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(54) **Title:** HLA-A*1101-RESTRICTED WT1 PEPTIDE AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

(54) 発明の名称: ＨＬＡ－Ａ*１１０１拘束性ＷＴ１ペプチド、およびそれを含む医薬組成物

(57) **Abstract:** Disclosed are: an HLA-A*-1101-restricted WT1 peptide, specifically a peptide which comprises an amino acid sequence composed of nine contiguous amino acid residues derived from WT1 protein, is capable of binding to an HLA-A* 1101 molecule and has a CTL-inducing ability; a peptide dimer which comprises two peptide monomers each comprising an amino acid sequence composed of nine contiguous amino acid residues containing at least one cysteine residue and derived from WT1 protein, wherein the two peptide monomers are linked to each other via a disulfide bond and the peptide dimer is capable of binding to an HLA-A* 1101 molecule and has a CTL-inducing ability; a polynucleotide encoding the peptide; a pharmaceutical composition for the treatment and/or prevention of cancer, which comprises the peptide or the peptide dimer; and others.

(57) 要約: 本発明は、ＨＬＡ－Ａ*１１０１拘束性ＷＴ１ペプチド、詳細には、ＷＴ１タンパク質由来の９個の連続するアミノ酸からなるアミノ酸配列を含むペプチドであって、ＨＬＡ－Ａ*１１０１分子と結合能を有し、かつＣＴＬ誘導能を有するペプチド、および少なくとも１個のシステイン残基を含むＷＴ１タンパク質由来の９個の連続するアミノ酸からなるアミノ酸配列を含む２個のペプチド単量体が、ジスルフィド結合により互いに結合しているペプチド二量体であって、ＨＬＡ－Ａ*１１０１分子と結合能を有し、かつＣＴＬ誘導能を有する、ペプチド二量体に関する。さらに本発明は、該ペプチドをコードするポリヌクレオチド、それらを含む癌治療および／または予防用医薬組成物などに関するものである。

WO 2008/081701 A1

DESCRIPTION

HLA-A*1101-RESTRICTED WT1 PEPTIDE AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

Technical Field

[0001]

The present invention relates to an HLA-A*1101-restricted WT1 peptide, specifically, a peptide comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, wherein the peptide has an ability to bind to an HLA-A*1101 molecule, and has an ability to induce a CTL. The present invention also relates to a peptide dimer having an ability to bind to an HLA-A*1101 molecule, and having an ability to induce a CTL, wherein two peptide monomers each comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein and comprising at least one cysteine residue are bound to each other through a disulfide bond. Furthermore, the present invention relates to a polynucleotide encoding the peptide, a pharmaceutical composition for the treatment and/or prevention of a cancer comprising the same and the like.

Background

[0002]

WT1 gene (Wilms' tumor 1 gene) was identified as a gene responsible for Wilms tumor which is a renal cancer in children (Non-patent Documents 1 and 2). WT1 is a transcription factor having a zinc finger structure. At the beginning, the WT1 gene was considered to be a tumor suppressor gene. However, subsequent studies (Non-patent Documents 3, 4, 5 and 6) showed that the WT1 gene rather functions as an oncogene in hematopoietic tumors and solid cancers.

[0003]

The WT1 gene is expressed at high levels in many types of malignant tumors. Then, it has been examined whether or not the WT1 gene product free of mutations, which is an autologous protein, has immunogenicity in a living body. The results revealed that the protein derived from the WT1 gene which is expressed at high levels in tumor cells is fragmented through intracellular processing, the resulting peptides form complexes with MHC class I molecules, and the complexes are presented on the surfaces of cells, and that CTLs recognizing such complexes can be induced by peptide vaccination (Non-patent Documents 7, 8 and 9). It was also shown that in a mouse immunized with a WT1 peptide or a WT1 cDNA, transplanted tumor cells expressing a WT1 gene are rejected with a high probability

(Non-patent Documents 7 and 10), while normal tissues expressing physiologically the WT1 gene are not damaged by the induced CTLs (Non-patent Document 7). It was shown in in vitro experiments using human cells that when Db126 peptide or WH187 peptide (amino acids 187-195 of SEQ ID No: 1, SLGEQQYSV) having a high ability to bind to an HLA-A*0201 molecule, which is one of human MHC class I molecules, is used to stimulate human peripheral blood mononuclear cells having HLA-A*0201, WT1-specific CTLs are induced, the induced CTLs have a cytotoxic activity specific for tumor cells expressing endogenously a WT1 gene at high levels, and the cytotoxic activity of such CTLs is HLA-A2-restricted (Non-patent Document 11). It was shown in in vitro experiments in human cells using WT1 peptide that matches HLA-A*2402 (which is found most frequently in Japanese people among HLA-A alleles) (WT1235; amino acids 235-243 of SEQ ID No: 1, CMTWNQMNL) that WT1-specific CTLs (TAK-1) are induced (Non-patent Document 12), and the induced CTLs do not suppress the colony-forming activity of normal hematopoietic stem cells which partially express physiologically a WT1 gene (Non-patent Documents 12 and 13). These reports strongly suggest that not only in mice but also in human beings, WT1-specific CTLs can be induced, such CTLs have a cytotoxic activity against tumor cells expressing a WT1 gene at high levels, but do not have a

cytotoxic activity against normal cells expressing physiologically a WT1 gene (Non-patent Documents 7, 10, 11, 12 and 13).

[0004]

The WT1 gene product is present as a nuclear protein, and is processed by proteasomes in cytoplasm to be fragmented into peptides. The fragmented peptides are transported into endoplasmic reticulum lumen by TAP (transporter associated with antigen processing) molecules, form complexes with MHC class I molecules, and are presented on the surfaces of cells. WT1-specific CTLs are induced as a result of recognition of WT1 peptide-MHC class I molecule complexes by CTL precursor cells via TCR, thereby exerting a cytotoxic effect on tumor cells presenting a WT1 gene product through MHC class I molecules (Non-patent Documents 7, 8 and 9). Then, it is required at least that a WT1 peptide used in cancer immunotherapy targeting a WT1 gene product is in the form that binds to an MHC class I molecule in a living body. However, MHC class I molecules are diverse and amino acid sequences of the WT1 peptides binding to respective MHC class I molecules are different from each other. Therefore, it is required to provide a peptide matching each subtype of MHC class I. However, only HLA-A*2402 molecule-, HLA-A*0201 molecule-, HLA-A*2601 molecule- and HLA-A*3303 molecule-

restricted peptides are known as HLA molecule-restricted WT1 peptides to date (Patent Document 1, Non-patent Document 11, Patent Document 2 and Patent Document 3, respectively). Thus, there is a need to find an HLA-A*1101-restricted WT1 peptide.

Patent Document 1: WO 2003/106682

Patent Document 2: WO 2005/095598

Patent Document 3: Japanese Patent Application No. 2006-45287

Non-patent Document 1: Daniel A. Haber et al., Cell. 1990 Jun 29; 61(7):1257-69.

Non-patent Document 2: Call KM et al., Cell. 1990 Feb 9; 60(3):509-20.

Non-patent Document 3: Menke AL et al., Int Rev Cytol. 1998; 181:151-212. Review.

Non-patent Document 4: Yamagami T et al., Blood. 1996 Apr 1; 87(7):2878-84.

Non-patent Document 5: Inoue K et al., Blood. 1998 Apr 15; 91(8):2969-76.

Non-patent Document 6: Tsuboi A et al., Leuk Res. 1999 May; 23(5):499-505.

Non-patent Document 7: Oka Y et al., J Immunol. 2000 Feb 15; 164(4):1873-80.

Non-patent Document 8: Melief CJ et al., Immunol Rev. 1995 Jun; 145:167-77.

Non-patent Document 9: Ritz J, J Clin Oncol. 1994 Feb; 12(2):237-8.

Non-patent Document 10: Tsuboi A et al., J Clin Immunol. 2000 May; 20(3):195-202.

Non-patent Document 11: Oka Y et al., Immunogenetics. 2000 Feb; 51(2):99-107.

Non-patent Document 12: Ohminami H et al., Blood. 2000 Jan 1; 95(1):286-93.

Non-patent Document 13: Gao L et al., Blood. 2000 Apr 1; 95(7):2198-203.

Disclosure of Invention

Problems to be Solved by the Invention

[0005]

The problems to be solved by the present invention are to provide a peptide that is an HLA-A*1101 molecule-restricted and comprises an amino acid sequence from a WT1 protein, and a polynucleotide encoding the same, as well as a pharmaceutical composition for the treatment and/or prevention of a cancer, comprising the same, and the like.

Means to Solve the Problems

[0006]

As a result of intensive studies in view of the

situation as described above, the present inventor has found that among peptides each comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, peptides each having an ability to bind to an HLA-A*1101 molecule can induce a WT1-specific CTL with a high rate. Thus, the present invention has been completed.

[0007]

The present invention provides:

(1) a peptide comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, wherein the peptide has an ability to bind to an HLA-A*1101 molecule, and has an ability to induce a CTL;

(2) the peptide according to (1), wherein the amino acid at position 9 of the amino acid sequence is Lys or Arg;

(3) the peptide according to (1), wherein the amino acid sequence is selected from the group consisting of:

Ala Ala Gly Ser Ser Ser Ser Val Lys (SEQ ID No: 2),

Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID No: 3),

Arg Ser Ala Ser Glu Thr Ser Glu Lys (SEQ ID No: 4),

Ser Ala Ser Glu Thr Ser Glu Lys Arg (SEQ ID No: 5),

Ser His Leu Gln Met His Ser Arg Lys (SEQ ID No: 6),

Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID No: 7),

Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID No: 8),

Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID No: 9), and Asn Met His Gln Arg Asn Met Thr Lys (SEQ ID No: 10);

(4) the peptide according to (3), wherein the amino acid sequence is Ala Ala Gly Ser Ser Ser Ser Val Lys (SEQ ID No: 2);

(5) a peptide dimer having an ability to bind to an HLA-A*1101 molecule and having an ability to induce a CTL, in which two peptide monomers each comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, and comprising at least one cysteine residue are bound to each other through a disulfide bond;

(6) the peptide dimer according to (5), wherein the amino acid sequence of the peptide monomer is selected from the group consisting of:
Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID No: 3),
Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID No: 7),
Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID No: 8), and
Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID No: 9);

(7) a pharmaceutical composition for the treatment or prevention of a cancer, comprising the peptide according to (1) and/or the peptide dimer according to (5);

(8) a method for the treatment or prevention of a cancer, comprising administering an effective amount of the peptide according to (1) and/or the peptide dimer according to (5) to an HLA-A*1101-positive subject;

(9) a polynucleotide encoding the peptide according to (1);

(10) an expression vector comprising the polynucleotide according to (9);

(11) a pharmaceutical composition for the treatment or prevention of a cancer, comprising the polynucleotide according to (9) or the vector according to (10);

(12) a method for the treatment or prevention of a cancer, comprising administering an effective amount of the polynucleotide according to (9) or the vector according to (10) to an HLA-A*1101-positive subject;

(13) a WT1-specific CTL, which is induced by the peptide according to (1) and/or the peptide dimer according to (5);

(14) a method for the induction of a WT1-specific CTL, comprising culturing a peripheral blood mononuclear cell in the presence of the peptide according to (1) and/or the peptide dimer according to (5) to induce the WT1-specific CTL from the peripheral blood mononuclear cell;

(15) a kit for the induction of a WT1-specific CTL, comprising the peptide according to (1) and/or the peptide dimer according to (5) as an essential component;

(16) an antigen-presenting cell presenting a WT1 peptide, which is induced by the peptide according to (1)

and/or the peptide dimer according to (5);

(17) a method for the induction of an antigen-presenting cell presenting a WT1 peptide, comprising culturing an immature antigen-presenting cell in the presence of the peptide according to (1) and/or the peptide dimer according to (5) to induce the antigen-presenting cell presenting a WT1 peptide from the immature antigen-presenting cell;

(18) a kit for the induction of an antigen-presenting cell presenting a WT1 peptide, comprising the peptide according to (1) and/or the peptide dimer according to (5) as an essential component; and

(19) a method for the diagnosis of a cancer, comprising using the CTL according to (13) or the antigen-presenting cell according to (16).

Effects of the Invention

[0008]

The present invention provides a peptide that is HLA-A*1101-restricted and comprises an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, and a polynucleotide encoding the same, as well as a pharmaceutical composition for the treatment and/or prevention of a cancer, comprising the same, and the like. Therefore, it is possible to induce in vivo and in vitro

WT1-specific CTLs in subjects having HLA-A*1101. Because the rate of HLA-A*1101-positive in Japanese people is high (about 17.9%), WT1-specific CTLs can be induced in a wide range of subjects.

Brief Description of Drawings

[0009]

Fig. 1 represents the cytotoxic activity of the CTL induced with $WT1_{251}$.

Fig. 2 represents the cytotoxic activity of the CTL induced with $WT1_{279}$.

Fig. 3 represents the cytotoxic activity of the CTL induced with $WT1_{312}$.

Fig. 4 represents the cytotoxic activity of the CTL induced with $WT1_{313}$.

Fig. 5 represents the cytotoxic activity of the CTL induced with $WT1_{338}$.

Fig. 6 represents the cytotoxic activity of the CTL induced with $WT1_{378}$.

Fig. 7 represents the cytotoxic activity of the CTL induced with $WT1_{386}$.

Fig. 8 represents the cytotoxic activity of the CTL induced with $WT1_{415}$.

Fig. 9 represents the cytotoxic activity of the CTL induced with $WT1_{436}$.

Fig. 10 represents the cytotoxic activity of the CTL induced with $WT1_{378}$ peptide (a, b and c represent the cytotoxic activities observed using PBMCs from HLA-A*1101-positive healthy donors 1, 2 and 3, respectively).

Fig. 11 represents the cytotoxic activity of the CTL induced with $WT1_{378}$ peptide dimer (a and b represent the cytotoxic activities observed using PBMCs from HLA-A*1101-positive healthy donors 1 and 2, respectively).

Fig. 12 represents the cytotoxic activity of the CTL induced with modified $WT1_{378}$ peptide (G → I) (a, b and c represent the cytotoxic activities observed using PBMCs from HLA-A*1101-positive healthy donors 1, 2 and 3, respectively).

Fig. 13 represents the cytotoxic activity of the CTL induced with modified $WT1_{378}$ peptide (G → V) (a, b and c represent the cytotoxic activities observed using PBMCs from HLA-A*1101-positive healthy donors 1, 2 and 3, respectively).

Fig. 14 represents the cytotoxic activity of the CTL induced with $WT1_{379}$ peptide (a, b and c represent the cytotoxic activities observed using PBMCs from HLA-A*1101-positive healthy donors 1, 2 and 3, respectively).

Best Mode for Carrying Out the Invention

[0010]

In one aspect, the present invention relates to a peptide comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein, wherein the peptide has an ability to bind to an HLA-A*1101 molecule, and has an ability to induce a CTL (herein also referred to as a "WT1 peptide"). The amino acid sequence of the human WT1 protein is shown in SEQ ID No: 1. The peptide of the present invention comprises an amino acid sequence consisting of 9 contiguous amino acids in the amino acid sequence shown in SEQ ID No: 1. When the peptide of the present invention comprises an amino acid sequence comprising cystein(s) such as the amino acid sequence of SEQ ID No: 3, 7, 8 or 9 as described below, the stability may be increased by substituting the cystein(s) in the amino acid sequence with another substance such as another amino acid (for example, serine, alanine and α-aminobutyric acid) or by modifying the SH group of the cystein(s) with a protecting group known in the art (for example, carboxymethyl group or pyridylethyl group). The peptide of the present invention is a cancer antigen peptide that can induce a CTL as a result of presentation, by an antigen-presenting cell, of a peptide generated by processing the peptide of the present invention in a cell.

[0011]

As described above, it is an object of the

present invention to obtain an HLA-A*1101-restricted peptide. Thus, the peptide of the present invention has an ability to bind to an HLA-A*1101 molecule. The ability to bind can be determined by a method known in the art. Examples of such methods include a computer-based method such as Rankpep, BIMAS or SYFPEITHI, and a competitive binding test with a known peptide having an ability to bind to an HLA-A*1101 molecule. For example, the determined ability to bind can be compared with that of a known HLA-A*1101-restricted peptide to judge whether or not the peptide of the present invention has an ability to bind. Examples of peptides having an ability to bind according to the present invention include a peptide of which the affinity score to an HLA-A*1101 molecule as determined by the method described in example 1 is 4 or more, preferably 5 or more, more preferably 6 or more.

[0012]

The peptide of the present invention further has an ability to induce a CTL. The WT1 gene is expressed in its native form at high levels, for example, in hematopoietic tumors such as leukemia, myelodysplastic syndrome, multiple myeloma or malignant lymphoma and solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germ cell cancer, hepatic cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer,

cervical cancer or ovarian cancer. Therefore, the peptide of the present invention can induce a CTL with a high rate in a subject suffering from such a disease. The ability to induce a CTL refers to an ability to induce a CTL in vivo or in vitro. Such an ability can be determined using a general method such as a method in which a cytotoxic activity of a CTL is determined using a Cr - release assay.

[0013]

The peptide of the present invention may have Lys or Arg at position 9 of the amino acid sequence. It is considered that by having such an amino acid, the ability of the peptide to bind to an HLA-A*1101 molecule becomes higher.

[0014]

The amino acid sequence consisting of 9 amino acids comprised in the peptide of the present invention is preferably, Ala Ala Gly Ser Ser Ser Ser Val Lys (SEQ ID No: 2), Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID No: 3), Arg Ser Ala Ser Glu Thr Ser Glu Lys (SEQ ID No: 4), Ser Ala Ser Glu Thr Ser Glu Lys Arg (SEQ ID No: 5), Ser His Leu Gln Met His Ser Arg Lys (SEQ ID No: 6), Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID No: 7), Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID No: 8), Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID No: 9) or Asn Met His Gln Arg Asn Met Thr Lys (SEQ ID No: 10). Most preferably, it is Thr Gly Val Lys Pro Phe Gln

Cys Lys (SEQ ID No: 7). Furthermore, it may have a substitution of one to several, preferably one to five amino acids with other amino acids in the nine amino acids of any of SEQ ID Nos: 2-10. Any one of the 9 amino acids or other substituted amino acids may be appropriately modified. In any cases, the peptide of the present invention retains an ability to bind to an HLA-A*1101 molecule.

[0015]

As described above, the peptide of the present invention may be any one as long as it comprises an amino acid sequence that is derived from a WT1 protein and consists of 9 contiguous amino acids. Thus, the peptide of the present invention may be, for example, a peptide consisting of only the amino acid sequence shown in any of SEQ ID Nos: 2-10, or a WT1 protein or a part thereof comprising the amino acid sequence shown in any of SEQ ID Nos: 2-10. The number of amino acids comprised in the peptide of the present invention is not specifically limited, and the number is, for example, 9-500, 9-300, 9-200, 9-100, 9-50, 9-30 and 9-12 amino acids. Various substances may be attached at the N-terminus and/or the C-terminus of the amino acid sequence consisting of 9 contiguous amino acids in the peptide of the present invention. For example, an amino acid, a peptide or an

analog thereof may be attached. If such a substance is attached to the peptide of the present invention, the substance can be processed, for example, by an enzyme in a living body or through a process such as intracellular processing, and finally the amino acid sequence consisting of 9 contiguous amino acids can be produced and presented as a complex with an HLA-A*1101 molecule on the surface of a cell, thereby resulting in the effect of inducing a CTL. The substance may be a substance that modulates the solubility of the peptide of the present invention, or increases its stability (resistance to protease, etc.). Alternatively, it may be a substance that delivers the peptide of the present invention specifically, for example, to a given tissue or organ, or it may have an action to increase the efficiency of uptake by an antigen-presenting cell or the like. The substance may be a substance that increases an ability to induce a CTL, such as a helper peptide or the like.

[0016]

The peptide of the present invention can be synthesized by methods generally used in the art or modifications thereof. Such methods are described, for example, in Peptide Synthesis, Interscience, New York, 1966 ; The Proteins, Vol 2, Academic Press Inc., New York, 1976 ; Peptide-Gosei, Maruzen Co., Ltd., 1975; Peptide-

Gosei No Kiso To Jikken, Maruzen Co., Ltd., 1985; and Iyakuhin No Kaihatsu (Zoku), Vol. 14, Peptide-Gosei, Hirokawa - Book store, 1991.

[0017]

The peptide of the present invention can also be prepared using genetic engineering techniques based on the information about the nucleotide sequence that encodes the peptide of the present invention. Such genetic engineering techniques are well known to a skilled person in the art.

[0018]

In a further aspect, the present invention relates to a peptide dimer having an ability to bind to an HLA-A*1101 molecule and having an ability to induce a CTL, in which two peptide monomers each comprising an amino acid sequence consisting of 9 contiguous amino acids from a WT1 protein and comprising at least one cystein residue are bound to each other though a disulfide bond (hereinafter also referred to as a "WT1 peptide dimer"). The stability of the peptide dimer of the present invention is increased as compared with that of the peptide monomer by forming a dimer. The peptide dimer of the present invention is a tumor antigen peptide dimer that can induce a CTL as a result of presentation, by an antigen-presenting cell, of a peptide generated by processing the peptide of the present invention in a cell.

[0019]

The peptide dimer of the present invention is formed by binding two peptide monomers through a disulfide bond between cystein residues present in the monomers. Thus, each of the peptide monomers comprised in the WT1 peptide dimer of the present invention is the WT1 peptide as described above and comprises at least one cystein residue. The WT1 peptide dimer of the present invention may be a homodimer or a heterodimer.

[0020]

In the WT1 peptide dimer of the present invention, the amino acid sequence comprised by the peptide monomer comprises is preferably, Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID No: 3), Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID No: 7), Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID No: 8) or Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID No: 9). Most preferably, it is Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID No: 7).

[0021]

The WT1 peptide dimer of the preset invention can be prepared using a method known in the art. For example, if the peptide monomers comprise one pair of cystein residues, the WT1 peptide dimer of the present invention can be prepared, for example, by removing all the protecting groups including the ones on the cystein side

chains, and then subjecting the resulting monomer solution to air-oxidation under alkaline conditions, or adding an oxidant under alkaline or acidic conditions to form a disulfide bond. Examples of the oxidants include iodine, dimethylsulfoxide (DMSO) and potassium ferricyanide.

[0022]

When each of the peptide monomers comprises two or more cystein residues, the WT1 peptide dimer of the present invention can also be prepared by the method as described above. In this case, isomers are obtained due to different types of disulfide bonds. Alternatively, the WT1 peptide dimer of the present invention can be prepared by selecting a combination of protecting groups for cystein side chains. Examples of the combinations of the protecting groups include combinations of MeBzl (methylbenzyl) group and Acm (acetamidemethyl) group, Trt (trityl) group and Acm group, Npys (3-nitro-2-pyridylthio) group and Acm group, and S-Bu-t (S-tert-butyl) group and Acm group. For example, in the case of the combination of MeBzl group and Acm group, the WT1 peptide dimer can be prepared by removing protecting groups other than the MeBzl group and the protecting group on the cystein side chain, subjecting the resulting monomer solution to air-oxidation to form a disulfide bond between the protected cystein residues, and then deprotecting and oxdizing using iodine

to form a disulfide bond between the cystein residues previously protected by Acm.

[0023]

In another aspect, the present invention relates to a pharmaceutical composition for the treatment or prevention of a cancer comprising the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer. The WT1 gene is expressed at high levels in various cancers and tumors including hematopoietic tumors such as leukemia, myelodysplastic syndrome, multiple myeloma or malignant lymphoma and solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germ cell cancer, hepatic cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical cancer or ovarian cancer. Therefore, the pharmaceutical composition of the present invention can be used for the treatment or prevention of a cancer. When the pharmaceutical composition of the present invention is administered to an HLA-A*1101-positive subject, WT1-specific CTLs are induced by the HLA-A*1101-restricted WT1 peptide or the WT1 peptide dimer comprised in the pharmaceutical composition, and cancer cells in the subject are damaged by such CTLs.

[0024]

The pharmaceutical composition of the present invention may comprise in addition to the HLA-A*1101-

restricted WT1 peptide and/or the WT1 peptide dimer as an active ingredient, for example, a carrier, an excipient or the like. The HLA-A*1101-restricted WT1 peptide or the WT1 peptide dimer comprised in the pharmaceutical composition of the present invention induces a WT1-specific CTL. Thus, the pharmaceutical composition of the present invention may comprise an appropriate adjuvant, or may be administered together with an appropriate adjuvant in order to enhance the induction efficiency. Examples of preferable adjuvants include, but are not limited to, complete or incomplete Freund's adjuvant and aluminum hydroxide.

[0025]

The method of the administration of the pharmaceutical composition of the present invention can be appropriately selected depending on conditions such as the type of disease, the condition of the subject or the target site. Examples of such methods include, but are not limited to, intradermal administration, subcutaneous administration, intramuscular administration, intravenous administration, nasal administration and oral administration. The amount of the peptide or the peptide dimer comprised in the pharmaceutical composition of the present invention, as well as the dosage form, the number of times of the administration and the like of the pharmaceutical composition of the present invention can be

appropriately selected depending on conditions such as the type of disease, the condition of the subject or the target site. The single dose of the peptide is usually, 0.0001 mg - 1000 mg, preferably, 0.001 mg - 1000 mg.

[0026]

In another aspect, the present invention relates to a method for the treatment or prevention of a cancer, comprising administering an effective amount of the WT1 peptide and/or the WT1 peptide dimer to an HLA-A*1101-positive subject. The cancer to be treated or prevented may be any one, and examples thereof include hematopoietic tumors such as leukemia, myelodysplastic syndrome, multiple myeloma or malignant lymphoma and solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germ cell cancer, hepatic cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical cancer or ovarian cancer.

[0027]

In a further aspect, the present invention relates to a method for the determination of the presence or amount of a WT1-specific CTL in an HLA-A*1101-positive subject, comprising:

(a) reacting a complex of a WT1 peptide and an HLA-A*1101 molecule with a sample from the subject; and

(b) determining the presence or amount of a CTL recognizing

the complex contained in the sample. The sample from a subject may be any one as long as there is a possibility that it contains a lymphocyte. Examples of the samples include body fluid such as blood or lymph and a tissue. The complex of a WT1 peptide and an HLA-A*1101 molecule may be prepared, for example, as a tetramer or pentamer using a method known to a skilled person in the art such as biotin-streptavidin method. The presence or amount of the CTL recognizing such a complex can be measured by a method known to a skilled person in the art. In this aspect of the present invention, the complex may be labeled. A known label such as a fluorescent label or a radioactive label can be used as a label. Labeling makes the determination of the presence or amount of a CTL easy and rapid.

[0028]

Thus, the present invention also provides a composition for the determination of the presence or amount of a WT1-specific CTL in an HLA-A*1101-positive subject comprising an HLA-A*1101-restricted WT1 peptide.

[0029]

Furthermore, the present invention provides a kit for the determination of the presence or amount of a WT1-specific CTL in an HLA-A*1101-positive subject, comprising an HLA-A*1101-restricted WT1 peptide.

[0030]

In a further aspect, the present invention relates to a method for the production of a WT1-specific CTL using a complex of a WT1 peptide and an HLA-A*1101 molecule, comprising:

(a) reacting the complex with a sample; and

(b) obtaining a CTL recognizing the complex contained in the sample. The complex of a WT1 peptide and an HLA-A*1101 molecule is described above. The sample may be any one as long as there is a possibility that it contains a lymphocyte. Examples of the samples include a sample from a subject such as blood, and a cell culture. The CTL recognizing the complex can be obtained using a method known to a skilled person in the art such as FACS or MACS. The present invention allows to culture the obtained WT1-specific CTL and use it for the treatment or prevention of various cancers.

[0031]

Thus, the present invention also relates to a WT1-specific CTL obtainable by a method for the production of a WT1-specific CTL using a complex of a WT1 peptide and an HLA-A*1101 molecule.

[0032]

In another aspect, the present invention relates to a polynucleotide encoding the HLA-A*1101-restricted WT1 peptide. The polynucleotide of the present invention may

be DNA or RNA. The base sequence of the polynucleotide of the present invention can be determined based on the amino acid sequence of the HLA-A*1101-restricted WT1 peptide. The polynucleotide can be prepared by a known method for the synthesis of DNA or RNA (for example, chemical synthetic method), PCR method or the like.

[0033]

In another aspect, the present invention relates to an expression vector comprising the polynucleotide. The type of the expression vector, the comprised sequence other than the sequence of the polynucleotide and the like can be appropriately selected depending on the type of a host into which the expression vector of the present invention is introduced, the purpose of use, or the like. It is possible to treat or prevent hematopoietic tumors or solid cancers by administering the expression vector of the present invention to an HLA-A*1101-positive subject to produce an HLA-A*1101-restricted WT1 peptide in a living body and induce a WT1-specific CTL, and damaging hematopoietic tumor cells or solid cancer cells in the subject.

[0034]

In a further aspect, the present invention relates to a pharmaceutical composition for the treatment or prevention of a cancer, comprising the polynucleotide or

the expression vector. The composition, method of the administration and the like of the pharmaceutical composition of the present invention in this aspect are described above.

[0035]

In another aspect, the present invention relates to a method for the treatment or prevention of a cancer, comprising administering an effective amount of the polynucleotide or the expression vector to an HLA-A*1101-positive subject. Examples of cancers to be treated or prevented include hematopoietic tumors such as leukemia, myelodysplastic syndrome, multiple myeloma or malignant lymphoma and solid cancers such as gastric cancer, colon cancer, lung cancer, breast cancer, germ cell cancer, hepatic cancer, skin cancer, bladder cancer, prostate cancer, uterine cancer, cervical cancer or ovarian cancer.

[0036]

In another aspect, the present invention relates to a cell comprising the expression vector. The cell of the present invention can be prepared, for example, by transforming a host cell such as E. coli, yeast, insect cell or animal cell with the expression vector. The method for the introduction of the expression vector into a host cell can be appropriately selected from various methods. By culturing the transformed cell, and recovering and

purifying the produced WT1 peptide, the peptide of the present invention can be prepared.

[0037]

In a further aspect, the present invention relates to a WT1-specific CTL, which is induced by the HLA-A*1101-restriceted WT1 peptide and/or the WT1 peptide dimer. The CTL of the present invention recognizes a complex of a WT1 peptide and an HLA-A*1101 molecule. Thus, the CTL of the present invention can be used to damage specifically a tumor cell positive for HLA-A*1101 and expressing WT1 at a high level.

[0038]

In another aspect, the present invention relates to a method for the treatment or prevention of a cancer, comprising administering a WT1-specific CTL to an HLA-A*1101-positive subject. The method of the administration of the WT1-specific CTL can be appropriately selected depending on conditions such as the type of the disease, the condition of the subject or the target site. Examples of such methods include, but are not limited to, intravenous administration, intradermal administration, subcutaneous administration, intramuscular administration, nasal administration and oral administration.

[0039]

In another aspect, the present invention relates

to a method for the induction of a WT1-specific CTL, comprising culturing a peripheral blood mononuclear cell in the presence of the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer to induce the WT1-specific CTL form the peripheral blood mononuclear cell. The subject from which the peripheral blood mononuclear cell is derived may be any one as long as it is positive for HLA-A*1101. By culturing the peripheral blood mononuclear cells in the presence of the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer, WT1-specific CTLs are induced from CTL precursor cells contained in the peripheral blood mononuclear cells. It is possible to treat or prevent hematopoietic tumors or solid cancers in an HLA-A*1101-positive subject by administering the WT1-specific CTL obtained according to the present invention to the subject.

[0040]

In another aspect, the present invention relates to a kit for the induction of a WT1-specific CTL, comprising an HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer as an essential component. Preferably, the kit is used in the method for the induction of a WT1-specific CTL. The kit of the present invention may comprise in addition to the HLA-A*1101-restriceted WT1 peptide and/or the WT1 peptide dimer, for example, a means of obtaining a peripheral blood mononuclear cell, an

adjuvant, a reaction vessel or the like. In general, an instruction manual is attached to the kit. By using the kit of the present invention, WT1-specific CTLs can be induced efficiently.

[0041]

In a further aspect, the present invention relates to an antigen-presenting cell (such as a dendritic cell) presenting a WT1 peptide through an HLA-A*1101 molecule, which is induced by the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer. By using the antigen-presenting cell of the present invention, WT1-specific CTLs are induced efficiently.

[0042]

In another aspect, the present invention relates to a method for the treatment or prevention of a cancer, comprising administering the antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule to an HLA-A*1101-positive subject. The method of the administration of the antigen-presenting cell can be appropriately selected depending on conditions such as the type of the disease, the condition of the subject or the target site. Examples of such methods include, but are not limited to, intravenous administration, intradermal administration, subcutaneous administration, intramuscular administration, nasal administration and oral

administration.

[0043]

In another aspect, the present invention relates to a method for the induction of an antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule, comprising culturing an immature antigen-presenting cell in the presence of the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer to induce the antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule from the immature antigen-presenting cell. The immature antigen-presenting cell refers a cell such as an immature dendritic cell that can be matured into an antigen-presenting cell. A subject from which the immature antigen-presenting cell is derived may be any one as long as it is positive for HLA-A*1101. Because the immature antigen-presenting cells are contained, for example, in peripheral blood mononuclear cells, such cells may be cultured in the presence of the WT1 peptide and/or the WT1 peptide diemr.

[0044]

In another aspect, the present invention relates to a kit for the induction of an antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule, comprising the HLA-A*1101-restricted WT1 peptide and/or the WT1 peptide dimer as an essential component. Preferably,

the kit is used in the method for the induction of an antigen-presenting cell. Another component to be comprised in the kit of the present invention and the like are described above. The kit of the present invention can be used to induce efficiently an antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule.

[0045]

In another aspect, the present invention relates to an antibody against an HLA-A*1101-restricted WT1 peptide or a polynucleotide encoding the peptide. The antibody of the present invention may be a polyclonal antibody or monoclonal antibody.

[0046]

In a further aspect, the present invention relates to a method for the diagnosis of a cancer, comprising using the WT1-specific CTL, the antigen-presenting cell presenting a WT1 peptide through an HLA-A*1101 molecule, or the antibody against an HLA-A-restricted WT1 peptide or a polynucleotide encoding the peptide. Preferably, the WT1-specific CTL is used in the method for the diagnosis of the present invention. For example, it is possible to diagnose a cancer by incubating the CTL, the antigen-presenting cell or the antibody with a sample from an HLA-A*1101-positive subject, or administering it to an HLA-A*1101-positive subject, and

determining, for example, the position, site or amount thereof. The CTL, the antigen-presenting cell or the antibody may be labeled. By attaching a label, the method for the diagnosis of the present invention can be practiced efficiently.

Examples

[0047]

The following examples illustrate the present invention in more detail, but are not to be construed to limit the scope thereof.

[0048]

Example 1: Selection of WT1 peptide

RANKPEP (http://bio.dfci.harvard.edu/Tools/rankpep.html) was used to select $WT1_{251}$, $WT1_{279}$, $WT1_{312}$, $WT1_{313}$, $WT1_{338}$, $WT1_{378}$, $WT1_{386}$, $WT1_{415}$ and $WT1_{436}$ having a high ability to bind to an HLA-A*1101 molecule derived from peptides from a WT1 protein (SEQ ID No: 1). Amino acid sequences, amino acid numbers in SEQ ID No: 1 and affinity scores to an HLA-A*1101 molecule of these peptides are shown in Table 1.

[0049]

[Table 1]

Peptide	Amino acid Number	Amino acid sequence	Affinity score
$WT1_{251}$	251-259	AAGSSSSVK	15.18

(SEQ ID No: 2)			
$WT1_{279}$	279-287	PILCGAQYR	11.47
(SEQ ID No: 3)			
$WT1_{312}$	312-320	RSASETSEK	14.96
(SEQ ID No: 4)			
$WT1_{313}$	313-321	SASETSEKR	6.87
(SEQ ID No: 5)			
$WT1_{338}$	338-346	SHLQMHSRK	13.72
(SEQ ID No: 6)			
$WT1_{378}$	378-386	TGVKPFQCK	11.33
(SEQ ID No: 7)			
$WT1_{386}$	386-394	KTCQRKFSR	13.82
(SEQ ID No: 8)			
$WT1_{415}$	415-423	SCRWPSCQK	10.29
(SEQ ID No: 9)			
$WT1_{436}$	436-444	NMHQRNMTK	14.19
(SEQ ID No: 10)			

[0050]

Preparation of B-LCL cell

Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll-Hypaque gradient density centrifugation method from peripheral blood that had been collected from an HLA-A*1101-positive healthy donor. The PBMCs were then seeded to a 24-well cell culture plate at the density of about 1×10^7 in RPMI 1640 medium containing 10% FCS, and a culture supernatant of B95-8 cells (cells producing EB virus) were added. They were cultured at 37°C with 5% CO_2 for about 1 month. B-LCL cells transformed with EB virus, which are B-cell tumor cells, were obtained. It was confirmed that the resulting B-LCL cells did not express WT1 gene. B-LCL cells were pulsed by incubating them with 20 μg/ml of $WT1_{251}$, $WT1_{279}$, $WT1_{312}$, $WT1_{313}$, $WT1_{338}$, $WT1_{378}$, $WT1_{386}$, $WT1_{415}$ or $WT1_{436}$ for 2 hours, and irradiated with 80 Gy of

radiation. The resulting B-LCL cells (hereinafter referred to as B-LCL cells pulsed with a WT1 peptide) were used as antigen-presenting cells for the following experiments.

[0051]

Induction of WT1-specific CTL

3×10^6 of autologous PBMCs were cultured in a 24-well cell culture plate in complete medium (45% RPMI, 45% AMI-V medium and 10% human AB serum) containing 20 μg/ml of $WT1_{251}$, $WT1_{279}$, $WT1_{312}$, $WT1_{313}$, $WT1_{338}$, $WT1_{378}$, $WT1_{386}$, $WT1_{415}$ or $WT1_{436}$ at 37°C with 5% CO_2 for 1 week to obtain responding cells. 2×10^6 of the resulting responding cells were cocultured with 1×10^6 of the B-LCL cells pulsed with the same WT1 peptide in complete medium for 1 week (first stimulation). The PBMCs were coculutured with the B-LCL cells pulsed with the WT1 peptide three more times (second to fourth stimulations) under the conditions under which 20 IU/ml (final concentration) of IL2 was added as follows: second stimulation: two times every other day from 3 days after the initiation of stimulation; third and fourth stimulations: three times at intervals of one day from the day after the initiation of stimulation. The resulting cells were concentrated using Negative Selection Columns Gravity Feed Kit (StemSp) so that the ratio of CD8-positive T cells became about 80%, and cocultured with the B-LCL cells pulsed with the WT1 peptide (fifth stimulation).

CD8-positive T cells (CTLs) obtained 5 days after the final stimulation were used for measurement of the cytotoxic activity.

[0052]

Cytotoxic activity of CTL

The cytotoxic activity of CTLs was measured using ^{51}Cr release assay. CTL cells (hereinafter referred to as effector cells) were mixed at the ratio (E/T ration) of 1:1 to 30:1 in 200 µl of medium with target cells into which ^{51}Cr had been incorporated, and cultured in a 96-well cell culture plate at 37°C with 5% CO_2 for 4 hours. B-LCL cells pulsed with the same WT1 peptide as that used for induction of CTLs (BLCL-Ps), and B-LCL cells without pulsing with a WT1 peptide (BLCL-NPs) were used as target cells. After the culture, the supernatants were collected by centrifugation. The amounts of ^{51}Cr released into the supernatants were measured using a liquid scintillation counter. The cytotoxic activity (%) was determined using the following formula:

(^{51}Cr release in supernatant of sample - Spontaneous ^{51}Cr release) / (Maximum ^{51}Cr release - Spontaneous ^{51}Cr release) x 100

wherein Spontaneous ^{51}Cr release is ^{51}Cr release observed when the target cells into which ^{51}Cr had been incorporated were cultured alone under the same condition, and Maximum

^{51}Cr release is ^{51}Cr release observed when the target cells into which ^{51}Cr had been incorporated were completely lysed using 1% Triton X-100. Results are shown in Figs. 1-9. In the figures, longitudinal axes represent specific lysis (%), and horizontal axes represent E/T ratios. BLCL-Ps are represented using full lines, and BLCL-NPs are represented using dotted lines. It was confirmed that CTLs induced with $WT1_{251}$, $WT1_{279}$, $WT1_{312}$, $WT1_{313}$, $WT1_{338}$, $WT1_{378}$, $WT1_{386}$, $WT1_{415}$ or $WT1_{436}$ damage specifically BLCL-Ps presenting the WT1 peptide as a complex with an HLA-A*1101 molecule as compared with BLCL-NPs. CTLs induced with $WT1_{251}$, $WT1_{279}$, $WT1_{313}$, $WT1_{338}$ or $WT1_{386}$ were used for additional experiments below.

[0053]

Cytotoxic activity of CTL against cell expressing WT1 endogenously

The cytotoxic activity of CTLs induced with $WT1_{251}$, $WT1_{279}$, $WT1_{313}$, $WT1_{338}$ or $WT1_{386}$ against B-LCLs expressing WT1 was determined using the method described above. A cell expressing WT1 refers to a B-LCL into which a human WT1 gene is introduced, and that expresses a WT1 protein in the cell, and presents a peptide consisting of about 9 amino acids resulting from processing on an HLA-A*1101 molecule. Results are shown in Figs. 1, 2, 4, 5 and 7. In the figures, B-LCLs expressing WT1 are represented using dashed

lines. It was confirmed that CTLs induced with $WT1_{251}$, $WT1_{279}$, $WT1_{313}$, $WT1_{338}$ or $WT1_{386}$ have a cytotoxic activity against cells expressing WT1 gene endogenously.

[0054]

Preparation of WT1 peptide dimer

A mixture of 227.5 mg of $WT1_{378}$ peptide monomer, 227.5 mg of N-methylglucamine (NMG) and 23 ml of water was air-oxidized by stirring at room temperature for about 2 days. To the resulting mixture, an aqueous solution of 2g of sodium acetate in 5 ml of water was added and the mixture was stirred at room temperature for about 20 minutes. To the resulting solution, 200 ml of water and about 200 ml of acetonitrile were added, and the mixture was filtered through Kiriyama funnel (filter paper No. 5C) and washed with water (about 50 ml x 3). To the residue, about 200 ml of water was added and the residue was lyophilized to obtain 158 mg of crude $WT1_{378}$ peptide dimer.

[0055]

Purification of crude WT1 peptide dimer

158 mg of the crude $WT1_{378}$ peptide dimer was dissolved in 9 ml of DMSO and injected into ODS C_{18} column (5 cm Φ x 50 cm L, YMC Co., LTD.) attached to HPLC (Shimadzu, LC8AD type) and equilibrated with solution 1 (H_2O/1% AcOH) using a HPLC pump. The column was left for about 30 minutes, and eluted with concentration gradient of

0% to 40% of solution 2 (CH_3CN/1% AcOH) over 360 minutes. The fractions containing WT1 peptide dimer were collected using an automatic fraction collector while monitoring UV absorbance at 220 nm. The collected fractions were combined, injected into ODS C_{18} column (4.6 mm Φ x 25 cm L, YMC Co., LTD.) attached to HPLC (Hitachi, L-4000 type) and equilibrated with 17% of solution 2, and eluted with concentration gradient of 0% to 47% of solution 2 over 30 minutes to obtain 46.6 mg of the purified $WT1_{378}$ peptide dimer at retention time of 20.51 minutes.

FAB.MS 2365.0 (theoretical value: 2342.70) Na^+ F = 0.25%

[0056]

Induction of CTL by WT1 peptide dimer

Abilities of the resulting $WT1_{378}$ peptide dimer, $WT1_{378}$ peptide, modified $WT1_{378}$ peptide(G → I) (SEQ ID No: 11) and modified $WT1_{378}$ peptide (G → V) (SEQ ID No: 12) as well as $WT1_{379}$ peptide (SEQ ID No: 13, as disclosed in WO 2002/28414) to induce a CTL were examined using PBMCs from HLA-A*1101-positive healthy donors 1-3 according to the method as described above. Results are shown in Figs. 10-14. In the figs, longitudinal axes represent specific lysis (%), and horizontal axes represent E/T ratios. BLCL-Ps are represented using full lines, and BLCL-NPs are represented using dotted lines. It was confirmed that $WT1_{378}$ peptide dimer has an ability to induce a CTL.

Furthermore, it was found that the ability of each $WT1_{379}$ peptide of which the amino acid sequence is different from that of $WT1_{378}$ peptide by one amino acid in the amino acid sequence of WT1 protein to induce a CTL is much lower than that of $WT1_{378}$ peptide and, thus, the WT1 peptide of the present invention has an excellent and unexpected effect as compared with the known peptide.

Industrial Applicability

[0057]

The present invention provides an HLA-A*1101-restricted WT1 peptide, a polynucleotide encoding the peptide, a pharmaceutical composition comprising the same and the like. Therefore, the present invention can be used in the fields of medicine and the like, for example, in the fields of development and preparation of a pharmaceutical composition for the prevention or treatment of various hematopoietic tumors and solid cancers that express WT1 gene at high levels.

Sequence listing free text

[0058]

SEQ ID NO: 11: Modified WT1 peptide

SEQ ID NO: 12: Modified WT1 peptide

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Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or method step or group of elements or integers or method steps but not the exclusion of any element or integer or method step or group of elements or integers or method steps.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

CLAIMS:

1. An isolated peptide consisting of an amino acid sequence selected from:

(1) Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID NO: 7); and

(2) an amino acid sequence of SEQ ID NO: 7, wherein one to five amino acids are substituted with other amino acid(s),

wherein the peptide has an ability to bind to an HLA-A*1101 molecule, and has an ability to induce a CTL.

2. An isolated peptide dimer comprising a first and a second peptide monomers bound through a disulfide bond, wherein:

the first peptide monomer comprises an amino acid sequence selected from:

(1) Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID NO: 7); and

(2) an amino acid sequence of SEQ ID NO: 7 wherein one to five amino acids are substituted with other amino acid(s); and

the second peptide monomer comprises an amino acid sequence selected from:

(1) Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID NO: 3);

(2) Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID NO: 7);

(3) Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID NO: 8);

(4) Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID NO: 9); and

(5) an amino acid sequence selected from SEQ ID NO: 3, 7, 8 and 9, wherein one to five amino acids are substituted with other amino acid(s),

and wherein the peptide dimer has an ability to bind to an HLA-A*1101 molecule, and has an ability to induce a CTL.

3. A pharmaceutical composition for the treatment or prevention of a cancer, comprising the peptide according to Claim 1 and/or the peptide dimer according to Claim 2.

4. A method for the treatment or prevention of a cancer, comprising administering an effective amount of the peptide according to Claim 1 and/or the peptide dimer according to Claim 2 to an HLA-A*1101-positive subject.

5. A polynucleotide encoding the peptide according to Claim 1.

6. An expression vector comprising the polynucleotide according to Claim 5.

7. A pharmaceutical composition for the treatment or prevention of a cancer, comprising the polynucleotide according to Claim 5 or the vector according to Claim 6.

8. A method for the treatment or prevention of a cancer, comprising administering an effective amount of the polynucleotide according to Claim 5 or the vector according to Claim 6 to an HLA-A*1101-positive subject.

9. A WT1-specific CTL, which is induced by the peptide according to Claim 1 and/or the peptide dimer according to Claim 2.

10. A method for the induction of a WT1-specific CTL, comprising culturing a peripheral blood mononuclear cell in the presence of the peptide according to Claim 1 and/or the peptide dimer according to Claim 2 to induce the WT1-specific CTL from the peripheral blood mononuclear cell.

11. A kit for the induction of a WT1-specific CTL, comprising the peptide according to claim 1 and/or the peptide dimer according to Claim 2 as an essential component.

12. An antigen-presenting cell presenting a WT1 peptide, which is induced by the peptide according to claim 1 and/or the peptide dimer according to Claim 2.

13. A method for the induction of an antigen-presenting cell presenting a WT1 peptide, comprising culturing an immature antigen-presenting cell in the presence of the peptide according to claim 1 and/or the peptide dimer according to Claim 2 to induce the antigen-presenting cell presenting a WT1 peptide from the immature antigen-presenting cell.

14. A kit for the induction of an antigen-presenting cell presenting a WT1 peptide, comprising the peptide according to Claim 1 and/or the peptide dimer according to Claim 2 as an essential component.

15. A method for the diagnosis of a cancer, comprising using the CTL according to Claim 9 or the antigen-presenting cell according to Claim 12.

16. An isolated peptide according to Claim 1 or 2 or a pharmaceutical composition according to Claim 3 or 7 or a method according to any one of Claims 4, 8, 10, 13 or 15 or a polynucleotide of Claim 5 or an expression vector of Claim 6 or a WTF-specific CTL of Claim 9 or a kit according to Claim 11 or 14 or an antigen-presenting cell of Claim 12 substantially as herein described with reference to the Figures and/or Examples.

Fig. 1

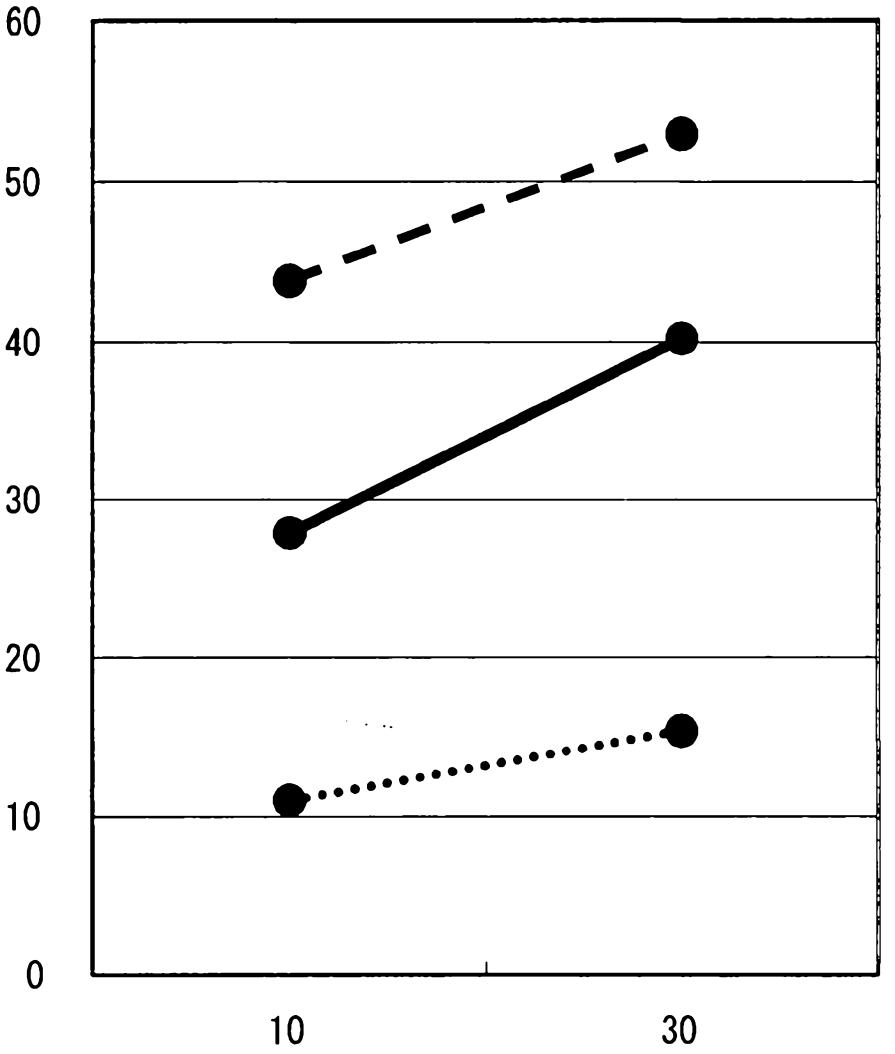


Fig. 2

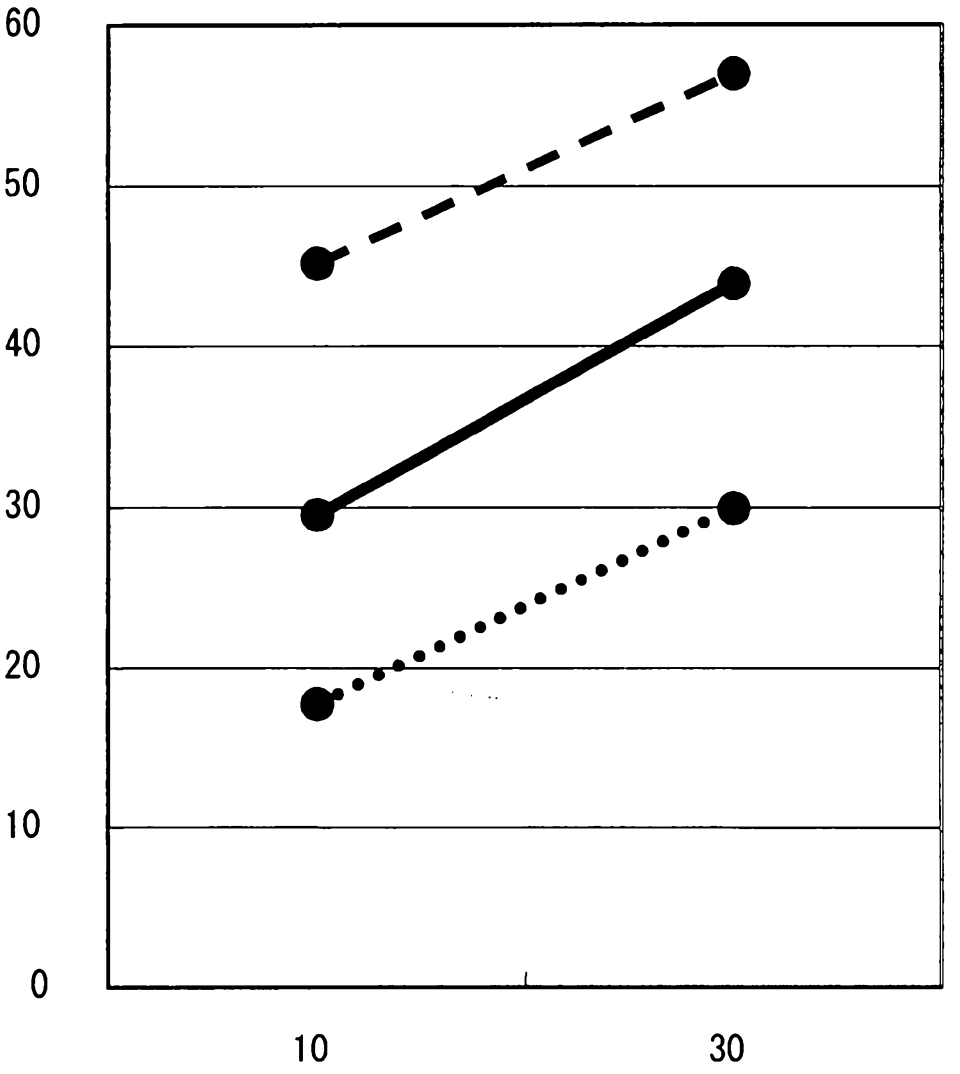


Fig. 3

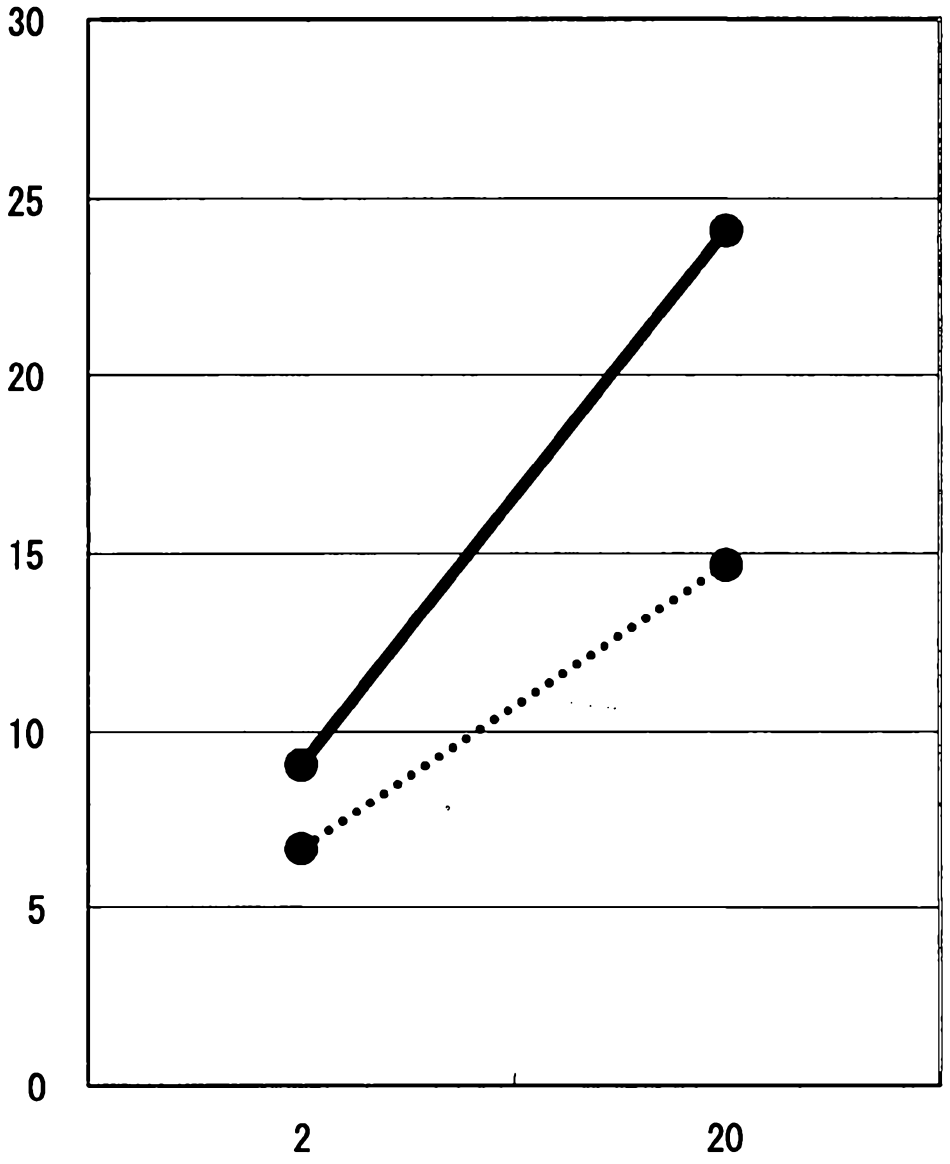


Fig. 4

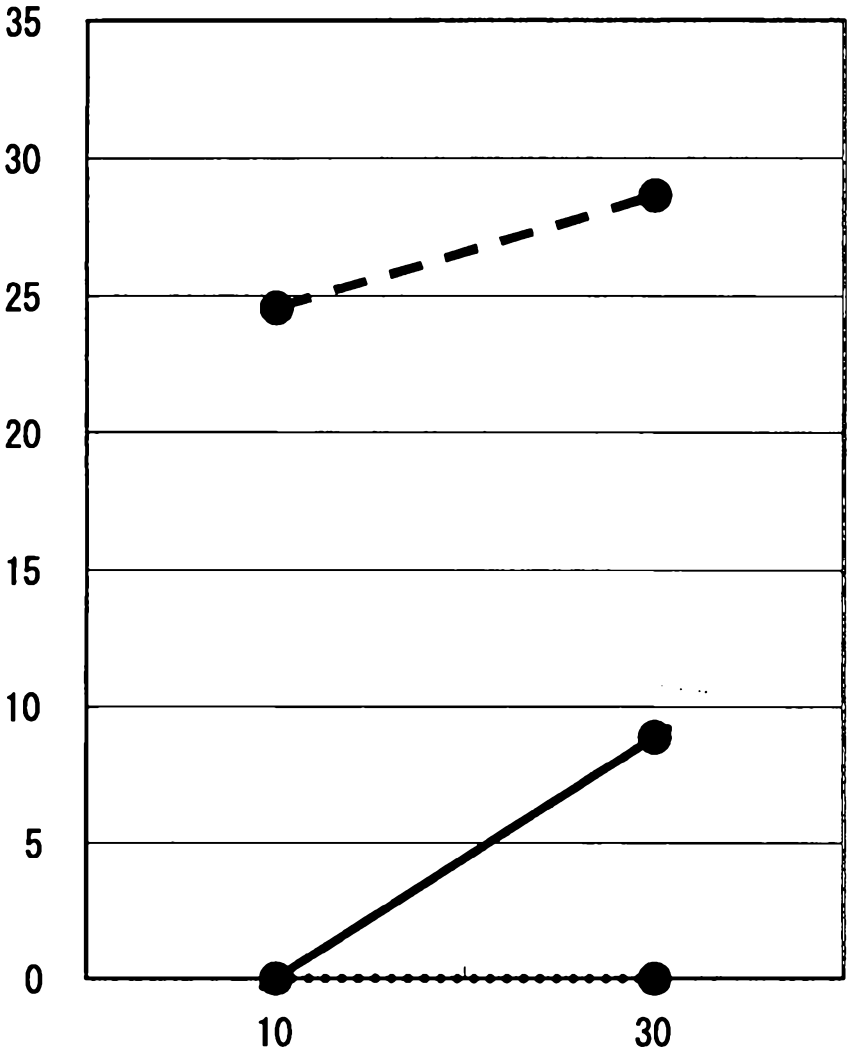
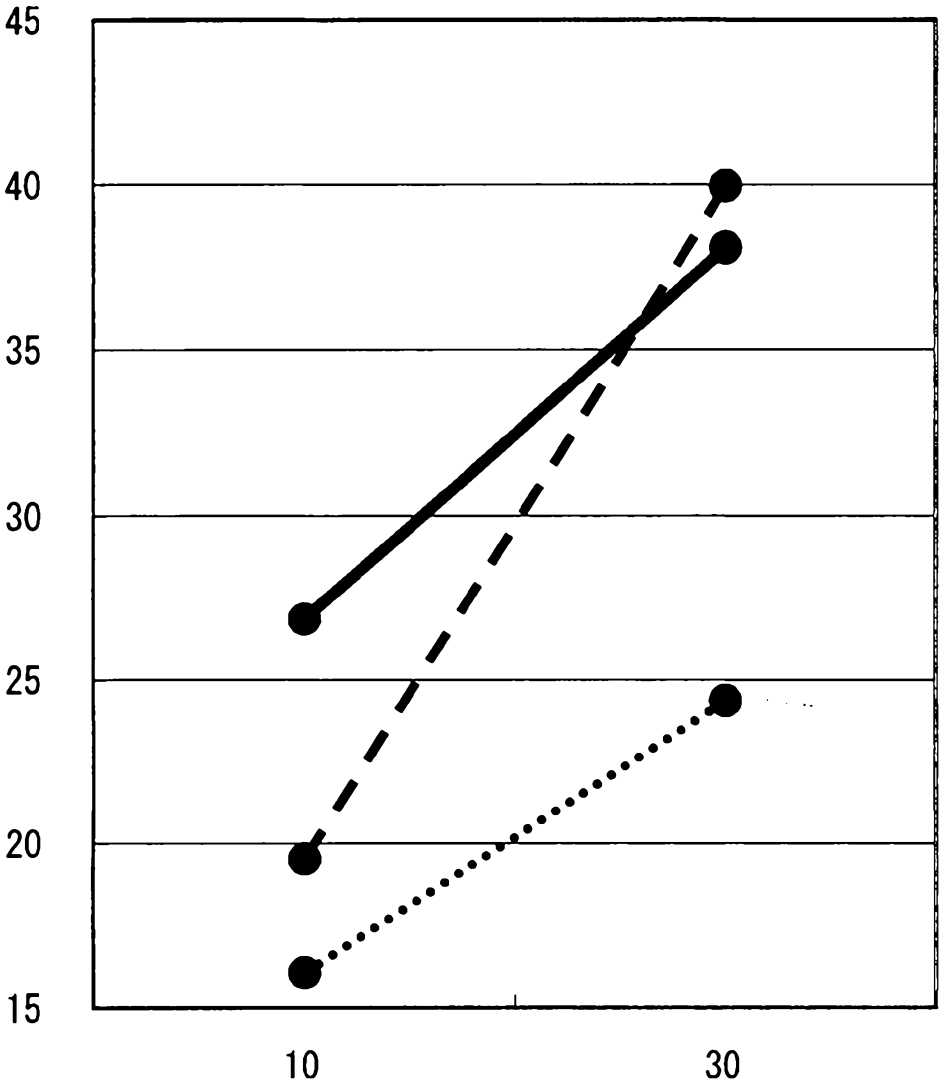


Fig. 5



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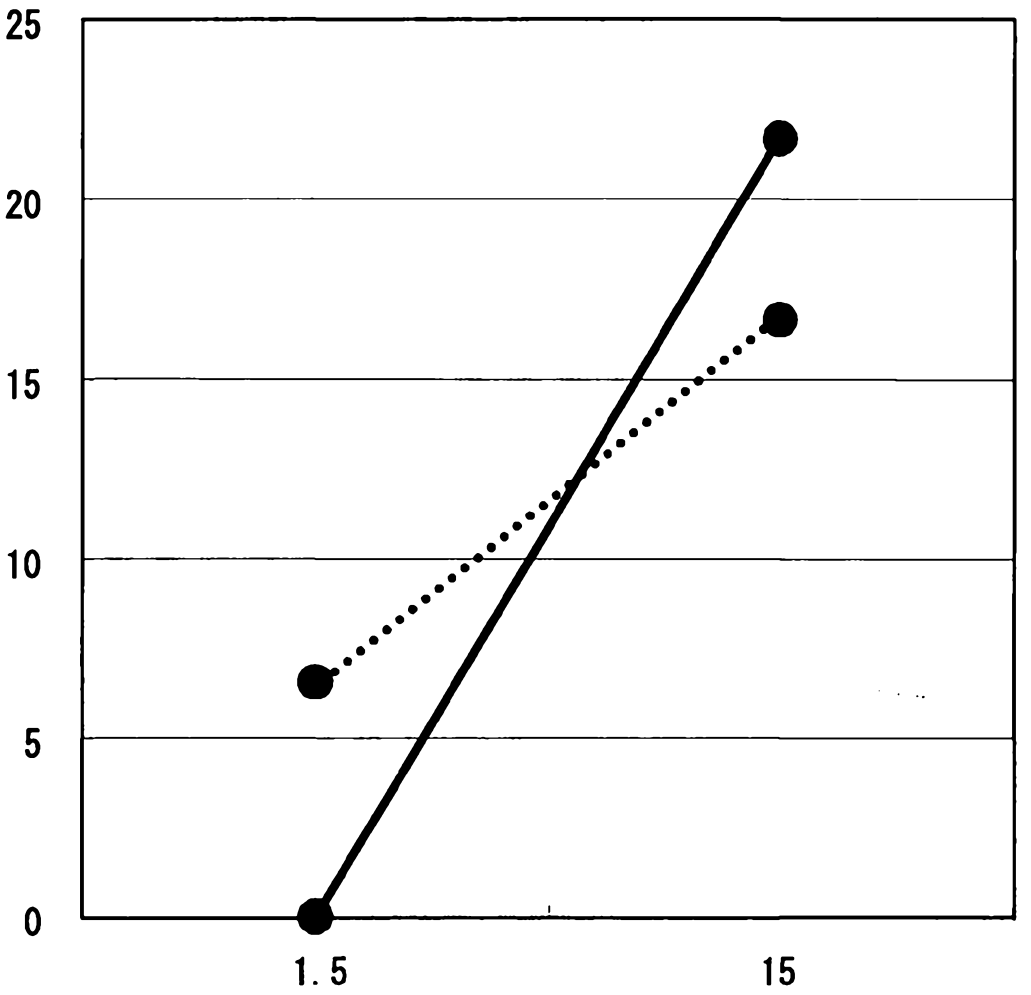

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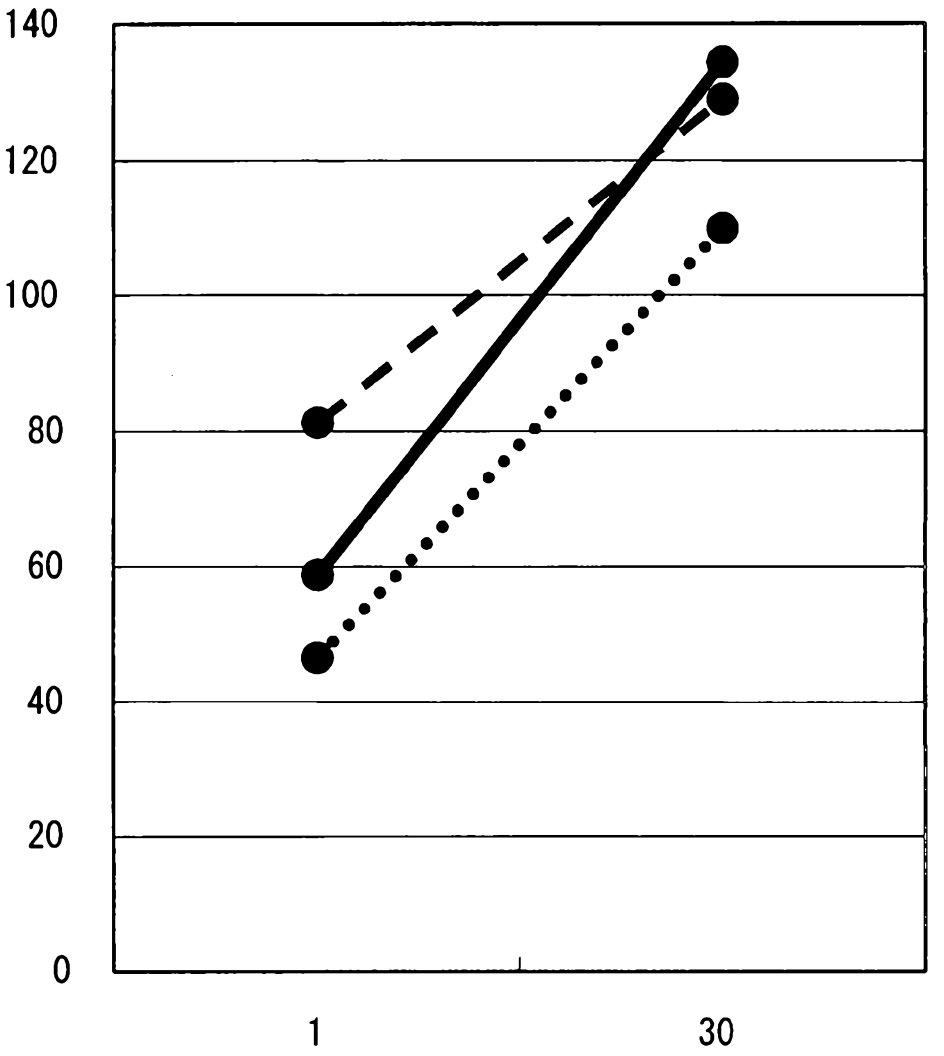

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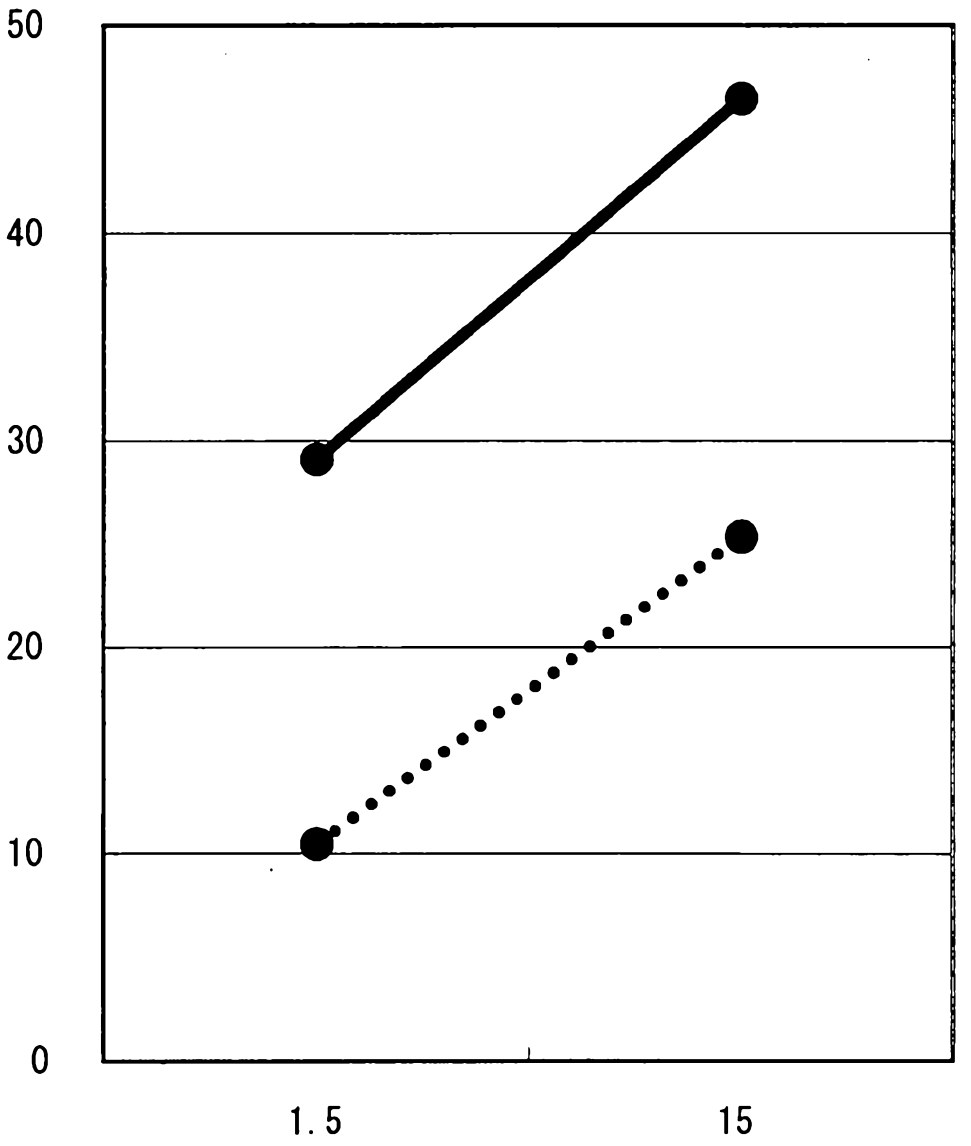

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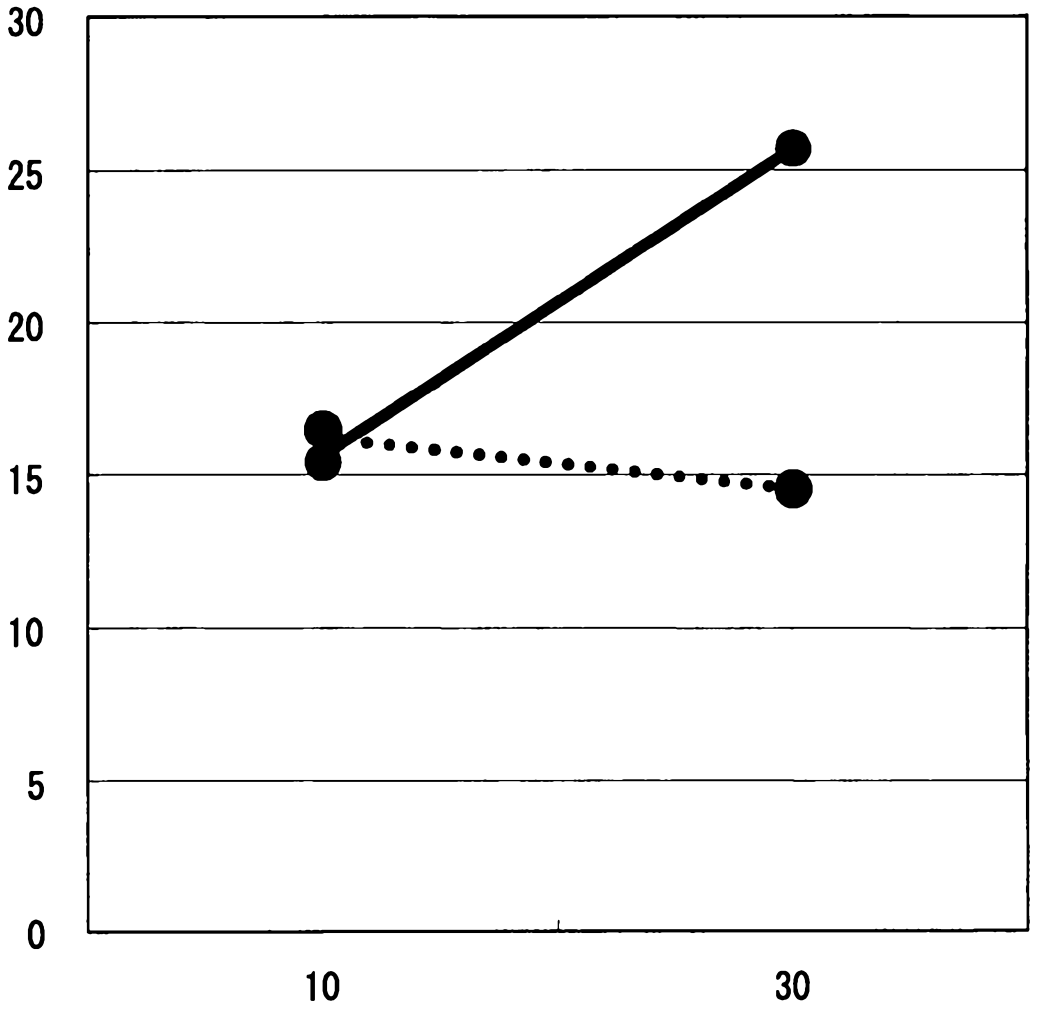

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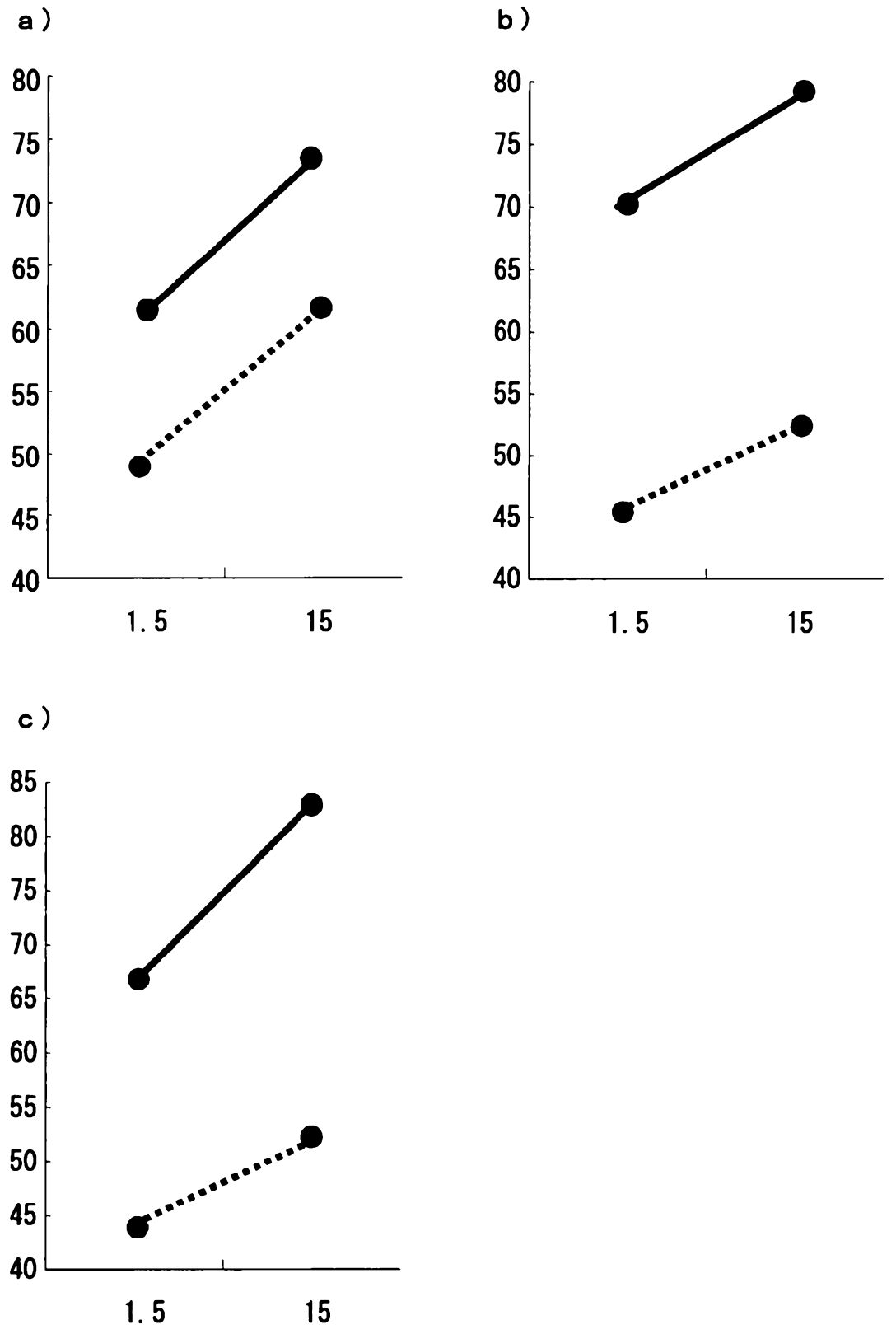
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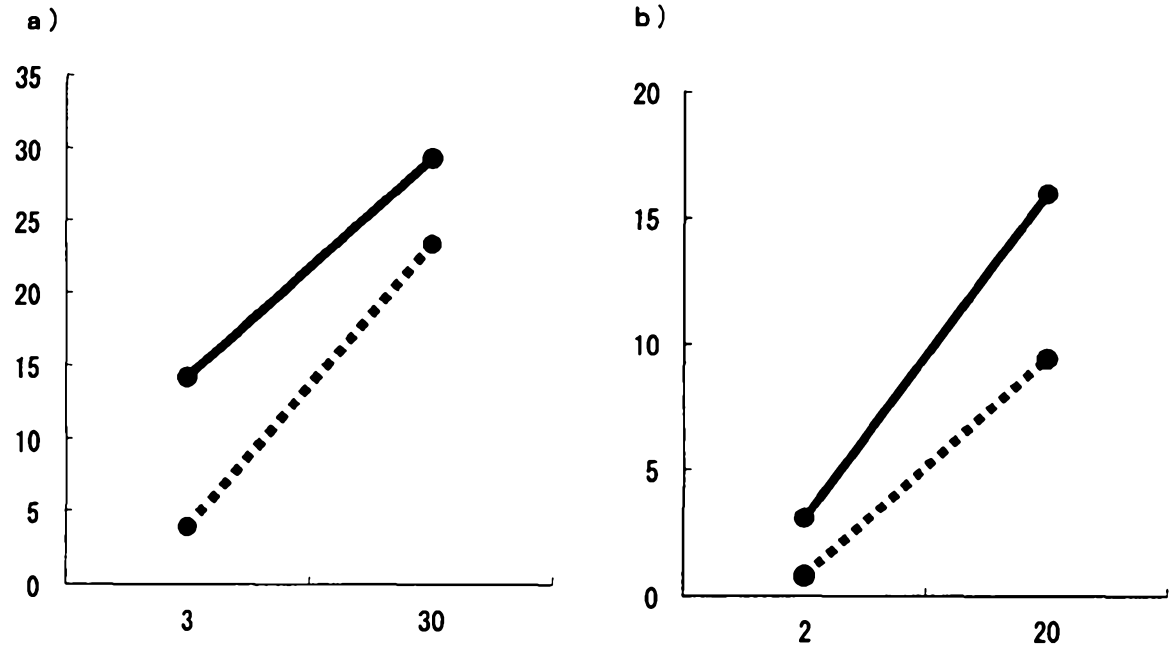

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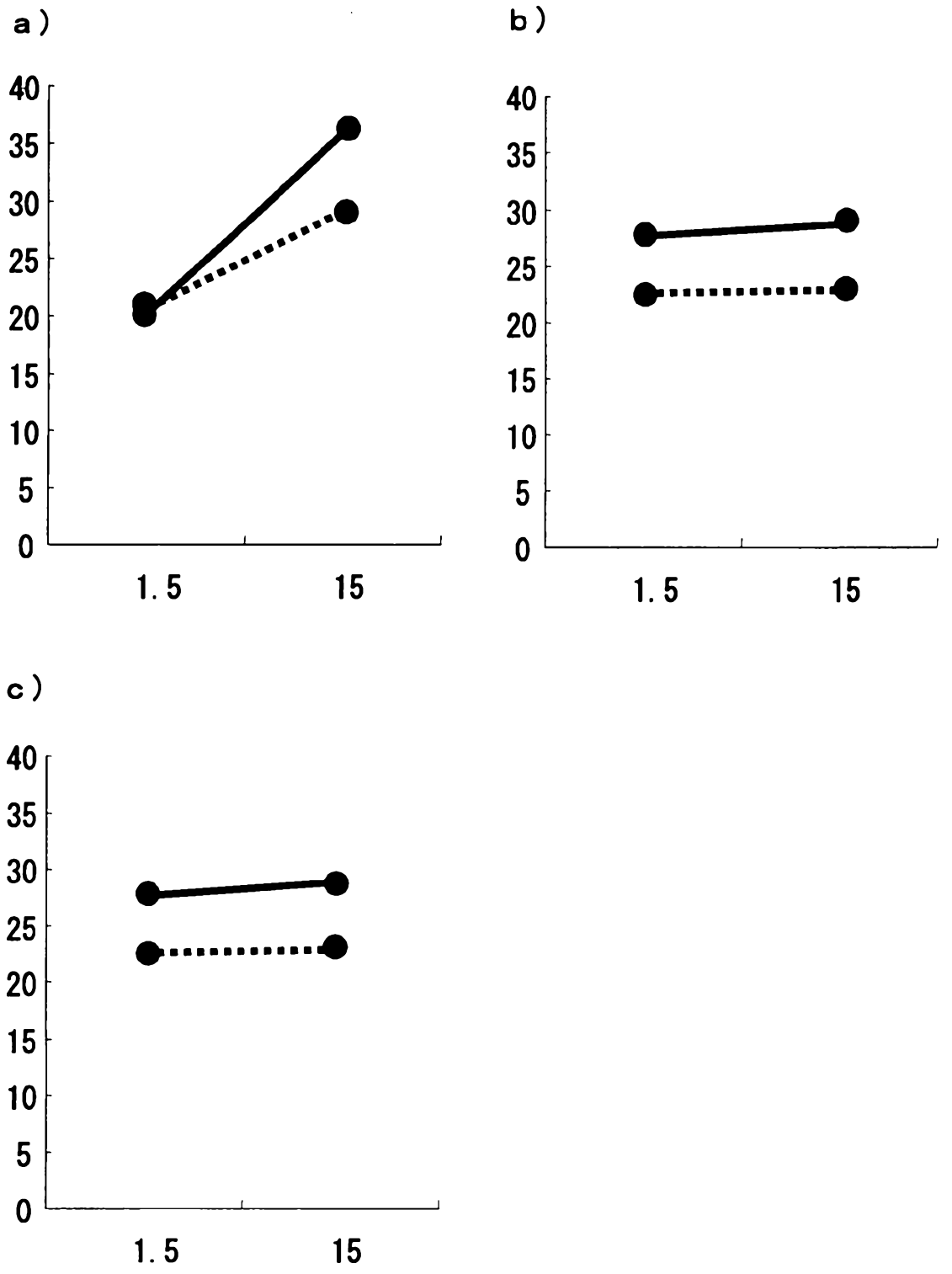
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Fig. 13

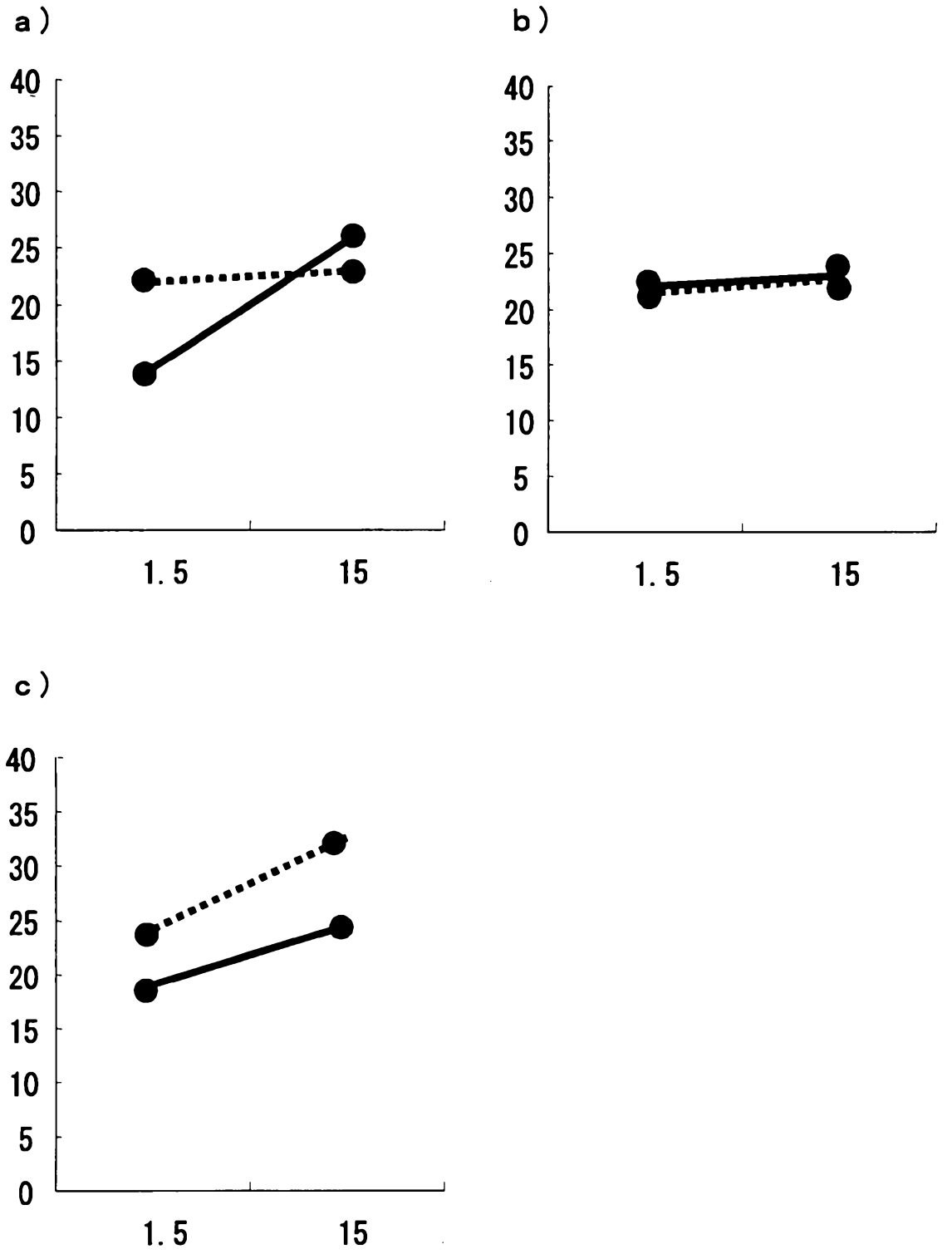
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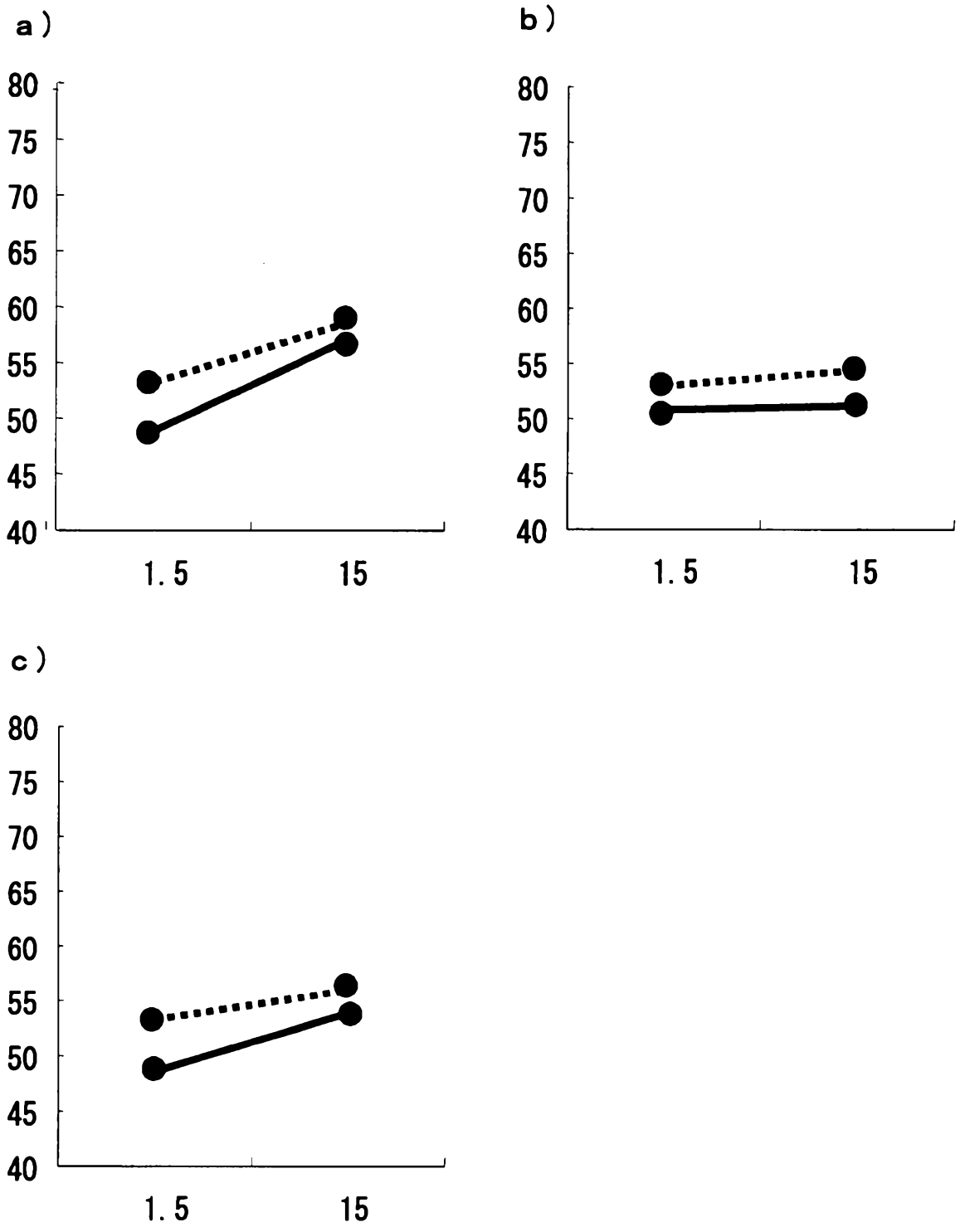
Fig. 14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2007340679 14 Jul 2009

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13 July, 2009

The Commissioner of Patents
WODEN ACT 2606

Amendment – Amending a Specification

Our Ref: 30789625/AJH/kxg

Re: International Institute of Cancer Immunology, Inc.
Australian Patent Application No. 2007340679
"HLA-A*1101-restricted WT1 peptide and pharmaceutical composition comprising the same"

Madam,

We ***enclose*** herewith a First Statement of Proposed Amendments.

We request that the Examiner takes the preliminary amendment into consideration during examination.

Yours respectfully,
DAVIES COLLISON CAVE

Dr Anne Hendtlass
Associate
ahendtlass@davies.com.au

Encl.

In association with:
Davies Collison Cave Solicitors
Intellectual Property Law

AUSTRALIAN PATENT APPLICATION NO. 2007340679

INTERNATIONAL INSTITUTE OF CANCER IMMUNOLOGY, INC.

FIRST STATEMENT OF PROPOSED AMENDMENTS

1. Insert new sequence listing pages 1 to 5 forwarded herewith.

13 July, 2009

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