(19) DANMARK

(10) DK/EP 2325305 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 12 N 9/12 (2006.01)* *C 12 Q 1/68 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2014-03-03**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2014-02-12**

(86) Europæisk ansøgning nr.: **10010438.9**

(86) Europæisk indleveringsdag: **2006-02-17**

(87) Den europæiske ansøgnings publiceringsdag: **2011-05-25**

(30) Prioritet: **2005-02-25 US 656857 P**

(62) Stamansøgningsnr: **06714493.1**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR**

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(54) Benævnelse: **Peptidvacciner til lungecancere, der udtrykker TTK-, URLC10- eller KOC1-polypeptid**

(56) Fremdragne publikationer:
WO-A2-2004/031413
DE NOOIJ-VAN DALEN A G ET AL: "Characterization of the human Ly-6 antigens, the newly annotated member Ly-6K included, as molecular markers for head-and-neck squamous cell carcinoma", INTERNATIONAL JOURNAL OF CANCER, JOHN WILEY & SONS, INC, UNITED STATES, SWITZERLAND, GERMANY, vol. 103, 1 January 2003 (2003-01-01), pages 768-774, XP003004048, ISSN: 0020-7136, DOI: DOI:10.1002/IJC.10903
DATABASE UniProt [Online] 23 November 2004 (2004-11-23), "Putative uncharacterized protein from Oryza sativa", XP002631665, retrieved from EBI accession no. UNIPROT:Q5ZED4 Database accession no. Q5ZED4
WANG T ET AL: "IDENTIFICATION OF GENES DIFFERENTIALLY OVER-EXPRESSED IN LUNG SQUAMOUS CELL CARCINOMA USING COMBINATION OF CDNA SUBTRACTION AND MICROARRAY ANALYSIS", ONCOGENE, BASINGSTOKE, HANTS, GB, vol. 19, no. 12, 16 March 2000 (2000-03-16), pages 1519-1528, XP000951444, ISSN: 0950-9232
KONO KOJI ET AL: "Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer.", CANCER SCIENCE AUG

Fortsættes ...

2009 LNKD- PUBMED:19459850, vol. 100, no. 8, August 2009 (2009-08), pages 1502-1509, XP002631666, ISSN: 1349-7006

Description

Technical Field

[0001] The invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, disclosed are novel peptides that are extremely effective as cancer vaccines, and drugs for treating and preventing tumors that contain these peptides.

Background Art

[0002] Lung cancer is one of the most commonly fatal human tumors. Many genetic alterations associated with the development and progression of lung cancer have been reported. Genetic changes can aid prognostic efforts and predictions of metastatic risk or response to certain treatment. (Mitsudomi T et al., (2000) Clin Cancer Res 6: 4055-63.; Niklinski et al., (2001) Lung Cancer. 34 Suppl 2: S53-8.; Watine J. (2000) Bmj 320: 379-80.). Non-small cell.lung cancer (NSCLC) is by far the most common form of lung cancer, accounting for nearly 80% of lung tumors (Society, A. C. Cancer Facts and Figures 2001, 2001.). The overall 10-year survival rate remains as low as 10%, despite recent advances in multi-modality therapy, because the majority ofNSCLCs are not diagnosed until advanced stages (Fry, W. A. et al., (1999) Cancer. 86: 1867-76.). Although chemotherapy regimens based on platinum are considered the reference standards for treatment of NSCLC, those drugs are able to extend survival of patients with advanced NSCLC only about six weeks (Non-small Cell Lung Cancer Collaborative Group, (1995) BMJ. 311: 899-909.). Numerous targeted therapies are being investigated for this disease, including tyrosine kinase inhibitors; however, to date promising results have been achieved in only a limited number of patients and some recipients suffer severe adverse reactions (Kris MG, et al., (2002) Proc Am Soc Clin OncoL 21: 292a(A1166).).

[0003] It has been demonstrated that $CD8^+$ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC class I molecules, and lyse the tumor cells. Since the discovery of the MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon T. (1993) Int J Cancer 54: 177-80.; Boon T. et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-47.; Brichard V et al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.). Some of them are now in clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen P et al., (1991) Science 254: 1643-7.), gp100 (Kawakami Y et al., (1994) J Exp Med 180: 347-52.), SART (Shichijo S et al., (1998) J Exp Med 187:277-88.), and NY-ESO-1 (Chen Y.T. et al., (1997) Proc.Natl.Acd Sci. USA, 94: 1914-8.). On the other hand, certain gene products demonstrated to be somewhat specifically over-expressed in tumor cells have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano Y et al., (2001) Br J Cancer, 84:1052-7.), HER2/neu (Tanaka H et al., (2001) Br J Cancer, 84: 94-9.), CEA (Nukaya I et al., (1999) Int. J. Cancer 80, 92-7.) and the like.

[0004] It has been shown in De Nooij et al. (2003), International Journal of Cancer 103:768-774, that human Ly-6 antigens, including Ly-6k, can be used as molecular markers for head-and-neck squamzus cell carcinoma.

[0005] Despite significant progress in basic and clinical research concerning TAAs (Rosenberg SA et al., (1998) Nature Med, 4: 321-7.; Mukherji B. et al., (1995) Proc Natl Acad Sci USA, 92: 8078-82.: Hu X et al., (1996) Cancer Res, 56: 2479-83.), only a very limited number of candidate TAAs suitable for treatment of adenocarcinomas, such as lung cancer, are available. TAAs that are abundantly expressed in cancer cells, and whose expression is restricted to cancer cells, would be promising candidates as immunotherapeutic targets.

[0006] It has been repeatedly shown in ^{51}Cr-release assays that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN-γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or A0201 restricted manner (Kawano K et al., (2000) Cancer Res 60: 3550-8.; Nishizaka et al., (2000) Cancer Res 60: 4830-7.; Tamura et al., (2001) Jpn J Cancer Res 92: 762-7.). However, both HLA-A24 and HLA-A0201 are common HLA alleles in Japanese and Caucasian populations (Date Y et al., (1996) Tissue Antigens 47: 93-101.; Kondo A et al., (1995) J Immunol 155: 4307-12.; Kubo RT et al., (1994) J Immunol 152: 3913-24.; Imanishi et al., Proceeding of the eleventh International Histocompatibility Workshop and Conference Oxford University Press, Oxford, 1065 (1992); Williams F et al., (1997) Tissue Antigen 49: 129.). Thus, antigenic peptides of cancers presented by these HLA alleles may be especially useful for the treatment of cancers among Japanese and Caucasian patients. Further, it is known that the induction of low-affinity CTL *in vitro* usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen-presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., (1996) Proc Natl Acad Sci USA 93: 4102-7.).

[0007] Recent developments in cDNA microarray technologies have enabled the constructions of comprehensive profiles of gene expression of malignant cells as compared to normal cells (Okabe, H. et al., (2001) Cancer Res., 61, 2129-37.; Lin YM. et al., (2002) Oncogene, 21;4120-8.; Hasegawa S. et al., (2002) Cancer Res 62:7012-7.). This approach enables an understanding of the complex nature of cancer cells and the mechanisms of carcinogenesis and facilitates

the identification of genes whose expression is deregulated in tumors (Bienz M. et al., (2000) Cell 103, 311-320.). Among the transcripts identified as commonly up-regulated in lung cancers, *URLC10* (cDNA for differentially expressed CO16 gene; GenBank Accession No. AB105187; SEQ ID Nos.3, 4) is of particular interest to the present inventors, being specifically up-regulated in tumor cells of the lung cancer tissues in more than 80 % of the cases analyzed. In contrast, Northern blot analysis demonstrated that these gene products are not found in normal vital organs (See WO2004/031413).

[0008] Thus, immunogenic peptides derived from *URLC10* may find utility in killing tumor cells expressing those antigens. The present invention addresses these and other needs.

Summary of the Invention

[0009] The gene *URLC10* (cDNA for differentially expressed CO16 gene) has been identified as up-regulated in lung cancer. The gene was identified using gene expression profiling with a genome-wide cDNA microarray containing 23,040 genes. As discussed above, expression of *TTK*, *URLC10* and *KOC1* is specifically up-regulated in tumor cells in more than 80% of the patients with lung cancer yet absent in other normal vital organs.

[0010] The present invention is based, at least in part, on the identification of epitope peptides of the gene products of the gene (URLC10) which elicits cytotoxic T lymphocytes (CTLs) specific to the corresponding molecules. As discussed in detail below, Peripheral Blood Mononuclear Cells (PBMC) of healthy donor were stimulated using HLA-A*2402 binding candidate peptides derived from URLC 10.

[0011] CTL clones were then established with specific cytotoxicity against the HLA-A24 positive target cells pulsed with this candidate peptide. Further analysis of the CTL clones showed the potent cytotoxic activity against, not only the peptide-pulsed target cells, but also tumor cells that endogenously express URLC 10. Furthermore, both a cold target inhibition assay and an antibody blocking assay revealed that CTL cell clones specifically recognized the MHC class I - peptide complex. These results demonstrate that these peptides are HLA-A24 restricted epitope peptides that can induce potent and specific immune responses against lung cancer cells expressing URLC 10.

[0012] Accordingly, the present invention provides methods for treating or preventing lung cancer in a subject comprising the step of administering to the subject the URLC10 polypeptides of the invention. Anti-tumor immunity is induced by the administration of this polypeptide. Thus, the present invention provides methods for inducing anti-tumor immunity in a subject comprising the step of administering to the subject the URLC10 polypeptide, as well as pharmaceutical compositions for treating or preventing lung cancer comprising the URLC10 polypeptide.

[0013] These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of preferred embodiments, and not restrictive of the invention or other alternate embodiments of the invention.

Brief Description of the Drawings

[0014]

Figure 1 is a graph showing that the CTL clone raised by TTK-567 has peptide-specific cytotoxicity. Specifically, the CTL clone showed high cytotoxic activity against target cells (A24LCL) pulsed with TTK-567, whereas it did not show significant cytotoxic activity against the same target cells (A24LCL) pulsed with no peptides.

Figure 2 is a graph showing that the CTL clone raised by URLC10-177 has peptide-specific cytotoxicity. Specifically, the CTL clone showed high cytotoxic activity against target cells (A24LCL) pulsed with URLC10-177, whereas it did not show significant cytotoxic activity against the same target cells (A24LCL) pulsed with no peptides.

Figure 3 is a graph showing that the CTL clone raised by KOCI-508 has peptide-specific cytotoxicity. Specifically, the CTL clone showed high cytotoxic activity against target cells (A24LCL) pulsed with KOC1-508, whereas it did not show significant cytotoxic activity against the same target cells (A24LCL) pulsed with no peptides.

Figure 4 is a graph showing that the CTL clone raised by TTK-567 recognizes and lyses tumor cells endogenously expressing *TTK* in an HLA restricted fashion. Cytotoxic activity against TE1 cells, which endogenously express *TTK* and HLA-A24, was tested using as effector cells the CTL clones raised by TTK-567. PC9 cells were used as the target cells that endogenously express *TTK* but do not express HLA-A24. The CTL clone showed high cytotoxic activity against TE1 cells that express both *TTK* and HLA-A24. On the other hand, it did not show significant cytotoxic activity against PC9 cells that express *TTK* but not HLA-A24.

Figure 5 is a graph showing that the CTL clone raised by URLC10-177 recognizes and lyses tumor cells endogenously

expressing *URLC10* in an HLA restricted fashion. Cytotoxic activity against TE1 cells, which endogenously express *URLC10* and HLA-A24, was tested using as effector cells the CTL clone raised by URLC10-177. TE13 cells were used as the target cells that endogenously express *URLC10* but do not express HLA-A24. The CTL clone showed high cytotoxic activity against TE1 cells that express both *URLC10* and HLA-A24. On the other hand, it did not show significant cytotoxic activity against TE13 cells that express *URLC10,* but not HLA-A24.

Figure 6 is a graph showing that the CTL clone raised by KOC1-508 recognizes and lyses the tumor cells endogenously expressing *KOC1* in an HLA restricted fashion. Cytotoxic activity against TE1 cells, which endogenously express *KOC1* and HLA-A24, was tested using as effector cells the CTL clone raised by KOC1-508. PC9 cells were used as the target cells that endogenously express *KOC1* but do not express HLA-A24. The CTL clone showed high cytotoxic activity against TE1 cells that express both *KOC1* and HLA-A24. On the other hand, it did not show significant cytotoxic activity against PC9 cells that express *KOC1,* but not HLA-A24.

Figure 7 is a graph showing that the CTL clone raised by TTK-567 specifically recognizes TTK-567 in an HLA-A24 restricted manner. TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while TTK-567 peptide-pulsed A24LCL cells were used as cold target (Inhibitor). The E/T ratio was fixed at 20. The cytotoxic activity of TTK-567 CTL clone against TE1 cells was inhibited by the addition of A24LCL cells pulsed with the identical peptide.

Figure 8 is a graph showing that the CTL clone raised by URLC10-177 specifically recognizes URLC10 in an HLA-A24 restricted manner. TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while URLC10-177 peptide-pulsed A24LCL cells were used as cold target (Inhibitor). The E/T ratio was fixed at 20. The cytotoxic activity of URLC10-177 CTL clone against TE1 was inhibited by the addition of A24LCL cells pulsed with the identical peptide.

Figure 9 is a graph showing that the CTL clone raised by KOC1-508 specifically recognizes KOC1 in an HLA-A24 restricted manner. TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while KOC1-508 peptide-pulsed A24LCL cells were used as cold target (Inhibitor). The E/T ratio was fixed at 20. The cytotoxic activity of KOC1-508 CTL clone against TE1 cells was inhibited by the addition of A24LCL cells pulsed with the identical peptide.

Figure 10 is a graph showing that the cytotoxic activity of the CTL clone raised by TTK-567 is specifically blocked by antibodies recognizing the T-cell surface antigens of HLA class I or CD8. The specificity of cytotoxicity of the CTL clone was confirmed by an antibody blocking assay. TE1 cells were co-cultured with monoclonal antibodies respectively, and used as a target. The CTL activity was clearly blocked the addition of antibodies that recognize HLA Class I or CD8 and was marginally affected the addition of antibodies to HLA Class II or CD4; however, it was not inhibited at all by the addition of an isotype-matched control antibody.

Figure 11 is a graph showing that the cytotoxic activity of the CTL clone raised by URLC10-177 is specifically blocked by antibodies recognizing the T-cell surface antigens of HLA class I or CD8. The specificity of cytotoxicity of the CTL clone was confirmed by an antibody blocking assay. TE1 cells were co-cultured with monoclonal antibodies respectively, and used as a target. The CTL activity was clearly blocked by the addition of antibodies that recognize HLA Class I or CD8 and was marginally affected by the addition of antibodies to HLA Class II or CD4; however, it was not inhibited at all by the addition of an isotype-matched control antibody.

Figure 12 is a graph showing that the cytotoxic activity of the CTL clone raised by KOC1-508 is specifically blocked by antibodies recognizing the T-cell surface antigens of HLA class I or CD8. The specificity of cytotoxicity of the CTL clone was confirmed by an antibody blocking assay. TE1 cells were co-cultured with monoclonal antibodies respectively, and used as a target. The CTL activity was clearly blocked by the addition of antibodies that recognize HLA Class I or CD8 and was marginally affected by the addition of antibodies to HLA Class II or CD4; however, it was not inhibited at all by the addition of an isotype-matched control antibody.

Detailed Description of the Invention

[0015] The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0016] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0017] Identification of new TAAs, particularly those that induce potent and specific anti-tumor immune responses, warrants further development of the clinical application of the peptide vaccination strategy in various types of cancer (Boon T et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-7.; Brichard V et al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.; Shichijo S et al., (1998) J Exp Med

187:277-88.; Chen YT et al., (1997) Proc.Natl.Acd. Sci.USA, 94: 1914-8.; Harris CC, (1996) J Natl Cancer Inst 88: 1442-5.; Butterfield LH et al., (1999) Cancer Res 59:3134-42.; Vissers JL et al., (1999) Cancer Res 59: 5554-9.; van der Burg SH et al., (1996) J. Immunol 156:3308-14.; Tanaka F et al., (1997) Cancer Res 57:4465-8.; Fujie T et al., (1999) Int J Cancer 80:169-72.; Kikuchi M et al., (1999) Int J Cancer 81 : 459-66.; Oiso M et al., (1999) Int J Cancer 81:387-94.).

As noted above, *TTK, URLC10* and *KOC1* were previously identified as over-expressed in lung cancer using cDNA microarray technologies. As discussed in WO2004/031413, TTK encodes an S_TKc domain. The protein encoded by the TTK gene phosphorylates proteins on serine, threonine and tyrosine, such phosphorylation likely associated with cell proliferation (Mills GB et al., (1992) J Biol Chem 267: 16000-6.; Schmandt R et al., (1994) J Immunol.;152(1):96-105.; Stucke VM et al., (2002) EMBO J.;21(7):1723-32.). *KOCI* encodes insulin-like growth factor 2 (*IGF2*) mRNA-binding protein 3 (*IMP-3*). *IMP-3* protein contains 2 functional RNA recognition motifs (RRM) in addition to the 4 KH domains. The protein associates specifically with the 5-prime UTR of the human 6.0-kb insulin-like growth factor II (IGF2) leader-3 mRNA, suggesting a role for *IMP-3* in the physiologic regulation of IGF2 production. (Nielsen, J. et al., (1999) Molec. Cell. Biol. 19: 1262-1270) *IMP-3* was also over-expressed in pancreatic cancers (Mueller-Pillasch, F. et al., (1997) Oncogene 14: 2729-2733).

[0018] Previous experiments demonstrated that *TTK*, *URLC10* and *KOC1* were over-expressed in lung cancer and show minimal expression in normal tissues. In addition, these genes were shown to have a significant function related to cell proliferation (See WO2004/031413).

[0019] In the present invention, peptides derived from *URLC10* are shown to be TAA epitopes restricted by HLA-A24, an HLA allele commonly found in the Japanese and Caucasian populations. Specifically, using their binding affinities to HLA-A24, candidates of HLA-A24 binding peptides derived from *URLC10* was identified. After the *in vitro* stimulation of T-cells by dendritic cells (DCs) loaded with these peptides, CTLs were successfully established using URLC10-177 (RYCNLEGPPI (SEQ ID No.67)).

[0020] These CTLs showed potent cytotoxic activity against the peptide-pulsed A24LCL cells. Furthermore, CTL clones derived from these cells also showed specific cytotoxicity against HLA-A24 positive lung carcinoma cell lines that endogenously over-express URLC10. However, these CTL clones did not show cytotoxic activity against cell lines lacking expression of either HLA-A24 or target TAA. The specific cytotoxic activities of these CTL clones were significantly inhibited by the cold target. These results demonstrate that *URLC10* is useful as TAA of lung cancer cells and that URLC 10-177 is a epitope peptide of each TAA restricted by HLA-A24. Since these antigens are over-expressed in most lung cancers and are associated with tumor cell proliferation, they find utility as immunotherapeutic targets against lung cancers.

[0021] Accordingly, the present invention further provides methods of treating or preventing lung cancer in a subject, said methods comprising the steps of administering an immunogenic peptide of less than 15 amino acids and comprising the amino acid sequence of SEQ ID NO: 67 to the subject in need thereof. Alternatively, the immunogenic peptide may comprise a sequence of SEQ ID NO:67 in which 1 or 2 amino acids are substituted, deleted or added, provided the resulting variant peptide retains the immunogenic activity (*i.e.*, the ability to induce CTLs specific to lung cancer cells). There is also disclosed that the number of residues to be substituted, deleted, or added is generally 5 amino acids or less, preferably 4 amino acids or less, more preferably 3 amino acids or less.

[0022] Variant peptides (*i.e.*, peptides comprising an amino acid sequence modified by substituting, deleting, or adding one, two or several amino acid residues to an original amino acid sequence) have been known to retain the original biological activity (Mark DF et al., (1984) Proc Natl Acad Sci USA 81: 5662-6.; Zoller MJ and Smith M, (1982) Nucleic Acids Res 10:6487-500.; Dalbadie-McFarland G et al., (1982) Proc Natl Acad Sci USA 79: 6409-13.). In the context of the present invention, the amino acid modification results in conservation of the properties of the original amino acid side-chain (a process known as conservative amino acid substitution). Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). Note, the parenthetic letters indicate the one-letter codes of amino acids.

[0023] The immunogenic peptide is a decapeptide (10-mer).

[0024] The present invention further provides a method of inducing anti-tumor immunity for lung cancer in a subject, said method comprising the steps of administering to the subject an immunogenic peptide of the invention, namely one comprising the amino acid sequence of SEQ ID NO:67 or a variant thereof (*i.e.*, 1 or 2 amino acid substitutions, deletions, or additions) to the subject in need thereof.

[0025] In the context of the present invention, the subject is preferably a mammal. Exemplary mammals include, but are not limited to, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

[0026] In the present invention, the peptide can be administered to a subject via *in vivo* or *ex vivo*. Furthermore, the present invention also provides use of decapeptide comprising the amino acid sequence of SEQ ID NO: 67 (and variants thereof) for manufacturing an immunogenic composition for treating or preventing lung cancer.

[0027] Homology analyses of URLC10-177 demonstrates that it does not have significant homology with the peptides derived from any known human gene products. Accordingly, the possibility of unknown or undesirable immune responses with immunotherapy against these molecules is significantly reduced.

[0028] Regarding HLA antigens, the use of an A-24 type that is highly expressed among the Japanese population is favorable for obtaining effective results, and the use of subtypes, such as A-2402, is even more preferable. Typically, in the clinic, the type of HLA antigen of the patient requiring treatment is investigated in advance, which enables the appropriate selection of peptides having high levels of binding affinity to this antigen, or having cytotoxic T cell (CTL) inducibility by antigen presentation. Furthermore, in order to obtain peptides showing high binding affinity and CTL inducibility, substitution, deletion, or addition of 1 or 2 amino acids may be performed based on the amino acid sequence of the naturally occurring URLC10 partial peptide.

[0029] Furthermore, in addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens is already known (Kubo RT, et al., (1994) J. Immunol., 152, 3913-24.; Rammensee HG, et al., (1995) Immunogenetics. 41:178-228.; Kondo A, et al., (1995) J. Immunol. 155:4307-12.), modifications based on such regularity can be performed on the immunogenic peptides of the invention. For example, peptides showing high HLA-24 binding affinity in which the second amino acid from the N terminus substituted with phenylalanine, tyrosine, methionine, or tryptophan may be favorably used. Likewise, peptides whose C-terminal amino acid is substituted with phenylalanine, leucine, isoleucine, tryptophan, or methionine may also be used favorably.

[0030] However, when the peptide sequence is identical to a portion of the amino acid sequence of an endogenous or exogenous protein having a different function, side effects such as autoimmune disorders or allergic symptoms against specific substances may be induced. Therefore, it is preferable to avoid the situation wherein the immunogenic sequence matches the amino acid sequence of a known protein. This situation may be avoided by performing a homology search using available databases. If homology searches confirm that peptides in which 1 or 2 different amino acids do not exist, then the danger that modifications of the above-mentioned amino acid sequence that, for example, increase the binding affinity with HLA antigens, and/or increase the CTL inducibility can be avoided.

[0031] Although peptides having high binding affinity to the HLA antigens as descried above are expected to be highly effective as cancer vaccines, the candidate peptides, which are selected according to the presence of high binding affinity as an indicator, must be examined for the actual presence of CTL inducibility. CTL inducibility may be confirmed by inducing antigen-presenting cells carrying human MHC antigens (for example, B-lymphocytes, macrophages, and dendritic cells), or more specifically dendritic cells derived from human peripheral blood mononuclear leukocytes, and, after stimulation with the peptide of interest, mixing with CD8-positive cells and measuring the cytotoxic activity against the target cells. As the reaction system, transgenic animals produced to express a human HLA antigen (for example, those described in BenMohamed L, et al., (2000) Hum. Immunol.; 61(8):764-79 Related Articles, Books, Linkout.) may be used. For example, the target cells can be radio-labeled with ^{51}Cr and such, and cytotoxic activity can be calculated from radioactivity released from the target cells. Alternatively, it can be examined by measuring IFN-Y produced and released by CTL in the presence of antigen-presenting cells that carry immobilized peptides, and visualizing the inhibition zone on the media using anti-IFN- Y monoclonal antibodies,

[0032] As a result of examining the CTL inducibility of peptides as described above, it was discovered that peptides having high binding affinity to an HLA antigen did not necessarily have high inducibility. However, decapeptides selected from peptides comprising the amino acid sequence indicated by RYCNLEGPPI (SEQ ID NO: 67) showed particularly high CTL inducibility.

[0033] As noted above, the present invention provides peptides having cytotoxic T cell inducibility, namely those comprising the amino acid sequence of SEQ ID NO:67 or a variant thereof (*i.e.*, those in which 1 or 2 amino acids are substituted, deleted, or added). It is preferably that the amino acid sequence comprise 10 amino acids indicated in SEQ ID NO:67 or a variant thereof do not match an amino acid sequence associated with another endogenous protein. In particular, amino acid substitution to phenylalanine, tyrosine, methionine, or tryptophan at the second amino acid from the N terminus, and to phenylalanine, leucine, isoleucine, tryptophan, or methionine at the C-terminal amino acid, and amino acid addition of 1 to 2 amino acids at the N terminus and/or C terminus are favorable examples.

[0034] Peptides disclosed herein can be prepared using well known techniques. For example, the peptides can be prepared synthetically, using either recombinant DNA technology or chemical synthesis. Peptides disclosed herein may be synthesized individually or as longer polypeptides comprising two or more peptides. The peptides disclosed herein are preferably isolated, *i.e.*, substantially free of other naturally occurring host cell proteins and fragments thereof.

[0035] The peptides disclosed herein may contain modifications, such as glycosylation, side chain oxidation, or phosphorylation; so long as the modifications do not destroy the biological activity of the peptides as described herein. Other modifications include incorporation of D-amino acids or other amino acid mimetics that can be used, for example, to increase the serum half life of the peptides.

[0036] The peptides disclosed herein can be prepared as a combination, which comprises two or more of peptides disclosed herein, for use as a cancer vaccine that may induce CTL *in vivo.* The peptides may be in a cocktail or may be conjugated to each other using standard techniques. For example, the peptides can be expressed as a single polypeptide

sequence. The peptides in the combination may be the same or different. By administering the peptides disclosed herein, the peptides are presented at a high density on the HLA antigens of antigen-presenting cells, which, in turn, induces CTLs that specifically react toward the complex formed between the displayed peptide and the HLA antigen. Alternatively, antigen-presenting cells having immobilized the peptides disclosed herein on their cell surface, obtained by removing dendritic cells from the subjects, may be stimulated by the peptides disclosed herein. Re-administration of these cells to the respective subjects induces CTL, and, as a result, aggressiveness towards the target cells can be increased.

[0037] More specifically, there are disclosed drugs for treating tumors or preventing proliferation, metastasis, and such of tumors, which comprise one or more of peptides disclosed herein. The peptides of this invention find particular utility in the treatment of lung cancer.

[0038] The peptides of this invention can be administered to a subject directly, as a pharmaceutical composition that has been formulated by conventional formulation methods. In such cases, in addition to the peptides of this invention, carriers, excipients, and such that are ordinarily used for drugs can be included as appropriate, without particular limitations. The pharmaceutical composition of this invention may be used for treatment and prevention of lung cancers.

[0039] The immunogenic compositions for treatment and/or prevention of tumors, which comprise as the active ingredient one ore more peptides disclosed herein, can further include an adjuvant so that cellular immunity will be established effectively. Alternatively, they may be administered with other active ingredients, such as anti-tumor agents. Suitable formulations include granules. Suitable adjuvants are described in the literature (Johnson AG. (1994) Clin. Microbiol. Rev., 7:277-89.). Exemplary adjuvants include, but are not limited to, aluminum phosphate, aluminum hydroxide, and alum. Furthermore, liposome formulations, granular formulations in which the drug is bound to few-μm diameter beads, and formulations in which a lipid is bound to the peptide may be conveniently used. The method of administration may be oral, intradermal, subcutaneous, intravenous injection, or such, and may include systemic administration or local administration to the vicinity of the targeted tumor. The dose of the peptide(s) disclosed herein can be adjusted appropriately according to the disease to be treated, age of the patient, weight, method of administration, and such. Though the dosage is ordinarily 0.001 mg to 1000 mg, preferably 0.01 mg to 100 mg, more preferably 0.1 mg to 10 mg, preferably administered once in a few days to few months, one skilled in the art can readily select the appropriate dose and method of administration, as, the selection and optimization of these parameters is well within routine skill.

[0040] The present invention further provides intracellular vesicles called exosomes, which present complexes formed between the peptides of this invention and HLA antigens on their surface. Exosomes can be prepared, for example, by using the methods described in detail in Published Japanese Translation of International Publication Nos. Hei 11-510507 and 2000-512161, and are preferably prepared using antigen-presenting cells obtained from subjects who are targets of treatment and/or prevention. The exosomes disclosed herein can be inoculated as cancer vaccines. similarly to the peptides disclosed herein.

[0041] The type of HLA antigens used must match that of the subject requiring treatment and/or prevention. For example, in the Japanese population, HLA-A24, particularly HLA-A2402, is often appropriate.

[0042] In some embodiments, the vaccine compositions disclosed herein include a component which primes cytotoxic T lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo* against viral antigens. For example, palmitic acid residues can be attached to the ε-and α-amino groups of a lysine residue and then linked to an immunogenic peptide of the invention. The lipidated peptide can then be administered either directly, in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant As another example of a lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P3CSS), can be used to prime CTL when covalently attached to an appropriate peptide (see, *e.g.*, Deres K, et al., (1989) Nature 342:561-4.).

[0043] The immunogenic compositions disclosed herein may also comprise nucleic acids encoding one or more of the immunogenic peptides disclosed here. See, *e.g.,* Wolff JA et al., (1990) Science 247:1465-8; U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, *e.g.*, U.S. Patent No. 5,922,687).

[0044] The immunogenic peptides disclosed herein can also be expressed by viral or bacterial vectors. Examples of suitable expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, *e.g.*, as a vector to express nucleotide sequences that encode the peptide. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunization protocols are described in, *e.g.*, U.S. Patent No. 4,722,848. Another suitable vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover CK, et al., (1991) Nature 351: 456-60. A wide variety of other vectors useful for therapeutic administration or immunization *e.g.*, adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like, are known in the art. See, *e.g.*, Shata MT, et al., (2000) Mol. Med. Today 6:66-71; Shedlock DJ and Weiner DB., et al., (2000) J. Leukoc. BioL 68:793-806; and Hipp JD, et al., (2000) In Vivo 14:571-85.

[0045] The present invention also provides methods of inducing antigen-presenting cells using one or more peptides of this invention. The antigen-presenting cells can be induced by inducing dendritic cells from the peripheral blood

monocytes and then contacting (stimulating) them with one or more peptides of this invention *in vitro*, *ex vivo* or *in vivo*. When peptides are administered to the subjects, antigen-presenting cells that have the peptides immobilized to them are induced in the body of the subject. Alternatively, after immobilizing to the antigen-presenting cells, the cells can be administered to the subject as a vaccine. For example, the *ex vivo* administration may comprise steps of:

a: collecting antigen-presenting cells from a subject, and
b: contacting the antigen-presenting cells of step a with a peptide.

[0046] The antigen-presenting cells obtained by step b can be administered to the subject as a vaccine.

[0047] This invention also provides a method for inducing antigen-presenting cells having a high level of cytotoxic T cell inducibility, in which the method comprises the step of transferring genes comprising polynucleotide(s) encoding one or more peptides of this invention to antigen-presenting cells *in vitro.* The introduced genes may be in the form of DNAs or RNAs. For the method of introduction, without particular limitations, various methods conventionally performed in this field, such as lipofection, electroporation, and calcium phosphate method may be suitably used. More specifically, transfection may be performed as described in Reeves ME, et al., (1996) Cancer Res., 56:5672-7.; Butterfield LH, et aL, (1998) J. Immunol., 161:5607-13.; Boczkowski D, et al., (1996) J. Exp. Med., 184:465-72.; Published Japanese Translation of International Publication No. 2000-509281. By transferring the gene into antigen-presenting cells, the gene undergoes transcription, translation, and such in the cell, and then the obtained protein is processed by MHC Class I or Class II, and proceeds through a presentation pathway to present partial peptides.

[0048] The present invention further provides an in vitro method for inducing CTL using one or more peptides of this invention. When the peptides disclosed herein are administered to a subject, CTL are induced in the body of the subject, and the strength of the immune system targeting the lung cancer cells in the tumor tissues is thereby enhanced. Alternatively, the peptides disclosed herein may be used in the context of an *ex vivo* therapeutic method, in which subject-derived antigen-presenting cells and CD8-positive cells or peripheral blood mononuclear leukocytes are contacted (stimulated) with one or more peptides disclosed herein *in vitro,* and, after inducing CTL, the cells are returned to the subject For example, the method may comprise steps of:

a: collecting antigen-presenting cells from a subject,
b: contacting the antigen-presenting cells of step a with a peptide.
c: mixing the antigen-presenting cells of step b with CD^{8+} T cells and co-culturing so as to induce cytotoxic T-cells:, and
d: collecting CD^{8+} T cells from the co-culture of step c.

[0049] The CD^{8+} T cells having cytotoxic activity obtained by step d can be administered to the subject as a vaccine.

[0050] The present invention further provides isolated cytotoxic T cells induced using the peptides of this invention, wherein the isolated cytotoxic T cell specifically acts against target cells presenting the peptide consisting of the amino acid sequence of SEQ ID NO: 67. The cytotoxic T cells, induced by stimulation with in antigen-presenting cell presenting one or more peptides disclosed herein, are preferably derived from subjects who are the target of treatment and/or prevention, and can be administered alone or in combination with other drugs, including one or more peptides disclosed herein or exosomes having anti-tumor activity. The obtained cytotoxic T cells act specifically against target cells presenting the peptides of this invention. The target cells may be cells that express URLC10 endogenously, or cells that are transfected with URLC 10 genes. Cells that present the peptides disclosed herein on the cell surface, due to stimulation with these peptides, can also become targets of attack.

[0051] The present invention also provides antigen-presenting cells presenting complexes formed between HLA antigens and a peptide of this invention. The antigen-presenting cells, obtained through contact with the peptides disclosed herein or the nucleotides encoding such peptides, are preferably derived from subjects who are the target of treatment and/or prevention, and can be administered as vaccines, alone or in combination with other drugs, including the peptides, exosomes, or cytotoxic T cells of the present disclosure.

[0052] In the context of the present invention, the term "vaccine" (also referred to as an immunogenic composition) refers to a substance that induces anti-tumor immunity or suppresses lung cancer upon inoculation into animals. According to the present invention, polypeptides comprising the amino acid sequence of SEQ ID NO: 67 were suggested to be HLA-A24 restricted epitope peptides that may induce potent and specific immune response against lung cancer cells expressing URLC10.

[0053] Thus, the present invention also encompasses a method of inducing anti-tumor immunity using polypeptides comprising the amino acid sequence of SEQ ID NO: 67 or a variant thereof (*i.e.,* including 1 or 2 amino acid substitutions, deletions, or additions). In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors comprising cells expressing URLC 10,
- induction of antibodies that recognize tumors comprising cells expressing URLC 10, and

- induction of anti-tumor cytokine production.

[0054] Therefore, when a certain peptide induces any one of these immune responses upon inoculation into an animal, the peptide is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a peptide can be detected by observing *in vivo* or *in vitro* the response of the immune system in the host against the peptide.

[0055] For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen-presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (also referred to as cytotoxic T lymphocytes or CTLs) due to stimulation by the antigen, and then proliferate; this process is referred to herein as "activation" of T cells. Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to a T cell by APC, and detecting the induction of CTL. Furthermore, APCs have the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils and NK cells. Since CD4+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

[0056] A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ^{51}Cr-labeled tumor cells as the indicator. Alternatively, it is well known to evaluate the degree of tumor cell damage using-^{3}H-thymidine-uptake activity or LDH (lactose dehydrogenase)-release as the indicator.

[0057] Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL has been shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

[0058] The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against lung cancer. Furthermore, APC that have acquired the ability to induce CTL against lung cancer by contacting with the polypeptides are useful as vaccines against lung cancer. Furthermore, CTL that have acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against lung cancer. Such therapeutic methods for lung cancer, using anti-tumor immunity due to APC and CTL, are referred to as cellular immunotherapy.

[0059] Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction can be increased by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

[0060] The induction of anti-tumor immunity by a polypeptide can be further confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and when growth, proliferation and/or metastasis of tumor cells is suppressed by those antibodies, the polypeptide is determined to induce anti-tumor immunity.

[0061] Anti-tumor immunity can be induced by administering a vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of lung cancer. Therapy against or prevention of the onset of lung cancer may include inhibition of the growth of lung cancer cells, involution of lung cancer cells and suppression of occurrence of lung cancer cells. Decrease in mortality of individuals having lung cancer, decrease of lung cancer markers in the blood, alleviation of detectable symptoms accompanying lung cancer and such are also included in the therapy or prevention of lung cancer. Such therapeutic and preventive effects are preferably statistically significant, for example, observed at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against lung cancer is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test or ANOVA may be used for determining statistical significance.

[0062] The above-mentioned peptide, having immunological activity, or a polynucleotide or vector encoding such a peptide, may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the peptide when administered together (or successively) with the peptide having immunological activity. Examples of suitable adjuvants include cholera toxin, salmonella toxin, alum and such, but are not limited thereto. Furthermore, a vaccine of this disclosure may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration or boosted by multiple administrations.

[0063] When using APC or CTL as the vaccine of this invention, lung cancer can be treated or prevented, for example, by the *ex vivo* method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, contacted

ex vivo with a peptide of the present invention. Following the induction of APC or CTL, the cells may be administered to the subject. APC can be also induced by introducing a vector encoding the peptide into PBMC *ex vivo.* APC or CTL induced *in vitro* can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against similar types of diseases in other individuals.

[0064] Aspects of the present invention are described in the following examples, which are presented only to illustrate the present invention and to assist one of ordinary skill in making and using the same.

[0065] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.

EXAMPLES

[0066] The present invention is illustrated, but not restricted, by following Examples.

MATERIALS AND METHODS

Cell lines

[0067] A24LCL cells (HLA-A24/24) and EHM (HLA-A3/3), human B-lymphoblastoid cell lines, were generous gifts from Takara Shuzo Co, Ltd. (Otsu, Japan). The A24LCL cells were used for peptide-mediated cytotoxicity assays. Lung carcinoma cell lines TE1 (HLA-A2402+), TE13 (HLA-A2402-) and PC9 (HLA-A2402-) were purchased from ATCC. Expression levels of *TTK*, *URLC10* and *KOC1* in the lung carcinoma cell lines were determined by cDNA microarray and RT-PCR that revealed strong expression of all three genes in TE1, *TTK* and *KOC1* expression in PC9, and *URLC10* expression in TE13 (data not shown).

Candidate selection of peptide derived from TTK, URLC10 and LOC1

[0068] 9-mer and 10-mer peptides derived from *TTK*, *URLC10* or *KOC1* that bind to HLA-A24 molecule were predicted by binding prediction software "BIMAS" (http://bimas.dcrt.nih.gov/cgi-bin/molbio/ken parker comboform) (Parker KC, et al., (1994) J Immunol.;152(1):163-75.; Kuzushima K, et al., (2001) Blood.;98(6):1872-81.). These peptides were synthesized by Mimotopes (San Diego, LA) according to the standard solid phase synthesis method and purified by reversed phase HPLC. The purity (>90%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide (DMSO) at 20 mg/ml and stored at -80 °C.

In vitro CTL Induction

[0069] Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells (APCs) to induce CTL responses against peptides presented on HLA. DCs were generated *in vitro* as described elsewhere (Nukaya I et al., (1999) Int. J. Cancer 80, 92-7., Tsai V et al., (1997) J. Immunol 158:1796-802.). Briefly, peripheral blood mononuclear cells (PBMCs) isolated from a normal volunteer (HLA-A*2402) by Ficoll-Paque (Pharmacia) solution were separated by adherence to a plastic tissue culture flask (Becton Dickinson) so as to enrich them for the monocyte fraction. The monocyte-enriched population was cultured in the presence of 1000 U/ml of GM-CSF (provided by Kirin Brewery Company) and 1000 U/ml of IL-4 (Genzyme) in AIM-V (Invitrogen) containing 2% heat-inactivated autologous serum (AS). After 7 days in the culture, the cytokine-generated DCs were pulsed with 20 μg/ml of HLA-A24-binding peptides in the presence of 3 μg/ml of β2-microglobulin for 4 his at 20°C in AIM-V These peptide-pulsed DCs were then irradiated (5500 rad) and mixed at a 1:20 ratio with autologous $CD8^+$ T cells, obtained by positive selection with Dynabeads M-450 CD8 (Dynal) and DETACHa BEAD™ (Dynal). These cultures were set up in 48-well plates (Corning); each well contained $1.5x10^4$ peptide-pulsed DCs, $3x10^5$ $CD8^+$ T cells and 10 ng/ml of IL-7 (Genzyme) in 0.5 ml of AIM-V/2% AS. Three days later, these cultures were supplemented with IL-2 (CHIRON) to a final concentration of 20 IU/ml. On day 7 and 14, the T cells were further restimulated with the autologous peptide-pulsed DCs. The DCs were prepared each time by the same way described above. Cytotoxicity was tested against peptide-pulsed A24LCL cells after the 3rd round of peptide stimulation on day 21.

CTL Expansion Procedure

[0070] CTLs were expanded in culture using the method similar to the one described by Riddell SR, *et al.,* (Walter EA et al., (1995) N Engl J Med 333:1038-44.;, Riddel et al., (1996) Nature Med. 2:216-23.). A total 5 x 10^4 of CTLs were

resuspended in 25 ml of AIM-V/5% AS with 25 x 10^6 irradiated (3300 rad) PBMC and 5 x 10^6 irradiated (8000 rad) EHM cells in the presence of 40 ng/ml of anti-CD3 monoclonal antibody (Pharmingen). One day after initiating the cultures, 120 IU/ml of IL-2 were added to the cultures. The cultures were fed with fresh AIM-V/5% AS containing 30 IU/ml of IL-2 on days 5, 8 and 11.

Establishment of CTL clones

[0071] The dilutions were made to have 0.3, 1, and 3 CTLs/well in 96 round-bottomed micro titer plate (Nalge Nunc International). CTLs were cultured with 7×10^4 cells/well of allogenic PBMCs, 1×10^4 cells/well of EHM, 30ng/ml of anti-CD3 antibody, and 125 U/ml of I-2 in total of 150μl/well of AIM-V containing 5%AS. 50 μl /well of IL-2 was added to the medium 10 days later so that IL-2 became 125 U/ml in the final concentration. Cytotoxic activity of CTLs was tested on the 14th day, and CTL clones were expanded using the same method above.

Cytotoxicity Assay

[0072] Target cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ for 1 hr at 37°C in a CO_2 incubator (Perkin Elmer Life Sciences). Peptide-pulsed targets were prepared by incubating the cells with 20μg/ml of the peptide for 16 hrs at 37°C before labeling Labeled target cells were rinsed and mixed with effector cells in a final volume of 0.2 ml in round-bottom microtiter plates. The plates were centrifuged (4 minutes at 800 x g) to increase cell-to-cell contact and placed in a CO_2 incubator at 37°C. After 4 hrs of incubation, 0.1 ml of the supernatant was collected from each well and the radioactivity was determined with a gamma counter.

[0073] The percentage of specific cytotoxicity was determined by calculating the percentage of specific ^{51}Cr-release by the following formula:

{(cpm of the test sample release - cpm of the spontaneous release) / (cpm of the maximum release - cpm of the spontaneous release)} × 100.

[0074] Spontaneous release was determined by incubating the target cells alone, in the absence of effector cells, and the maximum release was obtained by incubating the target cells with 1N HCl. All measurements were done in duplicate, and the standard errors of the means were consistently below 10% of the value of the mean.

[0075] Antigen specificity was confirmed by the cold target inhibition assay, which utilized unlabeled A24LCL cells that were pulsed with or without peptide (20μg/ml for 16 hrs at 37°C) to compete for the recognition of ^{51}Cr-labeled tumor cells.

[0076] Blocking assay of cytotoxicity using the monoclonal antibodies (mAbs) (mouse anti-MHC-class I mAb, anti-MHC-class II mAb, anti-CDBmAb, and anti-CD4mAb) was performed to confirm the HLA restriction manner. Anti-mouse IgG1, anti-mouse IgG2a mAbs were used as Isotype.

RESULTS

Prediction of HLA-A24 binding peptides derived from TTK, URLC10 or KOCI

[0077] Table 1 shows the HLA-A*2402 binding peptides for *TTK* (GenBank Accession No. NM_003318; SEQ ID Nos. 1, 2) in order of binding affinity. Table 1A shows 9-mer peptides derived from *TTK* and Table 1B shows 10-mer peptides derived from *TTK*. Table 2 shows the HLA-A*2402 binding peptides for *URLC10* (GenBank Accession No. AB105187; SEQ ID Nos.3, 4) in order of binding affinity. Table 2A shows 9-mer peptides derived from *URLCL10* and Table 2B shows 10-mer peptides derived from *URLC10.* Table 3 shows the HLA-A*2402 binding peptides for *KOC1* (GenBank Accession No. NM_006S47; SEQ ID Nos.5, 6) in order of binding affinity. Table 3A shows 9-mer peptides derived from *KOC1* and Table 3B shows 10-mer peptides derived from *KOC1.*

Table 1A HLA-A24 binding 9-mer peptides derived from *TTK*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
90	RYSQAIEAL	7	400	71	KLEKNSVPL	17	12	
567	SYRNEIAYL	8	200	201	KNLSASTVL	18	12	N.D
549	IYAIKYVNL	9	200	467	RTPVVKNDF	19	10.1	N.D

(continued)

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
590	DYEITDQYI	10	90	102	KYGQNESFA	20	10	N.D
652	NFLIVDGML	11	42	19	KVRDIKNKF	21	8.9	N.D
141	KFAFVHISF	12	28	777	KQRISIPEL	22	8.8	N.D
214	SFSGSLGHL	13	20	75	NSVPLSDAL	23	8.6	N.D
28	KNEDLTDEL	14	19	605	GNIDLNSWL	24	8.6	N.D
111	RIQVRFAEL	15	15.8	596	QYIYMVMEC	25	8.3	N.D
108	SFARIQVRF	16	14	535	GSSKVFQVL	26	8.1	N.D

Start position indicates the number of amino acid from N-terminal of TTK. Binding score is derived from "BIMAS" described in Materials and Methods.
N.D. indicates "not done"

Table 1B HLA-A24 binding 10-mer peptides derived from *TTK*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score
810	KYVLGQLVGL	27	600	168	KAVERGAVPL	37	14.4
725	YYMTYGKTPF	28	150	232	RGQTTKARFL	38	12
598	TYMVMECGNI	29	75	185	RNLNLQKKQL	39	12
728	TYGKTPFQQI	30	72	777	KQRISIPELL	40	11.2
755	EFPDIPEKDL	31	36	573	AYLNKLQQHS	41	10.8
490	CFQQQQHQIL	32	36	373	EYQEPEVPES	42	9.9
143	AFVHISFAQF	33	18	74	KNSVPLSDAL	43	9.6
569	RNEIAYLNKL	34	15.8	315	KPSGNDSCEL	44	8.8
359	KTESSLLAKL	35	15.8	61	NPEDWLSLLL	45	8.6
553	KYVNLEEADN	36	15	763	DLQDVLKCCL	46	8.6

Start position indicates the number of amino acid from N-terminal of TTK. Binding score is derived from "BIMAS" described in Materials and Methods.

Table 2A HLA-A24 binding 9-mer peptides derived from *URLC10*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score		Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
154	KPEEKRFLL	47	14.4		193	EYAGSMGES	57	5.5	N.D
48	RADPPWAPL	48	9.6		168	FFYLKCCKI	58	5.5	N.D
205	LWLAILLLL	49	8.4		128	AAVKIFPRF	59	5	N.D
57	GTMALLALL	50	7.2		58	TMALLALLL	60	4.8	N.D
203	GGLWLAILL	51	7.2		152	RPKPEEKRF	61	4.8	N.D
62	LALLLVVAL	52	7.2		197	SMGESCGGL	62	4.8	N.D
53	WAPLGTMA L	53	6		173	CCKIRYCNL	63	4	N.D
214	ASIAAGLSL	54	6		28	DPGRGARRL	64	4	N.D
54	APLGTMALL	55	6		31	RGARRLRRF	65	4	N.D
212	LLASIAAGL	56	5.6	N.D	202	CGGLWLAIL	66	4	N.D

Start position indicates the number of amino acid from N-terminal of URLC10.
Binding score is derived from "BIMAS" described in Materials and Methods.
N.D. indicates "not done"

Table 2B HLA-A24 binding 10-mer peptides derived from *URLC10*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score		Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
177	RYCNLEGPPI	67	100		196	GSMGESCGGL	77	6	N.D
159	RFLLEEPMPF	68	30		204	GLWLAILLLL	78	5.6	N.D
152	RPKPEEKRFL	69	9.6		123	PYCVIAAVKI	79	5.5	N.D
211	LLLASIAAGL	70	8.4		193	EYAGSMGESC	80	5	N.D
172	KCCKIRYCNL	71	8		61	LLALLLVVAL	81	4.8	N.S
169	FYLKCCKIRY	72	7.5		202	CGGLWLAILL	82	4.8	N.D
57	GTMALLALLL	73	7.2		56	LGTMALLALL	83	4.8	N.D
53	WAPLGTMALL	74	6		70	LPRVWTDANL	84	4	N.D
203	GGLWLAILLL	75	6	N.D	201	SCGGLWLAIL	85	4	N.D
198	MGESCGGLWL	76	6	N.D	213	LASIAAGLSL	86	4	N.D

Start position indicates the number of amino acid from N-terminal of URLC10.
Binding score is derived from "BIMAS" described as Materials and Methods.
N.D. indicates "not done". N.S. indicates "not synthesized"

Table 3A HLA-A24 binding 9-mer peptides derived from *KOC1*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
350	SYENDIASM	87	37.5	4	LYIGNLSEN	97	8.25	
141	GFQLENFTL	88	30	423	KQGQHIKQL	98	8	N.D
508	KTVNELQNL	89	14.4	561	KQHQQQKAL	99	8	N.D
26	KIPVSGPFL	90	12	310	ITISPLQEL	100	7.9	N.D
192	KPCDLPLRL	91	11.5	470	IYGKIKEEN	101	7.7	N.D
433	RFAGASIKI	92	11	356	ASMNLQAHL	102	7.2	N.D
505	KGGKTVNEL	93	10.6	93	QWEVLDSLL	103	7.2	N.D
190	KQKPCDLPL	94	9.6	43	DCPDESWAL	104	7.2	N.D
152	AYIPDEMAA	95	9	92	LQWEVLDSL	105	6.7	N.D
320	LYNPERTIT	96	9	55	EALSGKIEL	106	6.6	N.D

Start position indicates the number of amino acid from N-terminal of KOC1.
Binding score is derived from "BIMAS" described in Materials and Methods.
N.D. indicates "not done"

Table 3B HLA-A24 binding 10-mer peptides derived from *KOC1*

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score		Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
470	IYGKIKEENF	107	100		91	HLQWEVLDSL	117	8.4	N.D
272	KFTEEIPLKI	108	18.5		359	NLQAHLIPGL	118	7.2	N.D
290	RLIGKEGRNL	109	12		364	LIPGLNLNAL	119	7.2	N.D
309	KITISPLQEL	110	10.6		165	LQQPRGRRGL	120	7.2	N.D
350	SYENDIASMN	111	10.5		273	FTEEIPLKIL	121	7.2	N.D
192	KPCDLPLRLL	112	9.6		406	ETVHLFIPAL	122	6	N.D
320	LYNPERTITV	113	9	N.D	138	KLNGFQLENF	123	6	N.D
4	LYIGNLSENA	114	9	N.D	9	LSENAAPSDL	124	6	N.D

(continued)

Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score		Start Position	Amino Acid Sequence	SEQ ID No.	Binding Score	
548	VAQRKIQEIL	115	8.4	N.D	88	IPPHLQWEVL	125	6	N.D
83	LQIRNIPPHL	116	8.4	N.D	127	SSKDQARQAL	126	5.7	N.D

Start position indicates the number of amino acid from N-terminal of KOC1.
Binding score is derived from "BIMAS" described in Materials and Methods.
N.D. indicates "not done"

Stimulation of the T cells using the predicted peptides

[0078] CTLs for those peptides derived from *TTK, URLC10 or KOC1* were generated in the manner described in "Materials and Methods" section above. Resulting CTLs showing detectable cytotoxic activity were expanded, and those CTL clones that demonstrated higher cytotoxic activities against the peptide-pulsed target as compared to the activities against target without peptide pulse were established.

[0079] The CTL clones stimulated by the HLA-A24 binding peptides TTK-567 (SYRNEIAYL (SEQ ID No.8)) (Figure 1), URLC10-177 (RYCNLEGPPI (SEQ ID No.67)) (Figure 2) or by KOC1-508 (KTVNELQNL (SEQ ID No.89)) (Figure 3) showed potent cytotoxic activity against the peptide-pulsed target without showing any significant cytotoxic activity against targets not pulsed with any peptide.

Cytotoxic activity against lung cancer cell lines endogenously expressing TTK, URLC10 or KOC1

[0080] The established CTL clones described above were examined for their ability to recognize and kill tumor cells endogenously expressing *TTK*, *URLC10* or *KOC1.* Cytotoxic activity against TE1 cells, which endogenously express *TTK* and HLA-A24, was tested using as effector cells the CTL clone raised by TTK-567. PC9 cells were used as the target cells that endogenously express *TTK* but not HLA-A24. The CTL clone showed high cytotoxic activity against the TE1 cells that express both *TTK* and HLA-A24. On the other hand, it did not show significant cytotoxic activity against the PC9 cells that express *TTK* but not HL,A-A24 (Figure 4). Cytotoxic activity against TE1 cells, which endogenously express *URLC10* and HLA-A24, was tested using as effector cells the CTL clone raised by URLC10-177. TE13 cells were used as the target cells that endogenously express *URLC10* but not HLA-A24. The CTL clone showed high cytotoxic activity against the TE1 cells that express both *URLC10* and HLA-A24. On the other hand, it did not show significant cytotoxic activity against theTE13 cells that express *URLC10* but not HLA-A24 (Figure 5). Cytotoxic activity against TE1 cells, which endogenously express *KOC1* and HLA-A24, was tested using as effector cells the CTL clone raised by KOC1-508. PC9 cells were used as the target cells that endogenously express *KOC1* but not HLA-A24. The CTL clone showed high cytotoxic activity against the TE1 cells that express both *KOC1*, and HLA-A24. On the other hand, it did not show significant cytotoxic activity against the PC9 cells that express *KOC1* but not HLA-A24 (Figure 6).

[0081] The above-described CTL clones showed potent cytotoxic activity against the TE1 lung cancer cell line, a cell line that expresses *TTK*, *URLC10 and KOC1,* and HLA-A24. On the other hand, the CTL clones against TTK-567 or KOC1-508 showed no cytotoxic activity against the PC9 lung cancer cell line, a cell line that expresses *TTK* and *KOC1* but not HLA-A24; likewise, the CTL clone raised against URLC10-177 did not show a cytotoxic activity against the TE13 lung cancer cell line, a cell line that expresses URLC10 but not HLA-A24. These CTL clones also show no cytotoxic activity against A24LCL cells, cell that express HLA-A24, but do not express *TTK*, *URLC10* or *KOC1* (Figure 1, 2 and 3). These results clearly demonstrate that TTK-567, URLC10-177 and KOC1-508 were naturally expressed to the tumor cell surface with HLA-A24 molecule and recognized CTL.

Cold target inhibition assay

[0082] Cold target inhibition assay was performed to confirm the specificity of the CTL clones as described in "Materials and Methods" section above. TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while TTK-567 peptide-pulsed A24LCL cells were used as cold target (Inhibitor). The cytotoxic activity of the TTK-567 CTL clone against TE1 cells was specifically inhibited by the addition of A24LCL cells pulsed with the identical peptide (Figure 7). Regarding *URLC10*, TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while URLC10-177 peptide-pulsed A24LCL cells were used as cold target (Inhibitor). The cytotoxic activity of the URLC10-177 CTL clone against TE1 was specifically inhibited by the addition of A24LCL cells pulsed with the identical peptide (Figure 8). As above, TE1 cells labeled by $Na_2{}^{51}Cr\ O_4$ were prepared as hot target, while KOC1-508 peptide-pulsed A24LCL cells were used as cold target (In-

hibitor). The E/T ratio was fixed at 20. The cytotoxic activity of KOC1-508 CTL clone against TE1 cells was specifically inhibited by the addition of A24LCL cells pulsed with the identical peptide (Figure 9). Specific cytotoxicity against target TE1 cells was significantly inhibited when peptide-pulsed cold target was added in the assay at various ratios but not inhibited at all by the addition of cold target These results were indicated as a percentage of specific lysis inhibition at the E/T ratio of 20.

Blocking of CTL activity by antibodies that bind to T-cell surface antigens

[0083] To see whether the observed killing activity is mediated by the cytotoxic T-cells, effects of antibodies on the killing activities were investigated, in which antibodies recognizing T-cell surface antigens related to the function of CTL were used. CTL activities were clearly blocked by the addition of antibodies that recognize HLA Class I and CD8 but are affected little by the addition of antibodies to HLA Class II or CD4, as TTK-567 CTL clone shown in Figure 10, URLC10-177 CTL clone in Figure 11 and KOC1-508 CTL clone in Figure 12. These results show that the cytotoxic activities of CTL clones against the lung carcinoma cells are the HLA class I restricted and CD8 mediated cytotoxic activity.

Homology analysis of the antigen peptides

[0084] The CTL clones established against TTK-567, URLC10-177 or KOC1-508 showed potent cytotoxic activity. Thus, it is possible that the sequence of TTK-567, URLC10-177 or KOC1-508 is homologous to the peptides derived from other molecules, which are known to sensitize human immune system. To exclude this possibility, homology analysis was performed with the peptide sequences as queries using the BLAST algorithm (http//www.ncbi.nlm.nih.gov/blast/blast.cgi) (Altschul SF, et al., (1997) Nucleic Acids Res.;25(17):3389-402.; Altschul SF, et al., (1990) J Mol Biol.;215(3): 403-10.) and revealed no sequence with significant homology. These results indicate that the sequences of TTK-567, URLC10-177 and KOC1-508 are unique and there is little possibility that the peptides would raise umintended immunologic responses to any unrelated molecule.

DISCUSSION

[0085] Identification of new TAAs, particularly those that induce potent and specific anti-tumor immune responses, warrants further development of the clinical application of peptide vaccination strategies in various types of cancer (Boon T. et al., (1996) J Exp Med 183: 725-9.; van der Bruggen P et al., (1991) Science 254: 1643-7.; Brichard V et al., (1993) J Exp Med 178: 489-95.; Kawakami Y et al., (1994) J Exp Med 180: 347-52.; Shichijo S et al., (1998) J Exp Med 187: 277-88.; Chen Y.T. et al., (1997) Proc.Natl.Acd. Sci. USA, 94: 1914-8.; Harris CC., (1996) J Natl Cancer Inst 88:1442-5.; Butterfield LH et al., (1999) Cancer Res 59:3134-42.; Vissers JL et al., (1999) Cancer Res 59: 5554-9.; van der Burg SH et al., (1996) J. Immunol 156:3308-14.; Tanaka F et al., (1997) Cancer Res 57:4465-8.; Fujie T et al., (1999) Int J Cancer 80:169-72.; Kikuchi M et al., (1999) Int J Cancer 81 : 459-66.; Oiso M et al., (1999) Int J Cancer 81:387-94.).

[0086] cDNA microarray technologies can disclose comprehensive profiles of gene expression of malignant cells (Okabe H. et al., (2001) Cancer Res., 61, 2129-37.; Lin Y-M. et al., (2002) Oncogene, 21;4120-8.; Hasegawa S. et al., (2002) Cancer Res 62:7012-7.) and, find utility in the identification of potential TAAs. Among the transcripts that are up-regulated in lung cancers, three novel human genes, termed *TTK*, *URLC10* and *KOC1,* respectively, were identified using these technologies.

[0087] As demonstrated above, *TTK*, *URLC10* and *KOC1* are over-expressed in lung cancer and show minimal expression in normal tissues. In addition, these genes have been shown to have a significant function related to cell proliferation (See WO2004/031413). Thus, peptides derived from *TTK*, *URLC10* and *KOC1* can serve as TAA epitopes, which, in turn, can be used to induce significant and specific immune responses against cancer cells.

[0088] Thus, as TTK, URLC10 and KOC1 are novel TAAs, cancer vaccines using these epitope peptides may be useful as immunotherapeutics against lung carcinoma or other cancer expressing these molecules.

INDUSTRIAL APPLICABILITY

[0089] The present disclosure identifies a new TAA, particularly those which induce potent and specific anti-tumor immune responses. Such TAAs warrants further development of the clinical application of peptide vaccination strategy in lung cancer.

[0090] Furthermore, the present invention relates to the following items:

1. An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 8, 67, and 89.

2. A peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 67, and 89, wherein 1, 2, or several amino acids are substituted, deleted, or added.

3. The peptide of item 2, wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.

4. The peptide of item 2, wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.

5. A pharmaceutical composition for treating or preventing lung cancer, said composition comprising one or more peptides of item 1 or 2.

6. An exosome that presents on its surface a complex comprising a peptide of item 1 or 2 and an HLA antigen.

7. The exosome of item 6, wherein the HLA antigen is HLA-A24.

8. The exosome of item 7, wherein the HLA antigen is HLA-A2402.

9. A method of inducing antigen-presenting cells having a high cytotoxic T cell inducibility comprising the step of contacting an antigen-presenting cell with a peptide of item 1 or 2.

10. A method of inducing cytotoxic T cells by contacting a T cell with a peptide of item 1 or 2.

11. The method of inducing antigen-presenting cells having high cytotoxic T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of item 1 or 2 to an antigen-presenting cell.

12. An isolated cytotoxic T cell is induced by contacting a T cell with a peptide of item 1 or 2.

13. An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of item: 1 or 2.

14. The antigen-presenting cell of item 13, induced by the method of claim 9.

15. A vaccine for inhibiting proliferation of lung cancer cells, wherein the vaccine comprises a peptide of item 1 or 2 as the active ingredient

16. The vaccine of item 15, formulated for administration to a subject whose HLA antigen is HLA-A24.

17. The vaccine of item 15, said vaccine capable of suppressing the growth of lung cancer cells.

18. A method of treating or preventing lung cancer in a subject comprising administering to said subject a vaccine comprising a peptide of item 1 or 2, an immunologically active fragment thereof, or a polynucleotide encoding said peptide or immunologically active fragment.

SEQUENCE LISTING

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Phe Leu Val Lys Thr Gly Tyr Ala Phe Val Asp Cys Pro Asp Glu Ser
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Trp Ala Leu Lys Ala Ile Glu Ala Leu Ser Gly Lys Ile Glu Leu His
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Gly Lys Pro Ile Glu Val Glu His Ser Val Pro Lys Arg Gln Arg Ile
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Arg Lys Leu Gln Ile Arg Asn Ile Pro Pro His Leu Gln Trp Glu Val
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Leu Asp Ser Leu Leu Val Gln Tyr Gly Val Val Glu Ser Cys Glu Gln
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Val Asn Thr Asp Ser Glu Thr Ala Val Val Asn Val Thr Tyr Ser Ser
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Pro Cys Asp Leu Pro Leu Arg Leu Leu Val Pro Thr Gln Phe Val Gly
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Ala Ile Ile Gly Lys Glu Gly Ala Thr Ile Arg Asn Ile Thr Lys Gln
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Thr Gln Ser Lys Ile Asp Val His Arg Lys Glu Asn Ala Gly Ala Ala
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Glu Lys Ser Ile Thr Ile Leu Ser Thr Pro Glu Gly Thr Ser Ala Ala
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Cys Lys Ser Ile Leu Glu Ile Met His Lys Glu Ala Gln Asp Ile Lys
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Phe Thr Glu Glu Ile Pro Leu Lys Ile Leu Ala His Asn Asn Phe Val
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Gly Arg Leu Ile Gly Lys Glu Gly Arg Asn Leu Lys Lys Ile Glu Gln
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Asp Thr Asp Thr Lys Ile Thr Ile Ser Pro Leu Gln Glu Leu Thr Leu
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Tyr Asn Pro Glu Arg Thr Ile Thr Val Lys Gly Asn Val Glu Thr Cys
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Ala Lys Ala Glu Glu Glu Ile Met Lys Lys Ile Arg Glu Ser Tyr Glu
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Asn Asp Ile Ala Ser Met Asn Leu Gln Ala His Leu Ile Pro Gly Leu
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Val Gly Ala Ile Ile Gly Lys Gln Gly Gln His Ile Lys Gln Leu Ser
            420                 425                 430

Arg Phe Ala Gly Ala Ser Ile Lys Ile Ala Pro Ala Glu Ala Pro Asp
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Ala Lys Val Arg Met Val Ile Ile Thr Gly Pro Pro Glu Ala Gln Phe
    450                 455                 460

Lys Ala Gln Gly Arg Ile Tyr Gly Lys Ile Lys Glu Glu Asn Phe Val
465                 470                 475                 480

Ser Pro Lys Glu Glu Val Lys Leu Glu Ala His Ile Arg Val Pro Ser
                485                 490                 495

Phe Ala Ala Gly Arg Val Ile Gly Lys Gly Gly Lys Thr Val Asn Glu
            500                 505                 510

Leu Gln Asn Leu Ser Ser Ala Glu Val Val Val Pro Arg Asp Gln Thr
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Pro Asp Glu Asn Asp Gln Val Val Val Lys Ile Thr Gly His Phe Tyr
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Ala Cys Gln Val Ala Gln Arg Lys Ile Gln Glu Ile Leu Thr Gln Val
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<400> 126

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PATENTKRAV

1. Peptid ifølge (A) eller (B):

(A) et peptid med mindre end 15 aminosyrer, der omfatter aminosyresekvensen SEQ ID NO: 67;

(B) et peptid med mindre end 15 aminosyrer, der har cytotoksisk T-celle-inducerbarhed, hvor peptidet omfatter en aminosyresekvens ifølge SEQ ID NO: 67, i hvilken 1 eller 2 aminosyrer er blevet erstattet, fjernet eller tilføjet.

2. Peptid ifølge krav 1, idet peptidet er (A) eller (B):

(A) et peptid, der består af aminosyresekvensen SEQ ID NO: 67;

(B) et peptid med cytotoksisk T-celle-inducerbarhed, hvor peptidet består af aminosyresekvensen SEQ ID NO: 67, i hvilken 1 eller 2 aminosyrer er erstattet, fjernet eller tilføjet.

3. Peptid ifølge krav 1 eller 2, hvori den anden aminosyre fra N-terminus er phenylalanin, tyrosin, methionin eller tryptophan.

4. Peptid ifølge krav 1 eller 2, hvori den C-terminale aminosyre er phenylalanin, leucin, isoleucin, tryptophan eller methionin.

5. Farmaceutisk sammensætning omfattende ét eller flere peptider ifølge et hvilket som helst af kravene 1 til 4.

6. Farmaceutisk sammensætning til anvendelse ved behandling eller forebyggelse af lungecancer, hvilken sammensætning omfatter ét eller flere peptider ifølge et hvilket som helst af kravene 1 til 4.

7. Exosom, som på sin overflade præsenterer et kompleks, der omfatter et peptid ifølge et hvilket som helst af kravene 1 til 4 og et HLA-antigen.

8. Exosom ifølge krav 7, hvori HLA-antigenet er HLA-A24.

9. Exosom ifølge krav 8, hvori HLA-antigenet er HLA-A2402.

10. Fremgangsmåde in vitro til induktion af antigenpræsenterende celler med høj cytotoksisk T-celle-inducerbarhed omfattende det trin at bringe en antigenpræsenterende celle i kontakt med et peptid ifølge et hvilket som helst af kravene 1 til 4.

11. Fremgangsmåde in vitro til induktion af cytotoksiske T-celler ved at bringe en T-celle i kontakt med et peptid ifølge et hvilket som helst af kravene 1 til 4.

12. Fremgangsmåde til induktion af antigenpræsenterende celler med høj cytotoksisk T-celle-inducerbarhed ifølge krav 10, hvilken fremgangsmåde omfatter det trin at overføre et gen, der omfatter et polynucleotid, som koder for et peptid ifølge et hvilket som helst af kravene 1 til 4, til en antigenpræsenterende celle.

13. Cytotoksisk T-celle induceret ved, at en T-celle er blevet bragt i kontakt med et peptid ifølge et hvilket som helst af kravene 1 til 4, som specifikt virker mod målceller, der præsenterer det peptid, der består af aminosyresekvensen SEQ ID NO: 67.

14. Antigenpræsenterende celle, som omfatter et mellem et HLA-antigen og et peptid ifølge et hvilket som helst af kravene 1 til 4 dannet kompleks.

15. Antigenpræsenterende celle ifølge krav 14 induceret ved fremgangsmåden ifølge krav 10.

16. Vaccine omfattende ét eller flere peptider ifølge et hvilket som helst af kravene 1 til 4 eller et polynucleotid, som koder for dette/disse, som aktivt stof.

17. Vaccine til anvendelse ved inhibering af proliferation af lungecancerceller, idet vaccinen omfatter ét eller flere peptider ifølge et hvilket som helst af kravene 1 til 4 eller et polynucleotid, der koder for dette/disse, som aktivt stof.

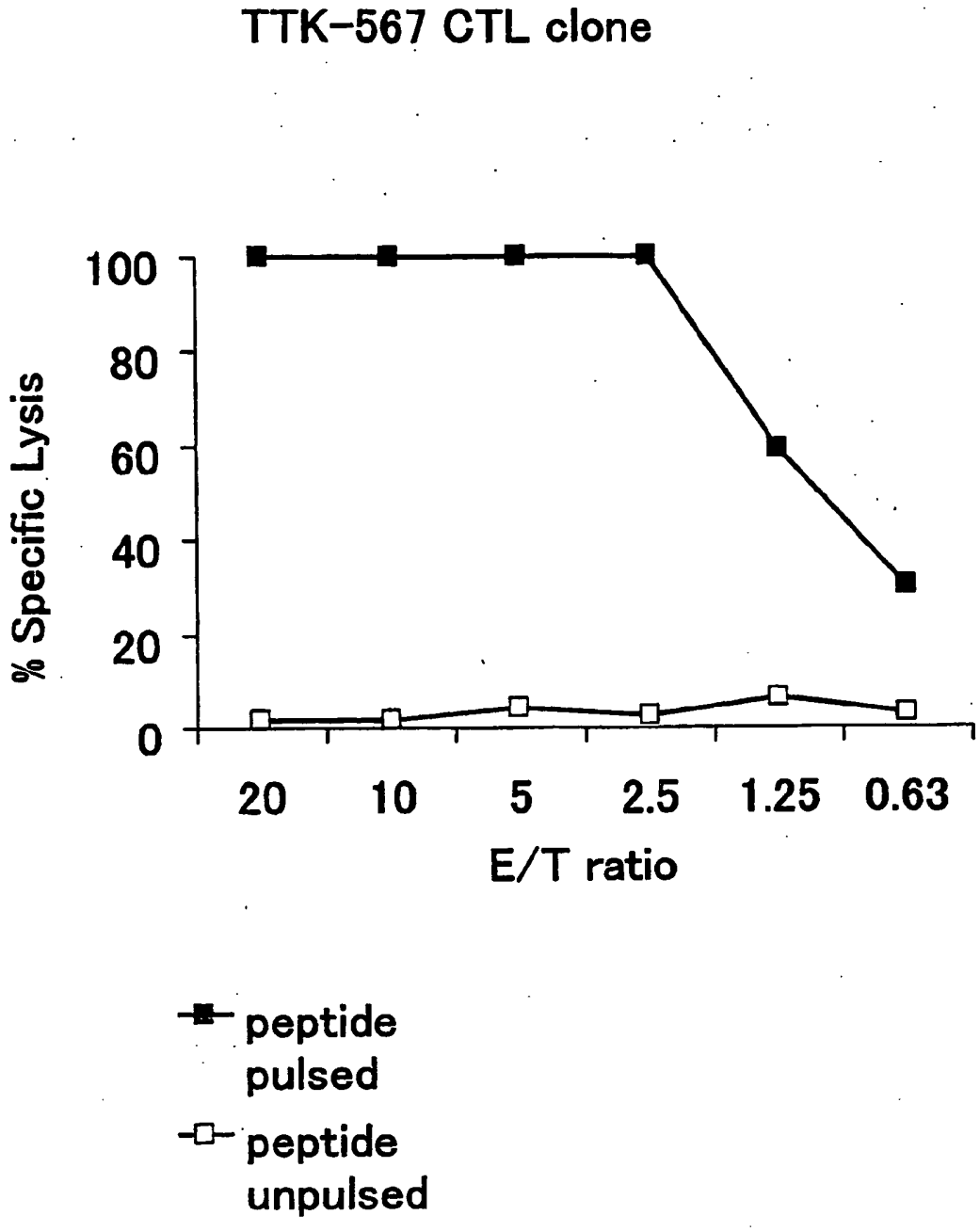
18. Vaccine til anvendelse ved behandling eller forebyggelse af lungecancer, idet vaccinen omfatter ét eller flere peptider ifølge et hvilket som helst af kravene 1 til 4 eller et polynucleotid, der koder for peptidet eller et immunologisk aktivt fragment.

19. Vaccine ifølge et hvilket som helst af kravene 16 til 18 eller farmeceutisk sammensætning ifølge et hvilket som helst af kravene 5 til 6, hvor vaccinen eller den farmaceu-

tiske sammensætning er formuleret til indgivelse til et individ, hvis HLA-antigen er HLA-A24.

20. Vaccine ifølge krav 19 eller farmaceutisk sammensætning ifølge krav 19, idet vaccinen eller den farmaceutiske sammensætning er formuleret til indgivelse til et individ, hvis HLA-antigen er HLA-A2402.

Fig. 1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1.25
0.63
E/T ratio
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peptide unpulsed

Fig. 2

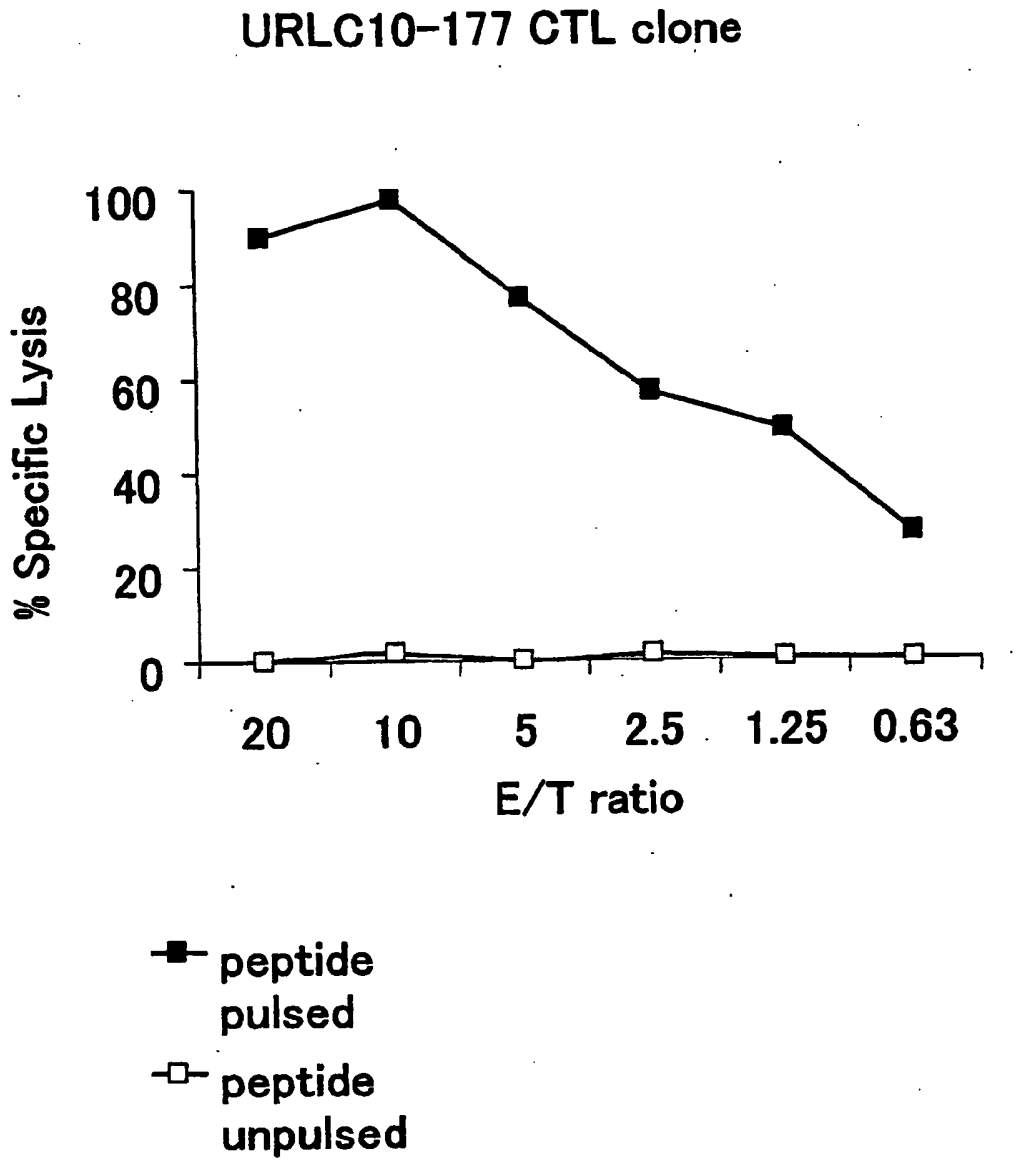
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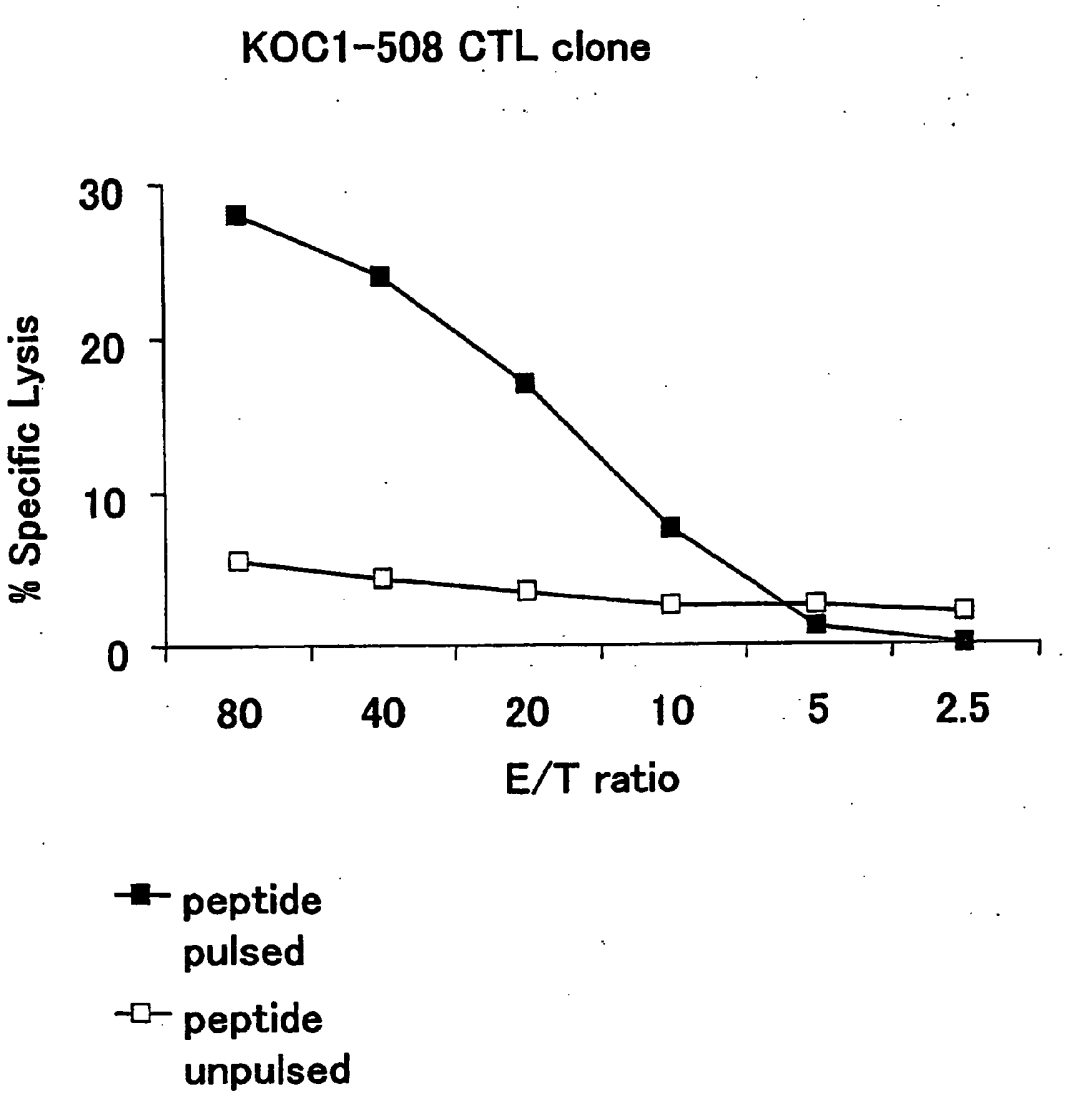
Fig. 3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peptide pulsed
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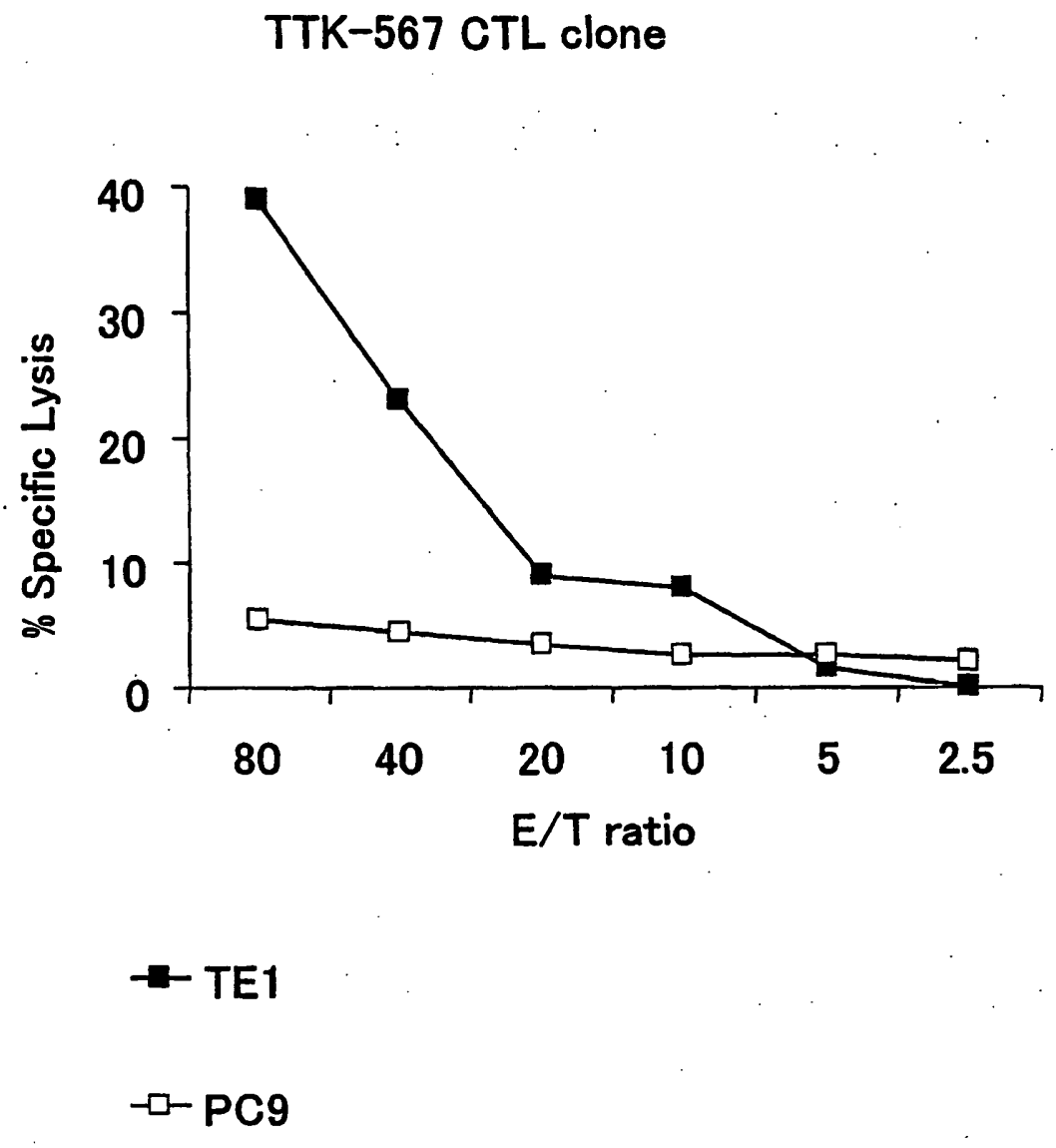
Fig. 4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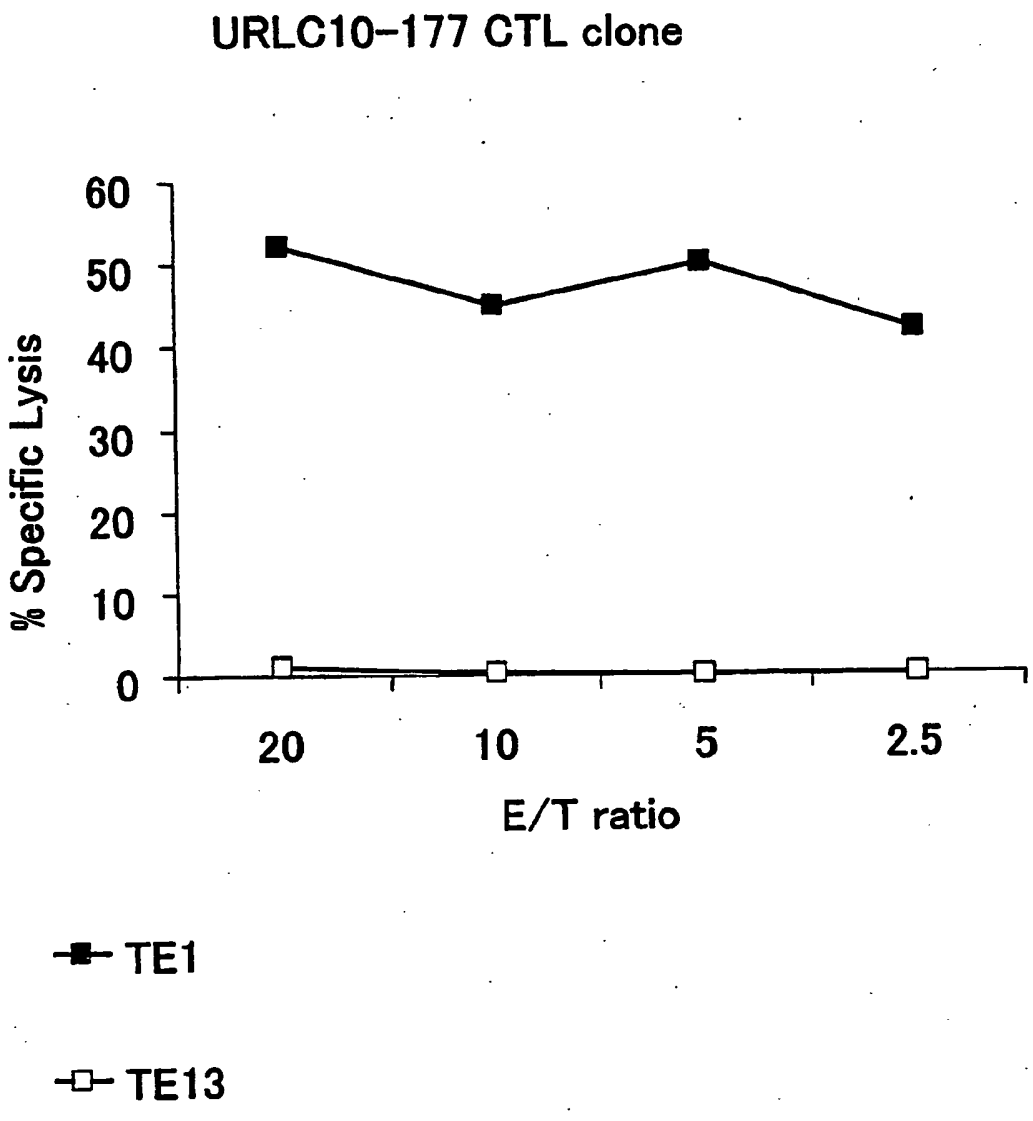
TE1
PC9

Fig. 5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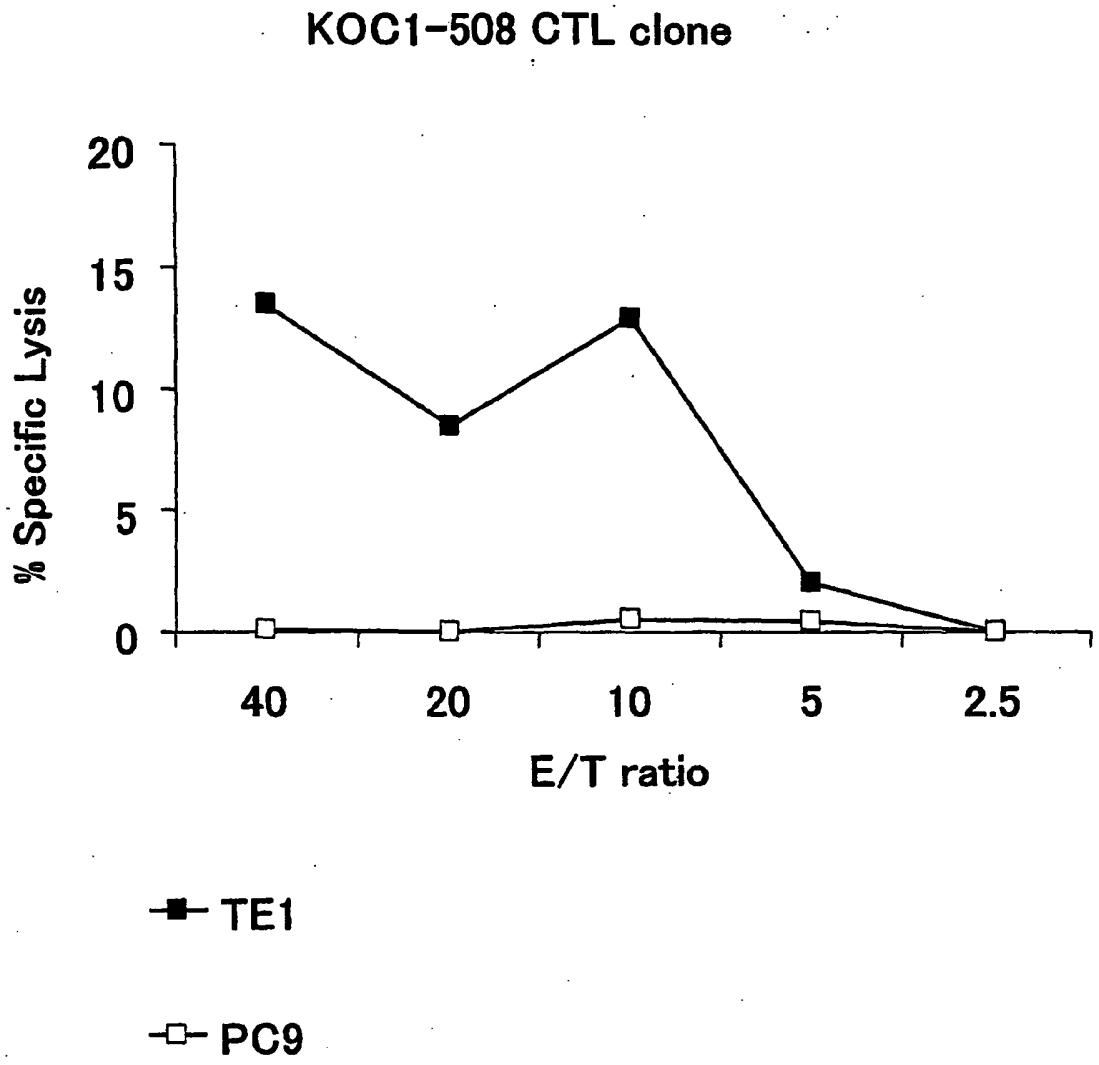
TE1
TE13

Fig. 6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TE1
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Fig. 7

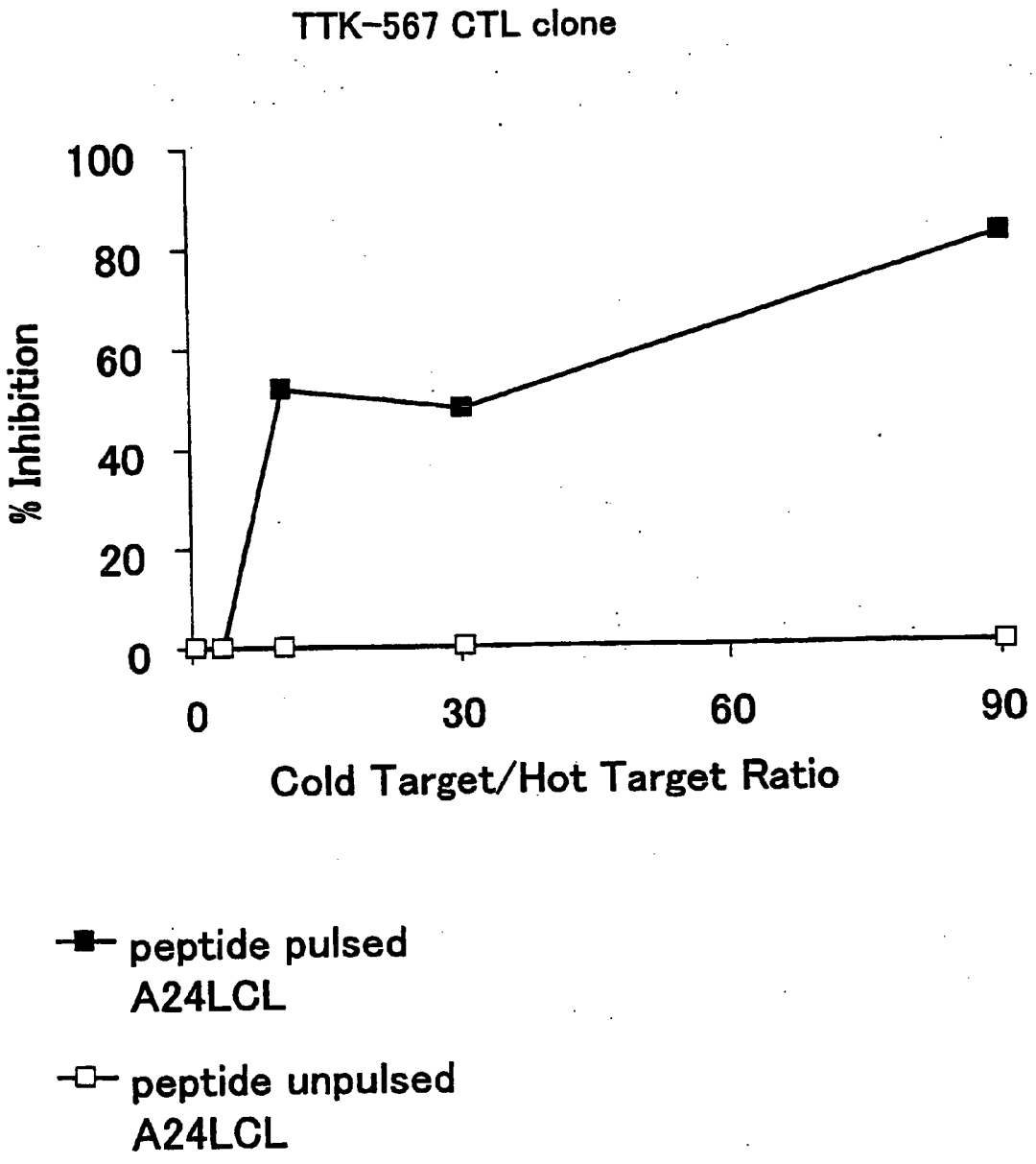
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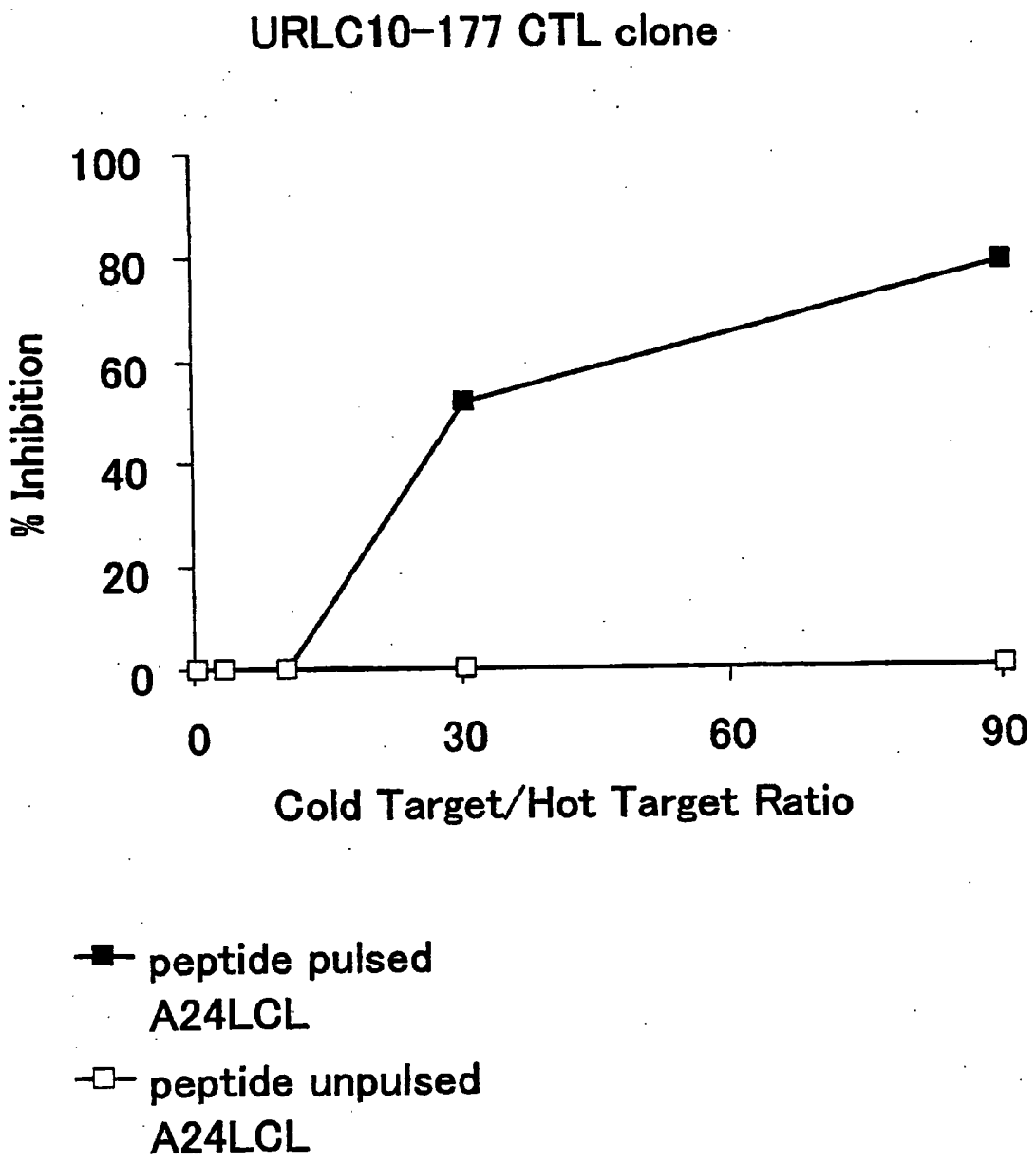
Fig. 8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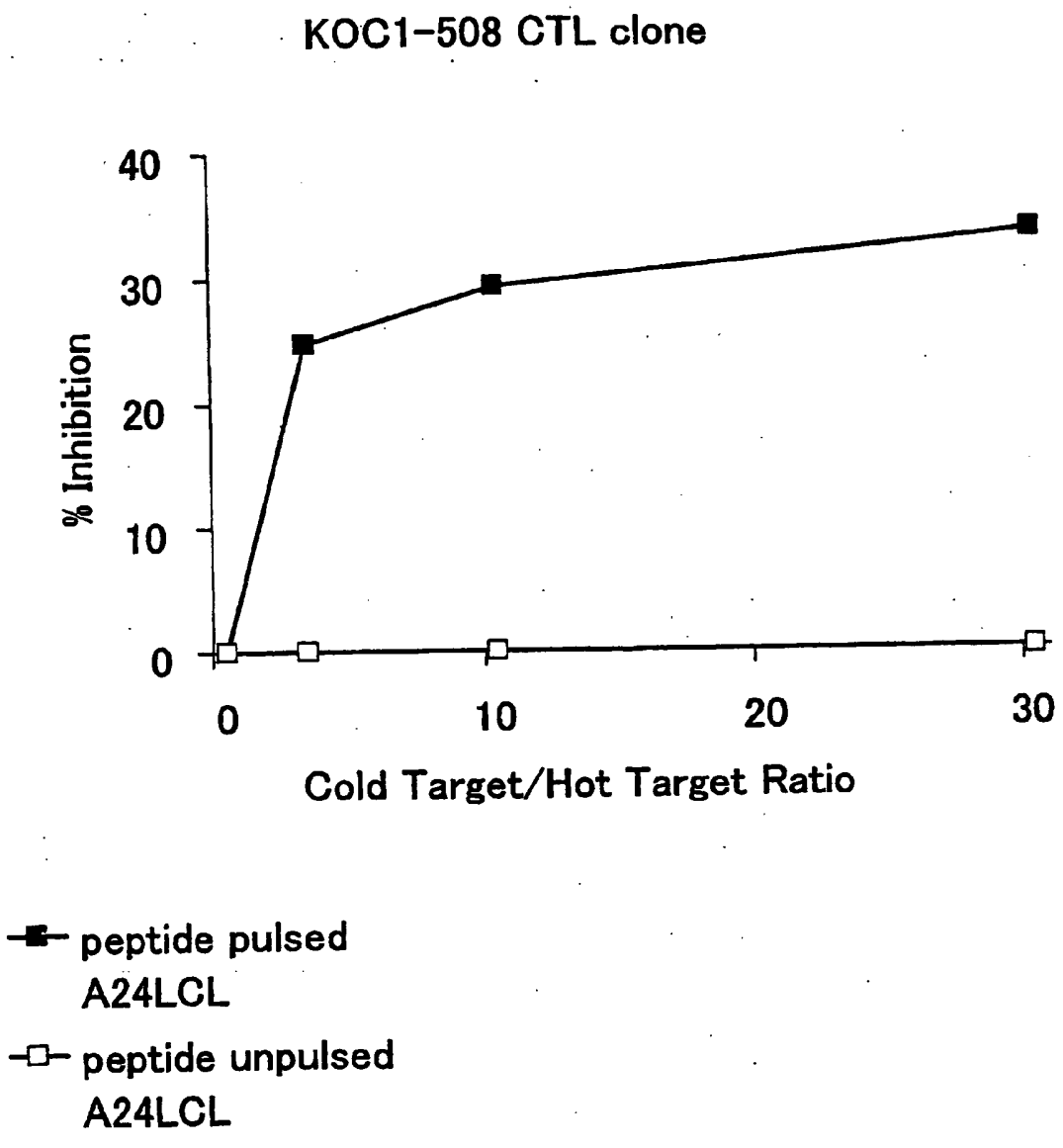
Cold Target/Hot Target Ratio
peptide pulsed A24LCL
peptide unpulsed A24LCL

Fig. 9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Cold Target/Hot Target Ratio
peptide pulsed A24LCL
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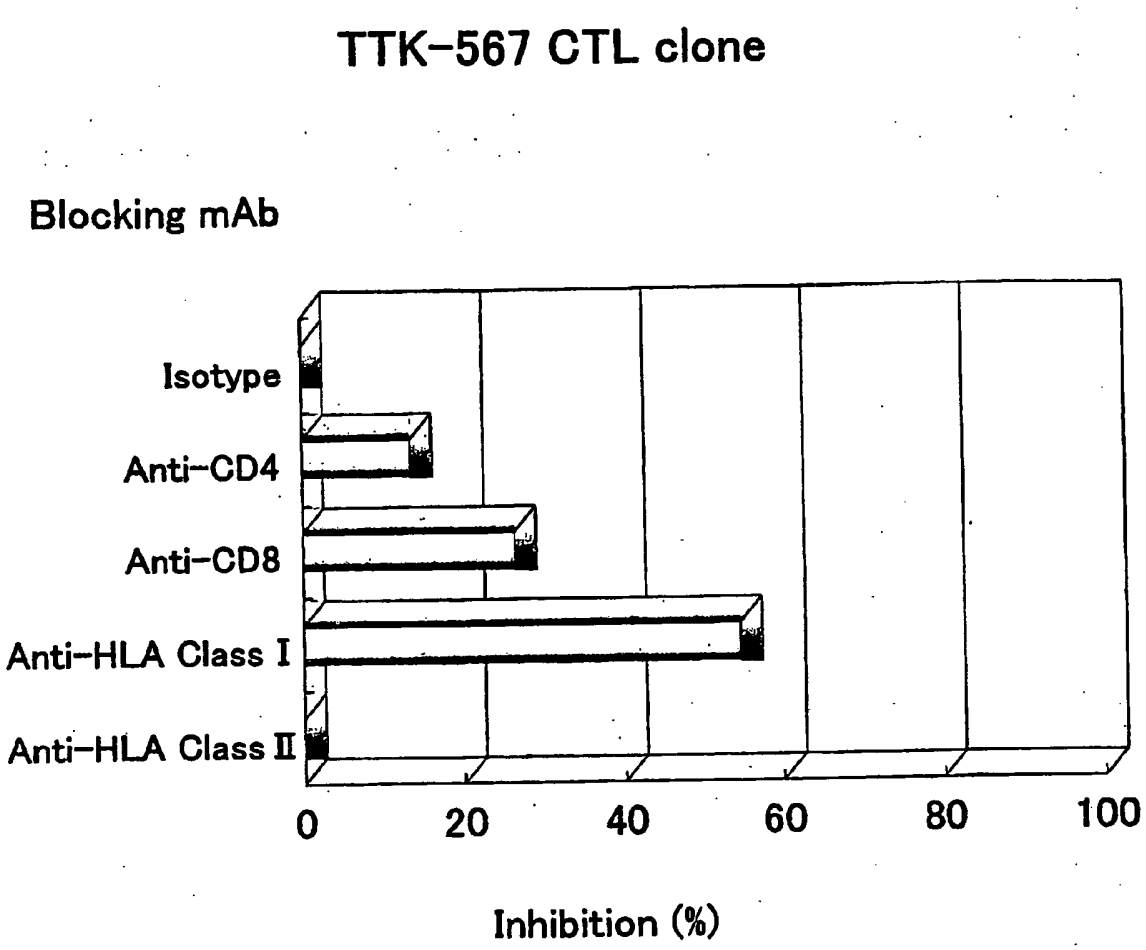
Fig. 10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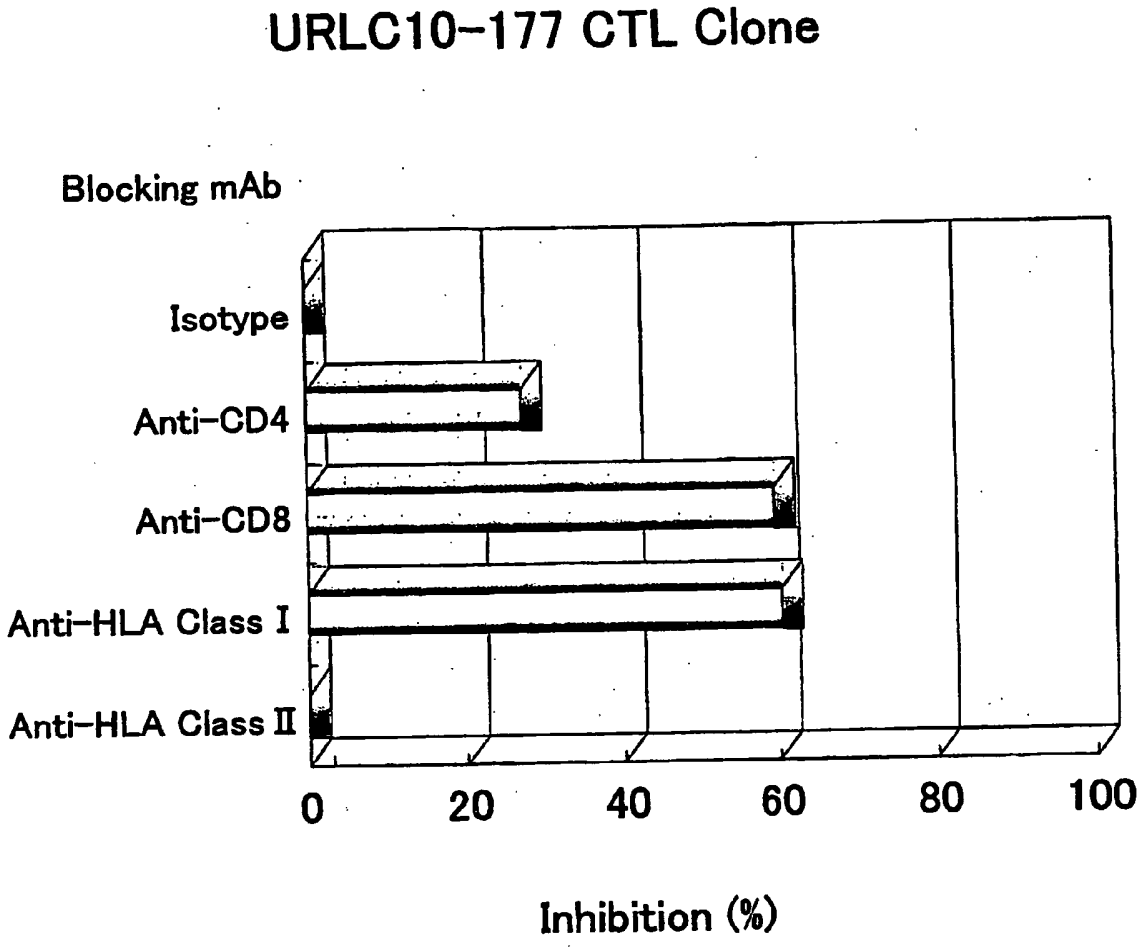
Inhibition (%)

Fig. 11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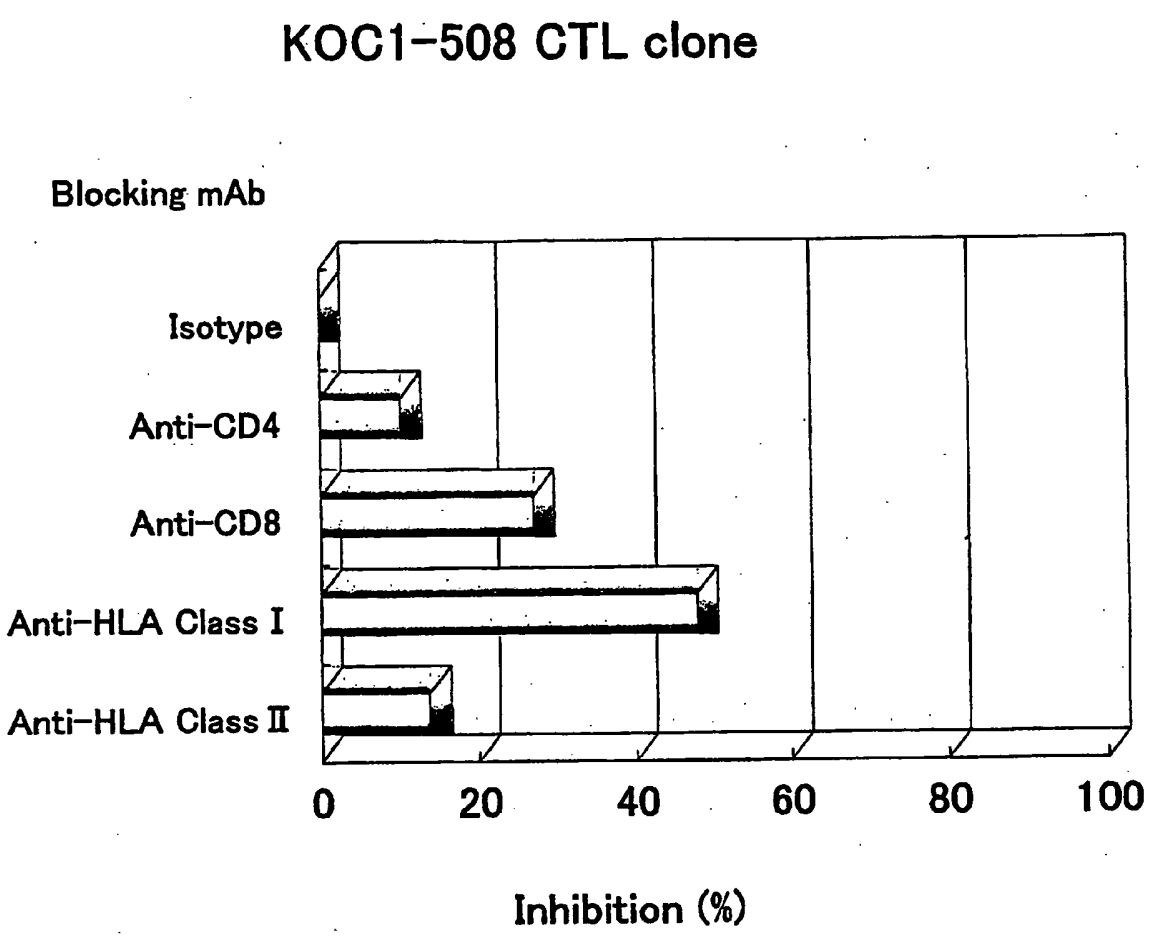
100
Inhibition (%)

Fig. 12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Anti-CD8
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20
40
60
80
100
Inhibition (%)