu Lys Pro
 50 55 60
 Pro Gln
 65

<210> 13
 <211> 2943
 <212> DNA
 <213> artificial

<220>

<223> synthetic sequence

<220>

<221> CDS

<222> (1)..(2943)

<400> 13

ctc tag act gcc atg gat ttt cag gtg cag att ttc agc ttc ctg cta	48
Leu Thr Ala Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu	
1 5 10 15	
atc agt gcc tca gtc ata atg tct aga atg gcc cag gtg caa ctg cag	96
Ile Ser Ala Ser Val Ile Met Ser Arg Met Ala Gln Val Gln Leu Gln	
20 25 30	
cag tca ggg gct gag ctg gct aga cct ggg gct tca gtg aag atg tcc	144
Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser	
35 40 45	
tgc aag gct tct ggc tac acc ttt act acc tac aca ata cac tgg gta	192
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val	
50 55 60	
aga cgg agg cct gga cac gat ctg gaa tgg att gga tac att aat cct	240
Arg Arg Arg Pro Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro	
65 70 75	
agc agt gga tgt tct gac tac aat caa aac ttc aag ggc aag acc aca	288
Ser Ser Gly Cys Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr	
80 85 90 95	
ttg act gca gac aag tcc tcc aac aca gcc tac atg caa ctg aac agc	336
Leu Thr Ala Asp Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser	
100 105 110	
ctg aca tct gag gac tct gcg gtc tat tac tgt gca aga aga gcg gac	384
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Ala Asp	
115 120 125	
tat ggt aac tac gaa tat acc tgg ttt gct tac tgg ggc caa ggg acc	432
Tyr Gly Asn Tyr Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr	
130 135 140	
acg gtc acc gtc tcc tca agt gga ggc ggt tca ggt gga ggt ggc tct	480
Thr Val Thr Val Ser Ser Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser	
145 150 155	
ggc ggt ggc gga tcg gtc atc gag ctc act cag tct cca aaa ttc atg	528
Gly Gly Gly Gly Ser Val Ile Glu Leu Thr Gln Ser Pro Lys Phe Met	
160 165 170 175	
tcc aca tca gta gga gac agg gtc aac gtc acc tac aag gcc agt cag	576
Ser Thr Ser Val Gly Asp Arg Val Asn Val Thr Tyr Lys Ala Ser Gln	
180 185 190	
aat gtg ggt act aat gta gcc tgg ttt caa caa aaa cca ggg caa tct	624
Asn Val Gly Thr Asn Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser	
195 200 205	
cct aaa gtt ctg att tac tcg gca tct tac cga tac agt gga gtc cct	672
Pro Lys Val Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro	
210 215 220	
gat cgc ttc aca ggc agt gga tct gga aca gat ttc act ctc acc atc	720
Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile	
225 230 235	
agc aat gtg cag tct gaa gac ttg gca gag tat ttc tgt cag caa tat	768
Ser Asn Val Gln Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr	
240 245 250 255	

cac acc tat cct ctc acg ttc gga ggg ggc acc aag ctg gaa atc aaa His Thr Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 260 265 270	816
cgg tca gat ccc gga ggc ggg ggt tct ggt gga ggc gga agc ggt ggc Arg Ser Asp Pro Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly 275 280 285	864
ggg ggt tca gga ggc ggg ggt tcc ggt ggt ggc ggc agt ggt ggt ggc Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly 290 295 300	912
ggc agt ggt ggt ggc ggc agt ggt ggt ggc ggc agt ggt gtc cac tcc Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Val His Ser 305 310 315	960
cag gtc caa ctg cag gag tca ggt cca ggt ctt gtg aga cct agc cag Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln 320 325 330 335	1008
acc ctg agc ctg acc tgc acc gtg tct ggc ttc acc atc agc agt ggt Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Ile Ser Ser Gly 340 345 350	1056
tat agc tgg cac tgg gtg aga cag cca cct gga cga ggt ctt gag tgg Tyr Ser Trp His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp 355 360 365	1104
att gga tac ata cag tac agt ggt atc act aac tac aac ccc tct ctc Ile Gly Tyr Ile Gln Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu 370 375 380	1152
aaa agt aga gtg aca atg ctg gta gac acc agc aag aac cag ttc agc Lys Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser 385 390 395	1200
ctg aga ctc agc agc gtg aca gcc gcc gac acc gcg gtc tat tat tgt Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys 400 405 410 415	1248
gca aga gaa gac tat gat tac cac tgg tac ttc gat gtc tgg ggc caa Ala Arg Glu Asp Tyr Asp Tyr His Trp Tyr Phe Asp Val Trp Gly Gln 420 425 430	1296
ggg acc acg gtc acc gtc tcc tca gga ggt ggt gga tcg ggc ggt ggc Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly 435 440 445	1344
ggg tcg ggt ggc ggc gga tct gac atc cag ctg acc cag agc cca agc Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser 450 455 460	1392
agc ctg agc gcc agc gtg ggt gac aga gtg acc atc acc tgt agt acc Ser Leu Ser Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Thr 465 470 475	1440
agc tcg agt gta agt tac atg cac tgg tac cag cag aag cca ggt aag Ser Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys 480 485 490 495	1488
gct cca aag ctg ctg atc tac agc aca tcc aac ctg gct tct ggt gtg Ala Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val 500 505 510	1536
cca agc aga ttc agc ggt agc ggt agc ggt acc gac ttc acc ttc acc Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr 515 520 525	1584
atc agc agc ctc cag cca gag gac atc gcc acc tac tac tgc cat cag Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln 530 535 540	1632
tgg agt agt tat ccc acg ttc ggc caa ggg acc aag gtg gag atc aaa Trp Ser Ser Tyr Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	1680

545	550	555	
gtg gat ccc gcc gag ccc aaa tct cct gac aaa act cac aca tgc cca			1728
Val Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro			
560	565	570	575
ccg tgc cca gca cct cca gtc gcg gga ccg tca gtc ttc ctc ttc ccc			1776
Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro			
	580	585	590
cca aaa ccc aag gac acc ctc atg atc gcc cgg acc cct gag gtc aca			1824
Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr			
	595	600	605
tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac			1872
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn			
	610	615	620
tgg tac gtg gac gcc gtg gag gtg cat aat gcc aag aca aag ccg cgg			1920
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg			
	625	630	635
gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc			1968
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val			
	640	645	650
ctg cac cag gac tgg ctg aat gcc aag gag tac aag tgc aag gtc tcc			2016
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser			
	660	665	670
aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa			2064
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys			
	675	680	685
ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat			2112
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp			
	690	695	700
gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa gcc ttc			2160
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe			
	705	710	715
tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag			2208
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu			
	720	725	730
aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac gcc tcc ttc			2256
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe			
	740	745	750
ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg			2304
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly			
	755	760	765
aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac			2352
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr			
	770	775	780
acg cag aag agc ctc tcc ctg tct ccg ggt aaa aaa gat ccc aaa ttt			2400
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe			
	785	790	795
tgg gtg ctg gtg gtg gtt ggt gga gtc ctg gct tgc tat agc ttg cta			2448
Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu			
	800	805	810
gta aca gtg gcc ttt att att ttc tgg gtg agg agt aag agg agc agg			2496
Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg			
	820	825	830
ctc ctg cac agt gac tac atg aac atg act ccc cgc cgc ccc ggg ccc			2544
Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro			
	835	840	845
acc cgc aag cat tac cag gcc tat gcc gcc gcc gcc gcc ttc gcc gcc			2592

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acc cgc aag cat cac cag gcc cat gcc gcc gca cgc gac ccc gca gcc      2572
Thr Arg Lys His Tyr Gln Ala Tyr Ala Ala Ala Arg Asp Phe Ala Ala
      850                      855                      860

tat cgc tcc ctg aga gtg aag ttc agc agg agc gca gac gcc ccc gcg      2640
Tyr Arg Ser Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala
      865                      870                      875

tac cag cag ggc cag aac cag ctc tat aac gag ctc aat cta gga cga      2688
Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
      880                      885                      890                      895

aga gag gag tac gat gtt ttg gac aag aga cgt ggc cgg gac cct gag      2736
Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
      900                      905                      910

atg ggg gga aag ccg aga agg aag aac cct cag gaa ggc ctg tac aat      2784
Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn
      915                      920                      925

gaa ctg cag aaa gat aag atg gcg gag gcc tac agt gag att ggg atg      2832
Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met
      930                      935                      940

aaa ggc gag cgc cgg agg ggc aag ggg cac gat ggc ctt tac cag ggt      2880
Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
      945                      950                      955

ctc agt aca gcc acc aag gac acc tac gac gcc ctt cac atg cag gcc      2928
Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
      960                      965                      970                      975

ctg ccc cct cgc taa      2943
Leu Pro Pro Arg

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<210> 14

<211> 978

<212> PRT

<213> artificial

<220>

<223> Synthetic Construct

<400> 14

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Thr Ala Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser
1          5          10          15

Ala Ser Val Ile Met Ser Arg Met Ala Gln Val Gln Leu Gln Gln Ser
20          25          30

Gly Ala Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys
35          40          45

Ala Ser Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val Arg Arg
50          55          60

Arg Pro Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser
65          70          75          80

Gly Cys Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr Leu Thr
85          90          95

Ala Asp Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr

```

100	105	110
Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Ala Asp Tyr Gly		
115	120	125
Asn Tyr Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val		
130	135	140
Thr Val Ser Ser Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly		
145	150	155
Gly Gly Ser Val Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr		
	165	170
Ser Val Gly Asp Arg Val Asn Val Thr Tyr Lys Ala Ser Gln Asn Val		
	180	185
Gly Thr Asn Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys		
	195	200
Val Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg		
	210	215
Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn		
225	230	235
Val Gln Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr His Thr		
	245	250
Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ser		
	260	265
Asp Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly		
	275	280
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser		
	290	295
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Val His Ser Gln Val		
305	310	315
Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln Thr Leu		
	325	330
Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Ile Ser Ser Gly Tyr Ser		
	340	345
Trp His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile Gly		
	355	360
Tyr Ile Gln Tyr Ser Gly Ile Thr Asn Tyr Asn Pro Ser Leu Lys Ser		
	370	375
Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu Arg		
385	390	395
Leu Ser Ser Val Thr Ala Ala Asn Thr Ala Val Tyr Tyr Cys Ala Arg		

Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg
 405 410 415
 Glu Asp Tyr Asp Tyr His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr
 420 425 430
 Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 435 440 445
 Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu
 450 455 460
 Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Thr Ser Ser
 465 470 475 480
 Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
 485 490 495
 Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ser
 500 505 510
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser
 515 520 525
 Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys His Gln Trp Ser
 530 535 540
 Ser Tyr Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Val Asp
 545 550 555 560
 Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys
 565 570 575
 Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
 580 585 590
 Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val
 595 600 605
 Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
 610 615 620
 Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
 625 630 635 640
 Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
 645 650 655
 Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
 660 665 670
 Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
 675 680 685
 Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
 690 695 700
 Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro

705	710										715										720												
Ser Asp Ile Ala Val	Glu Trp Glu Ser	Asn Gly Gln Pro Glu	Asn Asn	725	730										735																		
Tyr Lys Thr Thr	Pro Pro Val Leu Asp	Ser Asp Gly Ser Phe Phe Leu	740	745										750																			
Tyr Ser Lys Leu Thr Val	Asp Lys Ser Arg Trp Gln Gln Gly Asn Val	755	760										765																				
Phe Ser Cys Ser Val Met	His Glu Ala Leu His	Asn His Tyr Thr Gln	770	775										780																			
Lys Ser Leu Ser Leu Ser	Pro Gly Lys Lys Asp	Pro Lys Phe Trp Val	785	790										795										800									
Leu Val Val Val	Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr	805	810										815																				
Val Ala Phe Ile Ile Phe Trp	Val Arg Ser Lys Arg Ser Arg Leu Leu	820	825										830																				
His Ser Asp Tyr Met Asn Met	Thr Pro Arg Arg Pro Gly Pro Thr Arg	835	840										845																				
Lys His Tyr Gln Ala Tyr	Ala Ala Ala Arg Asp Phe Ala Ala Tyr Arg	850	855										860																				
Ser Leu Arg Val Lys Phe Ser Arg Ser Ala	Asp Ala Pro Ala Tyr Gln	865	870										875										880										
Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu	885	890										895																					
Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly	900	905										910																					
Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu	915	920										925																					
Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly	930	935										940																					
Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser	945	950										955										960											
Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro	965	970										975																					

<213> artificial

<220>

<223> synthetic peptide

<220>

<221> misc_feature

<222> (8)..(20)

<223> SVFLFPPKPKDTL

<400> 15

Pro Glu Leu Leu Gly Gly Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Met Ile Ser Arg Thr
20 25

<210> 16

<211> 24

<212> PRT

<213> artificial

<220>

<223> synthetic peptide

<220>

<221> misc_feature

<222> (7)..(19)

<223> SVFLFPPKPKDTL

<400> 16

Pro Pro Val Ala Gly Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Met Ile Ala Arg Thr
20

<210> 17

<211> 2901

<212> DNA

<213> artificial

<220>

<223> synthetic molecule

<220>

<221> CDS

<222> (1)..(2901)

<400> 17

atg gat ttt cag gtg cag att ttc agc ttc ctg cta atc agt gcc tca
Met Ser Phe Glu Val Glu Phe Phe Ser Phe Ser Ser Phe Ser Phe Ser

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser

1	5	10	15	
gtc ata atg tct aga cag gtg aag ctg cag cag tct ggg act gtg ctg Val Ile Met Ser Arg Gln Val Lys Leu Gln Gln Ser Gly Thr Val Leu	20	25	30	96
gca agg cct ggg gct tcc gtg aag atg tcc tgc aag gct tct ggc tac Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr	35	40	45	144
agg ttt acc aac tac tgg atg cac tgg gta aaa cag agg cct gga cag Arg Phe Thr Asn Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln	50	55	60	192
ggt cta gaa tgg att ggt gtt att tat cct gga aat agt gat act agc Gly Leu Glu Trp Ile Gly Val Ile Tyr Pro Gly Asn Ser Asp Thr Ser	65	70	75	240
tac aac cag aag ttc aag ggc aag gcc aaa ctg act gca gtc aca tcc Tyr Asn Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser	85	90	95	288
gcc agc act gcc tac atg gag ctc agc agc ctg aca aat gag gac tct Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Ser Leu Thr Asn Glu Asp Ser	100	105	110	336
gcg gtc tat tac tgt aca aga gag gga gaa ggc tct gac tac tgg ggc Ala Val Tyr Tyr Cys Thr Arg Glu Gly Glu Gly Ser Asp Tyr Trp Gly	115	120	125	384
caa ggg acc acg gtc acc gtc tcc tca ggt gga ggc ggt tca ggc gga Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly	130	135	140	432
ggt ggc tct ggc ggt ggc gga tgc caa att gtt ctc acc cag tct cca Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Thr Gln Ser Pro	145	150	155	480
gca acc atg gct gca tct ccc ggg gag aag atc act atc acc tgc agt Ala Thr Met Ala Ala Ser Pro Gly Glu Lys Ile Thr Ile Thr Cys Ser	165	170	175	528
gcc agc tca agt ata agt tcc aat tac ttg cat tgg tat cag cag aag Ala Ser Ser Ser Ile Ser Ser Asn Tyr Leu His Trp Tyr Gln Gln Lys	180	185	190	576
cca gga ttc tcc cct aaa ctc ttg att tat agg act tcc aat ctg gct Pro Gly Phe Ser Pro Lys Leu Leu Ile Tyr Arg Thr Ser Asn Leu Ala	195	200	205	624
tct gga gtc cca gct cgc ttc agt ggc agt ggg tct ggg acc tct tac Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr	210	215	220	672
tct ctc aca att ggc acc atg gag gct gaa gat gtt gcc act tac tac Ser Leu Thr Ile Gly Thr Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr	225	230	235	720
tgc cag cag ggt agt agt ata ccg tac acg ttc gga ggg ggg acc aag Cys Gln Gln Gly Ser Ser Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys	245	250	255	768
ctg gag ctg gcg gcc gca gga ggc ggg ggt tct ggt gga ggc gga agc				816
Leu Glu Leu Ala Ala Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser	260	265	270	
ggt ggc ggg ggt tca gga ggc ggg ggt tcc ggt ggt ggc ggc agt ggt Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly	275	280	285	864
ggt ggc ggc agt ggt ggt ggc ggc agt ggt ggt ggc ggc agt ggt gtc Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Val	290	295	300	912

cac tcc cag gtc caa ctg cag gag tca ggt cca ggt ctt gtg aga cct His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro 305 310 315 320	960
agc cag acc ctg agc ctg acc tgc acc gtg tct ggc ttc acc atc agc Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Ile Ser 325 330 335	1008
agt ggt tat agc tgg cac tgg gtg aga cag cca cct gga cga ggt ctt Ser Gly Tyr Ser Trp His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu 340 345 350	1056
gag tgg att gga tac ata cag tac agt ggt atc act aac tac aac ccc Glu Trp Ile Gly Tyr Ile Gln Tyr Ser Gly Ile Thr Asn Tyr Asn Pro 355 360 365	1104
tct ctc aaa agt aga gtg aca atg ctg gta gac acc agc aag aac cag Ser Leu Lys Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln 370 375 380	1152
ttc agc ctg aga ctc agc agc gtg aca gcc gcc gac acc gcg gtc tat Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr 385 390 395 400	1200
tat tgt gca aga gaa gac tat gat tac cac tgg tac ttc gat gtc tgg Tyr Cys Ala Arg Glu Asp Tyr Asp Tyr His Trp Tyr Phe Asp Val Trp 405 410 415	1248
ggc caa ggg acc acg gtc acc gtc tcc tca gga ggt ggt gga tcg ggc Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly 420 425 430	1296
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cca agc agc ctg agc gcc agc gtg ggt gac aga gtg acc atc acc tgt Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys 450 455 460	1392
agt acc agc tcg agt gta agt tac atg cac tgg tac cag cag aag cca Ser Thr Ser Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro 465 470 475 480	1440
ggt aag gct cca aag ctg ctg atc tac agc aca tcc aac ctg gct tct Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser 485 490 495	1488
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atc aaa gtg gat ccc gcc gag ccc aaa tct cct gac aaa act cac aca Ile Lys Val Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr 545 550 555 560	1680
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gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys 595 600 605	1824

ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys 610 615 620	1872
cgc cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu 625 630 635 640	1920
acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 645 650 655	1968
gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys 660 665 670	2016
gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 675 680 685	2064
cgc gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 690 695 700	2112
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cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn 755 760 765	2304
cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa aaa gat ccc His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro 770 775 780	2352
aaa ttt tgg gtg ctg gtg gtg gtt ggt gga gtc ctg gct tgc tat agc Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser 785 790 795 800	2400
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ggg ccc acc cgc aag cat tac cag gcc tat gcc gcc gca cgc gac ttc Gly Pro Thr Arg Lys His Tyr Gln Ala Tyr Ala Ala Arg Asp Phe 835 840 845	2544
gca gcc tat cgc tcc ctg aga gtg aag ttc agc agg agc gca gac gcc Ala Ala Tyr Arg Ser Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala 850 855 860	2592
ccc gcg tac cag cag ggc cag aac cag ctc tat aac gag ctc aat cta Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu 865 870 875 880	2640
gga cga aga gag gag tac gat gtt ttg gac aag aga cgt ggc cgg gac Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp 885 890 895	2688
cct gag atg ggg gga aag ccg aga agg aag aac cct cag gaa ggc ctg Pro Glu Met Glv Glv Lvs Pro Arg Arg Lvs Asn Pro Gln Glu Glv Leu	2736

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tac aat gaa ctg cag aaa gat aag atg gcg gag gcc tac agt gag att      2784
Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
          915          920          925

ggg atg aaa ggc gag cgc cgg agg ggc aag ggg cac gat ggc ctt tac      2832
Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
          930          935          940

cag ggt ctc agt aca gcc acc aag gac acc tac gac gcc ctt cac atg      2880
Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
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          965

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<211> 966

<212> PRT

<213> artificial

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<223> Synthetic Construct

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Val Ile Met Ser Arg Gln Val Lys Leu Gln Gln Ser Gly Thr Val Leu
          20          25          30

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

Arg Phe Thr Asn Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Gln
          50          55          60

Gly Leu Glu Trp Ile Gly Val Ile Tyr Pro Gly Asn Ser Asp Thr Ser
65          70          75          80

Tyr Asn Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser
          85          90          95

Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser
          100          105          110

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

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

Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Thr Gln Ser Pro
145          150          155          160

Ala Thr Met Ala Ala Ser Pro Gly Glu Lys Ile Thr Ile Thr Cys Ser
          165          170          175

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Ala Ser Ser Ser Ile Ser Ser Asn Tyr Leu His Trp Tyr Gln Gln Lys
 180 185 190
 Pro Gly Phe Ser Pro Lys Leu Leu Ile Tyr Arg Thr Ser Asn Leu Ala
 195 200 205
 Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr
 210 215 220
 Ser Leu Thr Ile Gly Thr Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr
 225 230 235 240
 Cys Gln Gln Gly Ser Ser Ile Pro Tyr Thr Phe Gly Gly Gly Thr Lys
 245 250 255
 Leu Glu Leu Ala Ala Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 260 265 270
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 275 280 285
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Val
 290 295 300
 His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro
 305 310 315 320
 Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Ile Ser
 325 330 335
 Ser Gly Tyr Ser Trp His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 340 345 350
 Glu Trp Ile Gly Tyr Ile Gln Tyr Ser Gly Ile Thr Asn Tyr Asn Pro
 355 360 365
 Ser Leu Lys Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn Gln
 370 375 380
 Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr
 385 390 395 400
 Tyr Cys Ala Arg Glu Asp Tyr Asp Tyr His Trp Tyr Phe Asp Val Trp
 405 410 415
 Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
 420 425 430
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser
 435 440 445
 Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
 450 455 460
 Ser Thr Ser Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro
 465 470 475 480

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Ala Ser
 485 490 495

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
 500 505 510

Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
 515 520 525

His Gln Trp Ser Ser Tyr Pro Thr Phe Gly Gln Gly Thr Lys Val Glu
 530 535 540

Ile Lys Val Asp Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr
 545 550 555 560

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
 565 570 575

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu
 580 585 590

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 595 600 605

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 610 615 620

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 625 630 635 640

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 645 650 655

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 660 665 670

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 675 680 685

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 690 695 700

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 705 710 715 720

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 725 730 735

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 740 745 750

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 755 760 765

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro
770 775 780

Lys Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser
785 790 795 800

Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg
805 810 815

Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro
820 825 830

Gly Pro Thr Arg Lys His Tyr Gln Ala Tyr Ala Ala Ala Arg Asp Phe
835 840 845

Ala Ala Tyr Arg Ser Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
850 855 860

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
865 870 875 880

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
885 890 895

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
900 905 910

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
915 920 925

Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
930 935 940

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
945 950 955 960

Gln Ala Leu Pro Pro Arg
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<211> 2937

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<213> artificial

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<223> synthetic molecule

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gtc ata atg tct aga atg gcc cag gtg caa ctg cag cag tca ggg gct Val Ile Met Ser Arg Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala 20 25 30	96
gag ctg gct aga cct ggg gct tca gtg aag atg tcc tgc aag gct tct Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser 35 40 45	144
ggc tac acc ttt act acc tac aca ata cac tgg gta aga cgg agg cct Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val Arg Arg Arg Pro 50 55 60	192
gga cac gat ctg gaa tgg att gga tac att aat cct agc agt gga tgt Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Gly Cys 65 70 75 80	240
tct gac tac aat caa aac ttc aag ggc aag acc aca ttg act gca gac Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr Leu Thr Ala Asp 85 90 95	288
aag tcc tcc aac aca gcc tac atg caa ctg aac agc ctg aca tct gag Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu 100 105 110	336
gac tct gcg gtc tat tac tgt gca aga aga gcg gac tat ggt aac tac Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Ala Asp Tyr Gly Asn Tyr 115 120 125	384
gaa tat acc tgg ttt gct tac tgg ggc caa ggg acc acg gtc acc gtc Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val 130 135 140	432
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tct gaa gac ttg gca gag tat ttc tgt cag caa tat cac acc tat cct Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr His Thr Tyr Pro 245 250 255	768
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gga gga tct ggt ggc ggt ggc tcc gtc gac atg gcg cag gtc cag etc	960

Gly 305	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Val	Asp	Met	Ala	Gln	Val	Gln	Leu	320	
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Gln	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	Ser	Leu	Lys	Leu	335	
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Pro	Ala	Glu	Pro	Lys	Ser	Pro	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	575	
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Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	590	
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Pro	Lys	Asp	Thr	Leu	Met	Ile	Ala	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	605	
			595				600										
gtg	gtg	gac	gtg	agg	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	1872	

Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr		
610						615					620						
gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cgg	gag	gag	1920	
Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu		
625					630					635					640		
cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	1968	
Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His		
				645					650					655			
cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	2016	
Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys		
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gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggg	cag	2064	
Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln		
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Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu		
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acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	ggc	ttc	tat	ccc	2160	
Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro		
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agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	ccg	gag	aac	aac	2208	
Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn		
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tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	tcc	ttc	ttc	ctc	2256	
Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu		
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tac	agc	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	cag	ggg	aac	gtc	2304	
Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val		
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ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	ctg	cac	aac	cac	tac	acg	cag	2352	
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln		
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Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	Lys	Pro	Lys	Phe	Trp	Val			
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ctg	gtg	gtg	gtt	ggt	gga	gtc	ctg	gct	tgc	tat	agc	ttg	cta	gta	aca	2448	
Leu	Val	Val	Val	Gly	Val	Leu	Ala	Cys	Tyr	Ser	Leu	Leu	Val	Thr			
				805				810					815				
gtg	gcc	ttt	att	att	ttc	tgg	gtg	agg	agt	aag	agg	agc	agg	ctc	ctg	2496	
Val	Ala	Phe	Ile	Ile	Phe	Trp	Val	Arg	Ser	Lys	Arg	Ser	Arg	Leu	Leu		
			820					825					830				
cac	agt	gac	tac	atg	aac	atg	act	ccc	cgc	cgc	ccc	ggg	ccc	acc	cgc	2544	
His	Ser	Asp	Tyr	Met	Asn	Met	Thr	Pro	Arg	Arg	Pro	Gly	Pro	Thr	Arg		
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aag	cat	tac	cag	gcc	tat	gcc	gcc	gca	cgc	gac	ttc	gca	gcc	tat	cgc	2592	
Lys	His	Tyr	Gln	Ala	Tyr	Ala	Ala	Ala	Arg	Asp	Phe	Ala	Ala	Tyr	Arg		
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tcc	ctg	aga	gtg	aag	ttc	agc	agg	agc	gca	gac	gcc	ccc	gcg	tac	cag	2640	
Ser	Leu	Arg	Val	Lys	Phe	Ser	Arg	Ser	Ala	Asp	Ala	Pro	Ala	Tyr	Gln		
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cag	ggc	cag	aac	cag	ctc	tat	aac	gag	ctc	aat	cta	gga	cga	aga	gag	2688	
Gln	Gly	Gln	Asn	Gln	Leu	Tyr	Asn	Glu	Leu	Asn	Leu	Gly	Arg	Arg	Glu		
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gag	tac	gat	gtt	ttg	gac	aag	aga	cgt	ggc	cgg	gac	cct	gag	atg	ggg	2736	
Glu	Tyr	Asp	Val	Leu	Asp	Lys	Arg	Arg	Gly	Arg	Asp	Pro	Glu	Met	Gly		
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Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu	
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cag aaa gat aag atg gcg gag gcc tac agt gag att ggg atg aaa ggc	2832
Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly	
930 935 940	
gag cgc cgg agg gcc aag ggg cac gat ggc ctt tac cag ggt ctc agt	2880
Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser	
945 950 955 960	
aca gcc acc aag gac acc tac gac gcc ctt cac atg cag gcc ctg ccc	2928
Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro	
965 970 975	
cct cgc taa	2937

Pro Arg

$\langle 210 \rangle$ 20

<211> 978

<212> PRT

<213> artificial

 $\langle 220 \rangle$

<223> Synthetic Construct

<400> 20

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Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser
35 40 45

Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val Arg Arg Arg Pro
50 55 60

Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Gly Cys
65 70 75 80

Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr Leu Thr Ala Asp
85 90 95

Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu
100 105 110

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

Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
130 135 140

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

Ser Val Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val
165 170 175

Gly Asp Arg Val Asn Val Thr Tyr Lys Ala Ser Gln Asn Val Gly Thr
180 185 190

Asn Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Val Leu
195 200 205

Ile Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr
210 215 220

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln
225 230 235 240

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

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

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
275 280 285

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
290 295 300

Gly Gly Ser Gly Gly Gly Gly Ser Val Asp Met Ala Gln Val Gln Leu
305 310 315 320

Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Lys Leu
325 330 335

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser Trp
340 345 350

Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Leu Val Ala Thr Ile Asn
355 360 365

Ser Asn Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe
370 375 380

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met Ser
385 390 395 400

Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg Asp Arg
405 410 415

Asp Gly Tyr Asp Glu Gly Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val
420 425 430

Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
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Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ser Ile Met Ser Ala
450 455 460

450	455	460
Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val 465 470 475 480		
Ser Tyr Met His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg 485 490 495		
Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe 500 505 510		
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Pro Pro Thr Leu Gly Arg Arg Thr Gln Leu Gln Leu Lys Arg Ala Asp 545 550 555 560		
Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys 565 570 575		
Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys 580 585 590		
Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val 595 600 605		
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr 610 615 620		
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu 625 630 635 640		
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His 645 650 655		
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys 660 665 670		
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln 675 680 685		
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu 690 695 700		
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro 705 710 715 720		
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn 725 730 735		
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu 740 745 750		
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val 755 760 765		

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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
 770                               775                   780

Lys Ser Leu Ser Leu Ser Pro Gly Lys Lys Asp Pro Lys Phe Trp Val
 785                               790                   795                   800

Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr
                        805                               810                   815

Val Ala Phe Ile Ile Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu
                        820                               825                   830

His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg
      835                               840                   845

Lys His Tyr Gln Ala Tyr Ala Ala Ala Arg Asp Phe Ala Ala Tyr Arg
 850                               855                   860

Ser Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
 865                               870                   875                   880

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
                        885                               890                   895

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
      900                               905                   910

Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu
      915                               920                   925

Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly
 930                               935                   940

Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser
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Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
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Pro Arg

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<211> 3006

<212> DNA

<213> artificial

<220>

<223> synthetic molecule

<220>

<221> CDS

<222> (1)..(2985)

<400> 21

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gtc ata atg tct aga atg gcc cag gtg caa ctg cag cag tca ggg gct	96
Val Ile Met Ser Arg Met Ala Gln Val Gln Leu Gln Gln Ser Gly Ala	
20 25 30	
gag ctg gct aga cct ggg gct tca gtg aag atg tcc tgc aag gct tct	144
Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser	
35 40 45	
ggc tac acc ttt act acc tac aca ata cac tgg gta aga cgg agg cct	192
Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val Arg Arg Arg Pro	
50 55 60	
gga cac gat ctg gaa tgg att gga tac att aat cct agc agt gga tgt	240
Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Gly Cys	
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Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr Leu Thr Ala Asp	
85 90 95	
aag tcc tcc aac aca gcc tac atg caa ctg aac agc ctg aca tct gag	336
Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu	
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Asp Ser Ala Val Tyr Tyr Cys Ala Arg Arg Ala Asp Tyr Gly Asn Tyr	
115 120 125	
gaa tat acc tgg ttt gct tac tgg ggc caa ggg acc acg gtc acc gtc	432
Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val	
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Ser Ser Ser Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly	
145 150 155 160	
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Ser Val Ile Glu Leu Thr Gln Ser Pro Lys Phe Met Ser Thr Ser Val	
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gga gac agg gtc aac gtc acc tac aag gcc agt cag aat gtg ggt act	576
Gly Asp Arg Val Asn Val Thr Tyr Lys Ala Ser Gln Asn Val Gly Thr	
180 185 190	
aat gta gcc tgg ttt caa caa aaa cca ggg caa tct cct aaa gtt ctg	624
Asn Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Val Leu	
195 200 205	
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Ile Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr	
210 215 220	
ggc agt gga tct gga aca gat ttc act ctc acc atc agc aat gtg cag	720
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln	
225 230 235 240	
tct gaa gac ttg gca gag tat ttc tgt cag caa tat cac acc tat cct	768
Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr His Thr Tyr Pro	
245 250 255	
ctc acg ttc gga ggg ggc acc aag ctg gaa atc aaa cgg gcg gcc gca	816
Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala	
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Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly	
275 280 285	
aac aag aat tcc aat aat aac aac aat aat aat aac aac aat aat aat	912

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99~ 999 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~ 99~
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290                               295                               300

ggc ggc agt ggt ggt ggc ggc agt gtc gac ggt gtc cac tcc cag gtg      960
Gly Gly Ser Gly Gly Gly Gly Ser Val Asp Gly Val His Ser Gln Val
305                               310                               315                               320

cag att ttc agc ttc ctg cta atc agt gcc tca gtc ata atg tct aga      1008
Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Met Ser Arg
                               325                               330                               335

atg gcc cag gtg aag ctg cag cag tct ggc gct gag ttg gtg aaa cct      1056
Met Ala Gln Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro
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ggg gct tca gtg aag ata tcc tgc aag gct tct ggc tac acc ttc act      1104
Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
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gac cat gct att cac tgg gcg aag cag aag cct gaa cag ggc ctg gaa      1152
Asp His Ala Ile His Trp Gln Lys Gln Lys Pro Glu Gln Gly Leu Glu
                               370                               375                               380

tgg att gga tat att tct ccc gga aat gat gat att aag tac aat gag      1200
Trp Ile Gly Tyr Ile Ser Pro Gly Asn Asp Asp Ile Lys Tyr Asn Glu
385                               390                               395                               400

aag ttc aag ggc aag gcc aca ctg act gca gac aaa tcc tcc agc act      1248
Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr

                               405                               410                               415

gcc tac atg cag ctc aac agc ctg aca tct gag gat tct gca gtg tat      1296
Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
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ttc tgt aaa aga tcg tac tac ggc cac tgg ggc caa ggc acc acg gtc      1344
Phe Cys Lys Arg Ser Tyr Tyr Gly His Trp Gly Gln Gly Thr Thr Val
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acc gtc tcc tca ggt gga ggc ggt tca ggc gga ggt ggc tct ggc ggt      1392
Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
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ggc gga tcg gac atc gag ctc act cag tct cca gcc tcc tta tct gta      1440
Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ser Val
465                               470                               475                               480

tct gtg gga gaa act gtc acc atc aca tgt cga gca agt gag aat att      1488
Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile
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tac agt aat tta gca tgg tac caa cag aaa cag gga aaa tct cct cag      1536
Tyr Ser Asn Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln
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ctc ctg gtc tat gct gca aca aac tta gca gat ggt gtg cca tca agg      1584
Leu Leu Val Tyr Ala Ala Thr Asn Leu Ala Asp Gly Val Pro Ser Arg
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ttc agt ggc agt gga tcg ggc aca cag tat tcc ctc aag atc aac agc      1632
Phe Ser Gly Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Ser
530                               535                               540

ctg cag tct gaa gat ttt ggg agt tat tac tgt caa cat ttt tgg ggt      1680
Leu Gln Ser Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His Phe Trp Gly
545                               550                               555                               560

act ccg tac acg ttc gga ggg ggg acc aag ctg gag ctg aaa gtg gat      1728
Thr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Leu Lys Val Asp
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ccc gcc gag ccc aaa tct cct gac aaa act cac aca tgc cca ccg tgc      1776
Pro Ala Glu Pro Lys Ser Pro Asp Lys Thr His Thr Cys Pro Pro Cys
                               580                               585                               590

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cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac	2016
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His 660 665 670	
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agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn 740 745 750	2256
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ctg gtg gtg gtt ggt gga gtc ctg gct tgc tat agc ttg cta gta aca Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu Leu Val Thr 820 825 830	2496
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cac agt gac tac atg aac atg act ccc cgc cgc ccc ggg ccc acc cgc His Ser Asp Tyr Met Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg 850 855 860	2592
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tcc ctg aga gtg aag ttc agc agg agc gca gac gcc ccc gcg tac cag Ser Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln 885 890 895	2688

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	900			905		910			
gag tac	gat gtt	ttg gac	aag aga	cgt ggc	cgg gac	cct gag	atg ggg	2784	
Glu Tyr	Asp Val	Leu Asp	Lys Arg	Arg Gly	Arg Asp	Pro Glu	Met Gly		
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gga aag	ccg aga	agg aag	aac cct	cag gaa	ggc ctg	tac aat	gaa ctg	2832	
Gly Lys	Pro Arg	Arg Lys	Asn Pro	Gln Glu	Gly Leu	Tyr Asn	Glu Leu		
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Gln Lys	Asp Lys	Met Ala	Glu Ala	Tyr Ser	Glu Ile	Gly Met	Lys Gly		
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gag cgc	cgg agg	ggc aag	ggg cac	gat ggc	ctt tac	cag ggt	ctc agt	2928	
Glu Arg	Arg Arg	Gly Lys	Gly His	Asp Gly	Leu Tyr	Gln Gly	Leu Ser		
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aca gcc	acc aag	gac acc	tac gac	gcc ctt	cac atg	cag gcc	ctg ccc	2976	
Thr Ala	Thr Lys	Asp Thr	Tyr Asp	Ala Leu	His Met	Gln Ala	Leu Pro		
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Glu Leu Ala Arg Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser
35 40 45

Gly Tyr Thr Phe Thr Thr Tyr Thr Ile His Trp Val Arg Arg Arg Pro
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Gly His Asp Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Gly Cys
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Ser Asp Tyr Asn Gln Asn Phe Lys Gly Lys Thr Thr Leu Thr Ala Asp
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Lys Ser Ser Asn Thr Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu
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Glu Tyr Thr Trp Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
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 145 150 155 160
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 Gly Asp Arg Val Asn Val Thr Tyr Lys Ala Ser Gln Asn Val Gly Thr
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 Asn Val Ala Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Lys Val Leu
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 210 215 220
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln
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 Ser Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr His Thr Tyr Pro
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 290 295 300
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 Ala Tyr Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
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Phe Cys Lys Arg Ser Tyr Tyr Gly His Trp Gly Gln Gly Thr Thr Val
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Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
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Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ser Val
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Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile
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Pro Lys Asp Thr Leu Met Ile Ala Arg Thr Pro Glu Val Thr Cys Val
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Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
660 665 670

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
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690 695 700

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
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Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
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Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
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 Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
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 965 970 975
 Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro
 980 985 990
 Pro Arg

REFERENCES CITED IN THE DESCRIPTION

Cited references

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Patentkrav

5 **1.** Rekombinant polypeptid, som er en kimær antigenreceptor (CAR) indeholdende mindst følgende domæner i rækkefølgen, startende fra den N-terminale ende til den C-terminale ende:

a.) et første domæne indeholdende i) anti-CD30-enkeltkædeantistof-enheden af HRS3 scFv med SEQ ID No. 2 og ii) en antistof-enhed, som er en scFv-antistof-enhed, der binder specifikt til et tumorassocieret antigen, der er til stede på overfladen af en forudbestemt målcelle;

10 b.) eventuelt et spacer-domæne;

c.) et transmembrandomæne; og

d.) et cytoplasmatisk signaleringsdomæne.

15 **2.** Rekombinant polypeptid, som er en CAR indeholdende mindst følgende domæner i rækkefølgen, startende fra den N-terminale ende til den C-terminale ende:

a.) et første domæne indeholdende i) en antistof-enhed, som er en scFv-antistof-enhed, der binder specifikt til et tumorassocieret antigen, der er til stede på overfladen af en forudbestemt målcelle; og ii) en anti-CD30-enkeltkædeantistof-enhed af HRS3 scFv med SEQ ID No. 2,

20

b.) eventuelt et spacer-domæne;

c.) et transmembrandomæne; og

d.) et cytoplasmisk signaleringsdomæne.

25 **3.** Rekombinant polypeptid, som er en CAR ifølge krav 1 eller 2, yderligere omfattende en leader-sekvens, der befinder sig N-terminalt for det første domæne.

30 **4.** Rekombinant polypeptid, som er en CAR ifølge et hvilket som helst af de foregående krav, hvor spacer-domænet er et IgG1 CH2 CH3-domæne af SEQ ID No. 6 eller homologer deraf med mindst 95 % identitet dermed.

5. Rekombinant polypeptid, som er en CAR ifølge et hvilket som helst af de foregående krav, hvor transmembrandomænet er afledt af CD28.

35

- 6.** Rekombinant polypeptid, som er en CAR ifølge et hvilket som helst af de foregående krav, hvor det intracellulære domæne indeholder en CD3zeta eller en Fc-epsilon-receptor I-gamma-signalkæde eller en co-stimulerende enhed.
- 5 **7.** Rekombinant polypeptid, som er en CAR ifølge et hvilket som helst af de foregående krav, hvor det intracellulære domæne er et CD28-signaleringsdomæne.
- 10 **8.** Rekombinant polypeptid, som er en CAR ifølge et hvilket som helst af de foregående krav, hvor scFv-antistof-enheden binder til et tumorassocieret antigen udvalgt blandt carcinoembryonalt antigen (CEA), CA19-9, CA72-4 (TAG-72), PSCA, Muc-1, HMW-MAA, p97 melanotransferrin, fetal acetylcholinreceptor, ErbB2 (Her2/neu), multiresistens-protein (MDR), CD19, CD20 og TOSO.
- 15 **9.** Nukleinsyremolekyle omfattende en nukleinsyresekvens, der koder for polypeptidet ifølge et hvilket som helst af kravene 1 til 8.
- 10.** Vektor omfattende nukleinsyresekvensen ifølge krav 9.
- 20 **11.** Isoleret celle indeholdende en vektor ifølge krav 10 eller et nukleinsyremolekyle ifølge krav 9.
- 12.** Isoleret celle ifølge krav 11, som er en T-celle, der på sin overflade eksprimerer CAR'en ifølge et hvilket som helst af kravene 1 til 8.
- 25 **13.** Isoleret T-celle, der på sin overflade eksprimerer
a.) en første kimær antigenreceptor, der i sit ekstracellulære domæne har anti-CD30-enkeltkædeantistoffet af HRS3 scFv med SEQ ID No. 2 og
b.) en anden kimær antigenreceptor, der i sit ekstracellulære domæne har en
30 antistof-enhed, som er en scFv-antistof-enhed, der binder specifikt til et tumorassocieret antigen, der er til stede på overfladen af en forudbestemt målcelle.
- 14.** Isoleret T-celle ifølge et hvilket som helst af kravene 12 til 13 til anvendelse ved behandling af cancer, hvor canceren er en cancer, der eksprimerer det
35 antigen, som anti-tumor-antigenantistoffet er rettet mod.

DRAWINGS

Modularly composed CAR with dual CEA/CD30 specificity

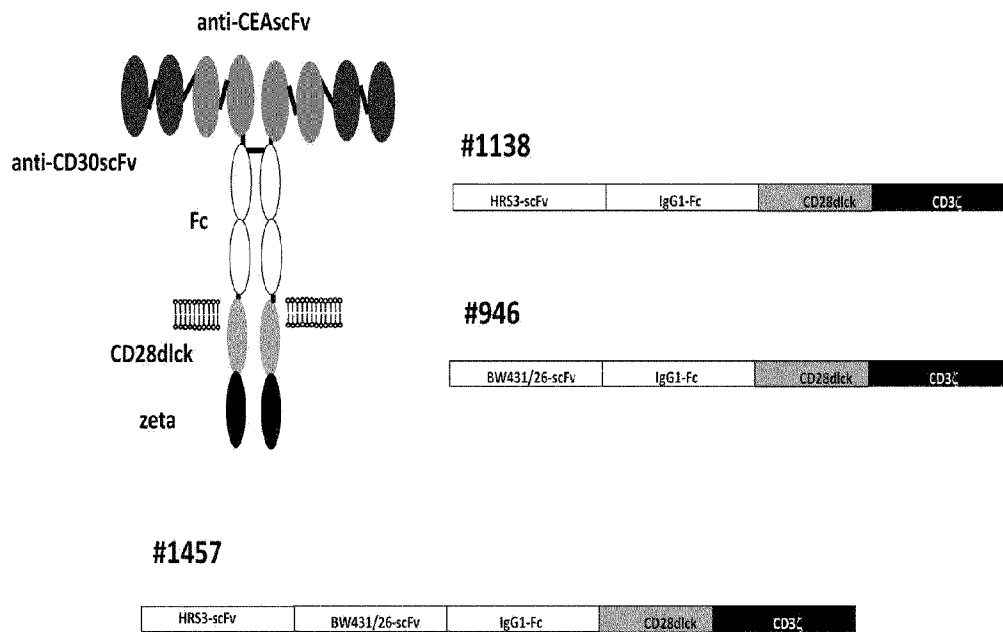


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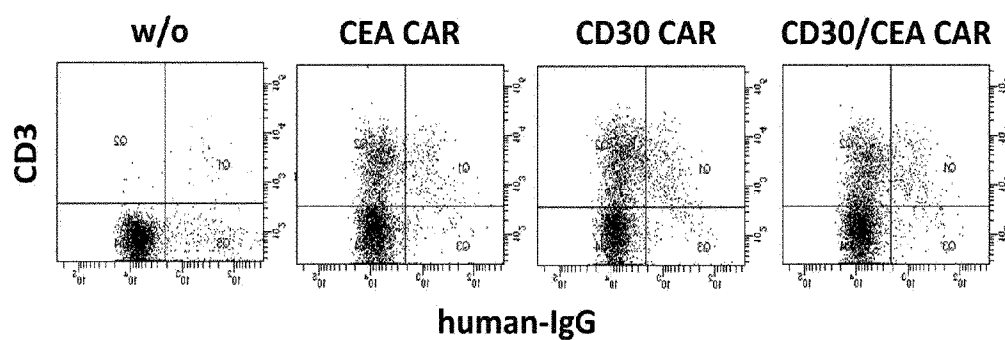


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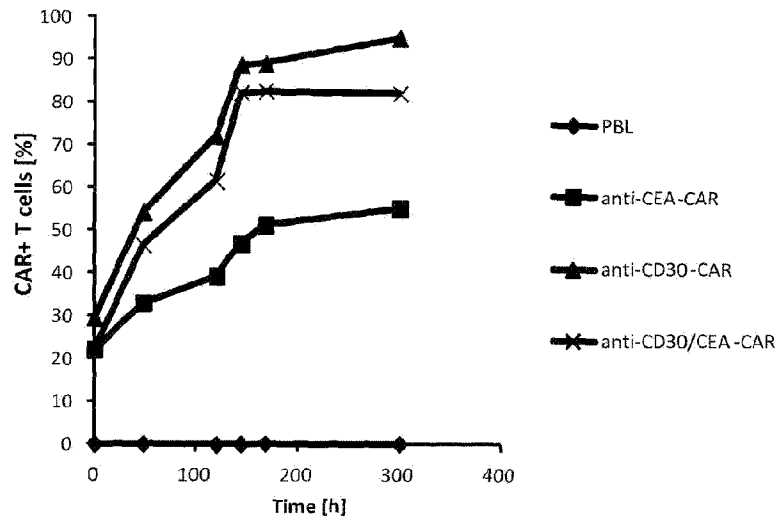


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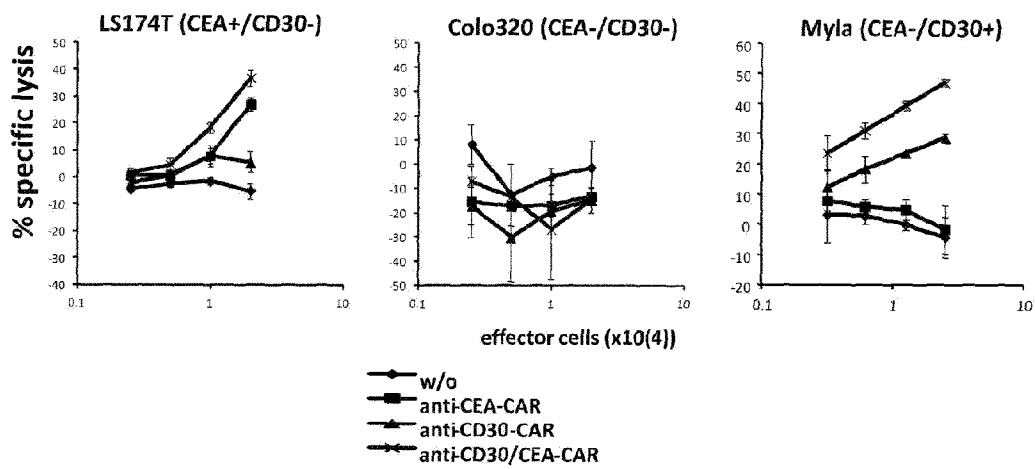


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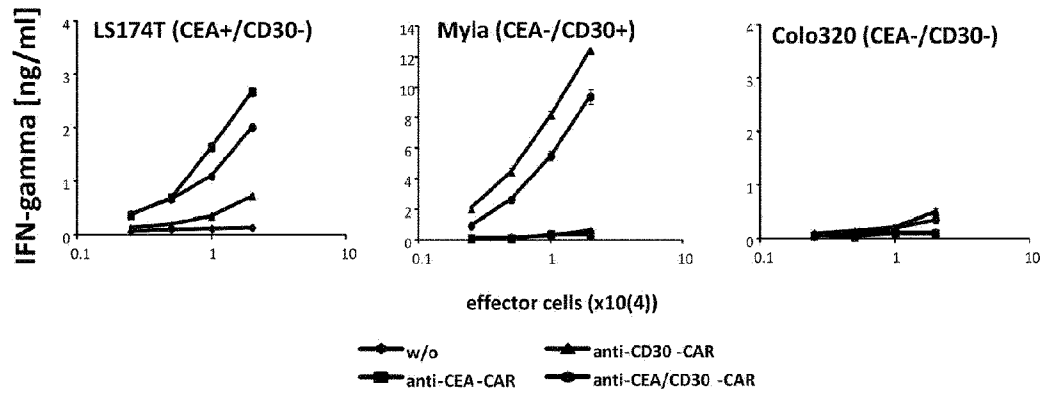


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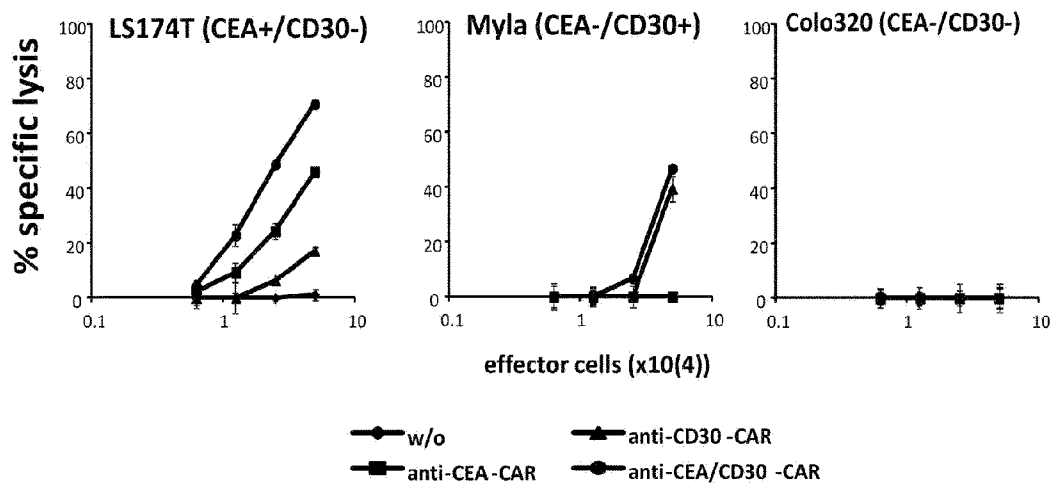


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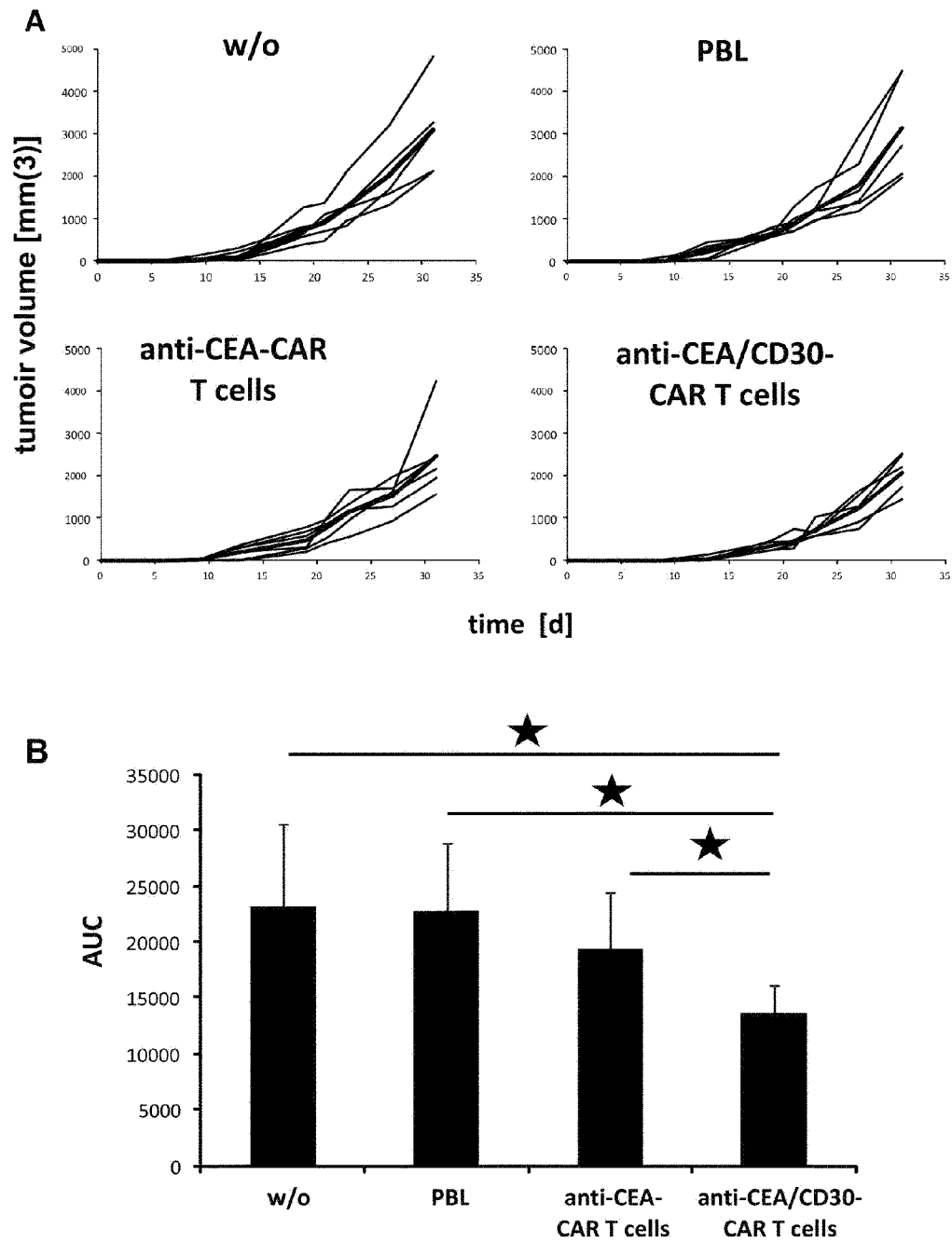


Figure 7

A

#946: anti-CEA CAR

BW431/26-scFv	IgG1-Fc	CD28dlck	CD3 ζ
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#1457: anti-CD30/CEA CAR

HRS3-scFv	BW431/26-scFv	IgG1-Fc	CD28dlck	CD3 ζ
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#1576: anti-CD25/CEA CAR

anti-CD25	BW431/26-scFv	IgG1-Fc	CD28dlck	CD3 ζ
-----------	---------------	---------	----------	-------------

B

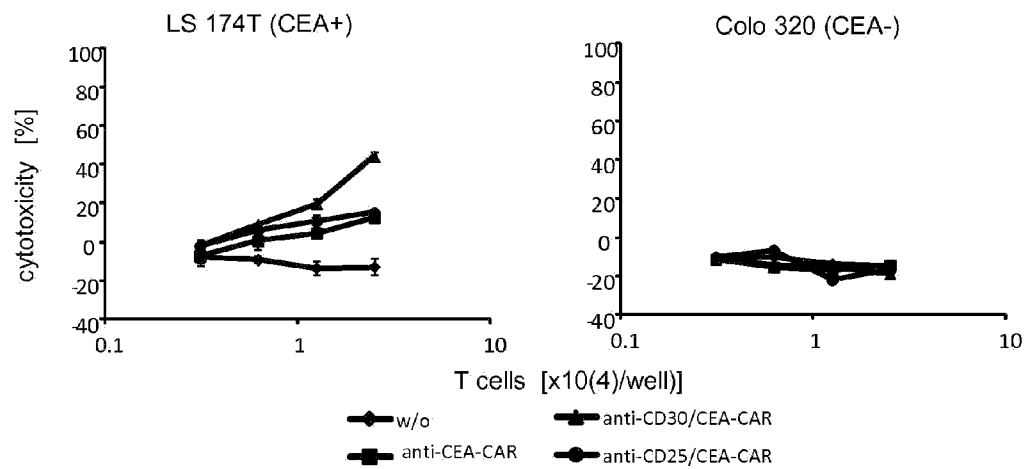


Figure 8

A

#1587: anti-CD30/Muc1 CAR

HRS3-scFv	C595-scFv	IgG1-Fc	CD28d1ck	CD3 ζ
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#1650: anti-CD30/TAG-72 CAR

HRS3-scFv	B72.3-scFv	IgG1-Fc	CD28d1ck	CD3 ζ
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B

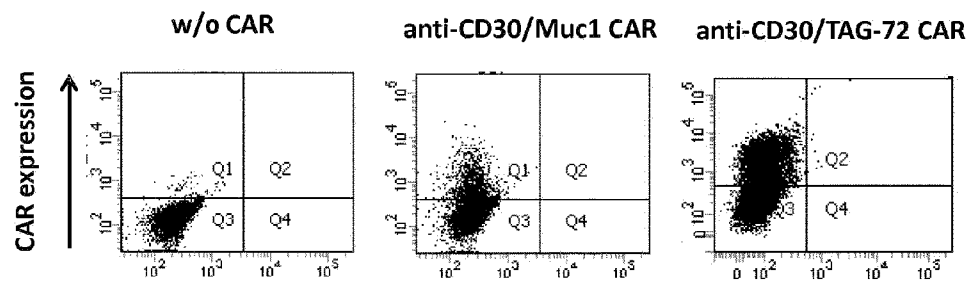


Figure 9