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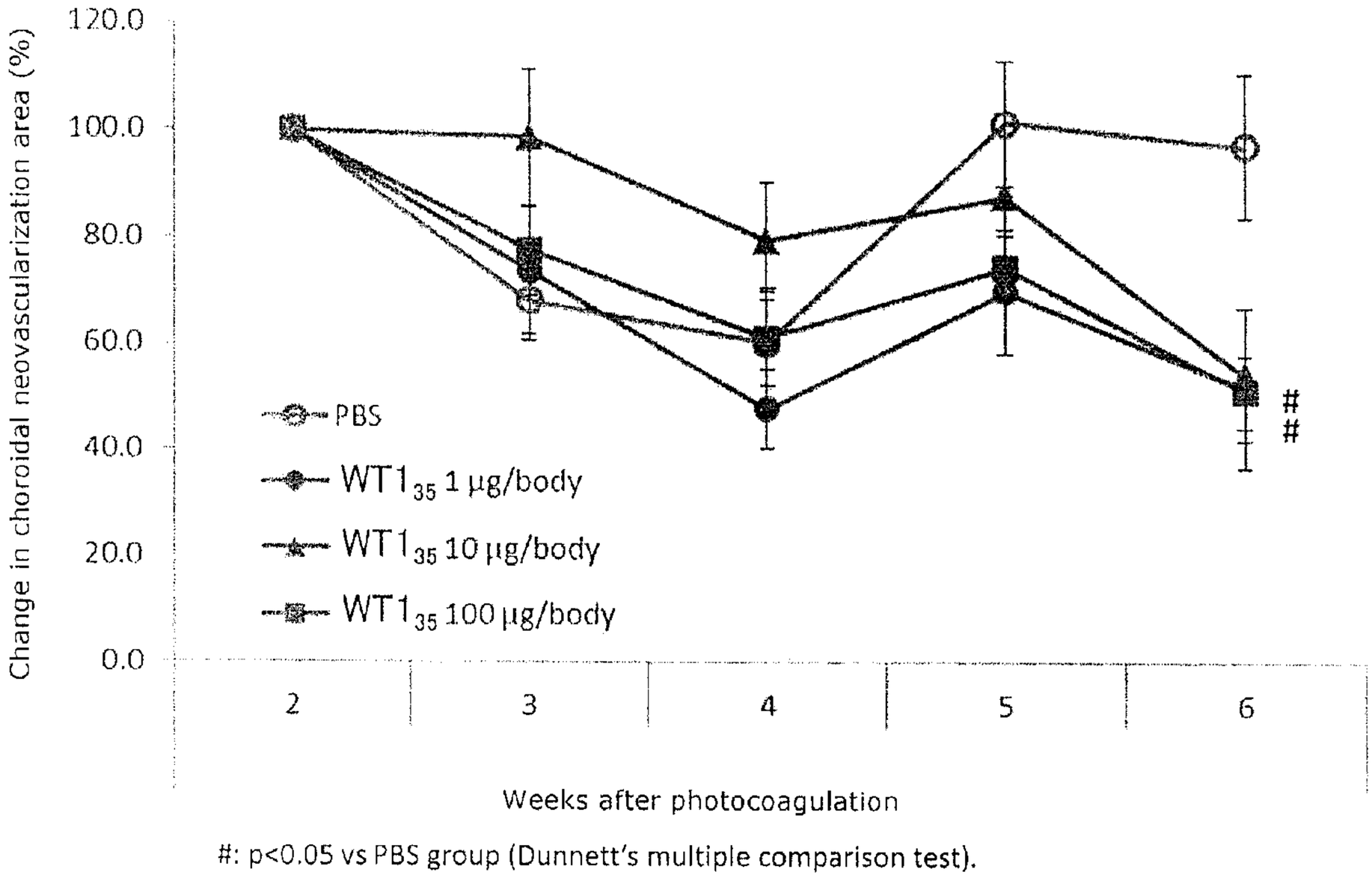

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(57) Abrégé/Abstract:
Provided is a medicinal composition for treating and preventing an angiogenic disease, said medicinal composition comprising a cancer antigen peptide.

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ABSTRACT

The present invention provides a pharmaceutical composition containing a cancer antigen peptide for treatment and prevention of angiogenic diseases.

DESCRIPTION

IMMUNOTHERAPY FOR ANGIOGENIC DISEASE

TECHNICAL FIELD

[0001]

The present invention relates to immunotherapy for angiogenic diseases.

BACKGROUND ART

[0002]

For angiogenic diseases such as various types of cancers, multiple myeloma, articular rheumatism, psoriasis, intraocular angiogenic diseases such as wet-type age-related macular degeneration, atherosclerotic plaque, vascular malformation, vascular agglutination, ovarian hypertrophy syndrome, polycystic ovary, granuloma, angioma, a hypertrophic scar, keloid, scleroderma, verruca, treatments are carried out using protamine, a combination of a steroid and heparin, a combination of a steroid and hexuronyl hexosaminoglycan sulfate, mitoxantrone, endostatin, a heparin-binding fragment of fibronectin, a prostaglandin synthetase inhibitor, γ interferon, a gold compound, lymphotoxin, D-penicillamine, a combination of a steroid and β-cyclodextrin tetradecasulfate, a protease inhibitor, methotrexate, interferon α-2a, an anti-VEGF drug, and the like.

[0003]

Typical types of angiogenic diseases include intraocular angiogenic

diseases such as wet-type age-related macular degeneration. A common method of treatment for intraocular angiogenic diseases uses an anti-VEGF drug as an intraocular injection. However, the method of treatment using an anti-VEGF drug takes a lot of money, needs repeated injections regularly, provides a great deal of psychological fear to patients of insertion of injection needles into their eye. Furthermore, there is a great risk of serious complications such as intraocular infections with great difficulty in treatment, endophthalmitis caused by stimulation of injection, and the like, and induction of retinal detachment. Further, there is a report that eyesight is improved for a certain period of time by a treatment using anti-VEGF drug in most cases, whereas the eyesight is worsened again in the long term even with continued treatment. That is, although an anti-VEGF drug used in the present treatment costs a lot, it has a danger, and thus sufficient treatment satisfaction is not necessarily achieved in view of its effects. Other treatments include surgical methods such as photodynamic therapy, laser photocoagulation, and neovascular extraction, however these methods have problems also in terms of costs and treatment satisfaction, have high invasiveness, and sometimes require hospitalization. Consequently, no conventional method of treatment for intraocular angiogenic diseases has been satisfactory yet, and thus it is considered that many problems to be overcome in the present treatments.

[0004]

Recently, it was reported that a WT1 protein, which is a cancer antigenic protein, is also developed in blood vessels of cancer tissues (Non-Patent Literature 1: N. Wagner et al. Oncogene (2008) 27, 3662-3672).

[0005]

There have already been many reports on experiments using a cancer antigen peptide as a cancer vaccine, however, there was no report on treatment and prevention of angiogenic diseases using a cancer antigen peptide as a vaccine.

[0006]

Under the above circumstances, a novel complete curative therapy for angiogenic disease is desired.

CITATION LIST

NON-PATENT LITERATURE

[0007]

Non-Patent Literature 1: N. Wagner et al. Oncogene (2008) 27, 3662-3672

SUMMARY OF INVENTION

PROBLEMS TO BE SOLVED

[0008]

An object of the present invention is to provide a novel preparation for treatment and prevention of angiogenic diseases. Further, it is also an object of the present invention to provide a minimally invasive preparation which can be self-administered by the patient with a relatively low cost.

MEANS TO SOLVE PROBLEMS

[0009]

The present inventors, after pursuing a diligent study to solve the problems mentioned above, have found that angiogenic diseases can be treated or prevented by using a cancer antigen peptide as a vaccine. Further, the present inventors also have found that angiogenic diseases can be treated by systemic administration of a WT1 peptide. The present inventors have completed the present invention based on the findings.

[0010]

Accordingly, the present invention provides the following.

(1) A pharmaceutical composition containing a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease.

(2) A pharmaceutical composition containing antigen presentation cells, killer T cells, or helper T cells induced or activated with a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of angiogenic disease.

(3) The pharmaceutical composition according to (1) or (2), wherein the cancer antigen peptide is a WT1 peptide or variant WT1 peptide.

(4) The pharmaceutical composition according to (3), wherein the WT1 peptide or variant WT1 peptide has a binding ability to an HLA molecule.

(5) The pharmaceutical composition according to (3) or (4), wherein the WT1 peptide or variant WT1 peptide activates killer T cells or helper T cells.

(6) The pharmaceutical composition according to any of (3) to (5), wherein the WT1 peptide has a length of 7 to 30 amino acids.

(7) The pharmaceutical composition according to any of (3) to (6), wherein the WT1 peptide comprises the following amino acid sequence:

(a) any of the amino acid sequence represented by SEQ ID NO: 1 to SEQ ID NO: 39, or

(b) an amino acid sequence, wherein one or several amino acids are replaced, deleted, or added in the amino acid sequence according to the above-described (a).

(8) The pharmaceutical composition according to any of (3) to (7), wherein the WT1 peptide comprises the following amino acid sequence:

(a) Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1),

(b) Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19),

(c)Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO: 27),

(d)Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32), or

(e) an amino acid sequence, wherein one or several amino acids are replaced, deleted, or added in any of the amino acid sequences according to (a) to (d).

(9) The pharmaceutical composition according to any of (1) to (8), wherein the angiogenic disease is an intraocular angiogenic disease.

(10) The pharmaceutical composition according to (9), wherein the intraocular angiogenic disease is selected from the group consisting of wet-type age-related macular degeneration, myopic macular degeneration, angioid streaks, central serous chorioretinopathy, various types of retinal pigment epitheliopathy, choroidal atrophy, choroideremia, choroidal osteoma,

diabetic retinopathy, retinopathy of prematurity, rubeotic glaucoma, and corneal neovascularization.

(11) The pharmaceutical composition according to any of (1) to (10), which is administered by intracutaneous administration, percutaneous administration, or transmucosal administration.

(12) The pharmaceutical composition according to (1) to (11), which is used in combination with a drug for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease.

EFFECTS OF INVENTION

[0011]

The present invention provides a pharmaceutical composition containing a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease. Further, the present invention provides a pharmaceutical composition containing antigen presentation cells, killer T cells, or helper T cells induced or activated with a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease. The pharmaceutical composition according to the present invention is minimally invasive because systemic administration, such as intracutaneous administration, subcutaneous administration, percutaneous administration, and transmucosal administration, as well as topical administration are available. Further, the pharmaceutical composition according to the present invention can be self-administered by the patient. Furthermore, the pharmaceutical composition according to the present invention has a high therapeutic effect,

and a high treatment satisfaction of patients. Moreover, when an intraocular angiogenic disease is treated with the pharmaceutical composition according to the present invention, treating or preventing effect on the other eye can also be expected.

BRIEF DESCRIPTION OF DRAWINGS

[0012]

Fig. 1 is a scheme showing a summary of observations, measurements, inspection items, and the like in animal experiments.

Fig. 2 is a typical fluorescein fluorescent ocular fundus image showing ocular fundus antiangiogenic effect of a WT1 peptide vaccine. Two pictures on the left show results of PBS intracutaneous administration (control), and two pictures on the right show results of intracutaneous administration with a WT1 peptide ($WT1_{126}$: Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1)).

Fig. 3 is a graph showing changes of choroidal neovascular areas obtained by time-course analysis with intracutaneous administration at 1 μg/body, 10 μg/body, and 100 μg/body of a WT1 peptide ($WT1_{126}$: Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1)).

Fig. 4 is a graph showing changes of choroidal neovascular areas obtained by time-course analysis with intracutaneous administration at 1 μg/body, 10 μg/body, and 100 μg/body of a WT1 peptide ($WT1_{35}$: Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO: 27)).

Fig. 5 is a graph showing changes of choroidal neovascular areas

obtained by time-course analysis with intracutaneous administration at 200 μg/body of a WT1 peptide ($WT1_{235m}$: Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19), and $WT1_{332}$: Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32)).

Fig. 6 is a graph showing changes of choroidal neovascular areas obtained by time-course analysis when WT1 peptides ($WT1_{126}$, $WT1_{235m}$, and $WT1_{332}$) and Aflibercept are used in combination.

DESCRIPTION OF EMBODIMENTS

[0013]

In one aspect, the present invention relates to a pharmaceutical composition containing a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease. The cancer antigen peptide is a part of cancer antigenic proteins. Various cancer antigenic proteins including WT1 are known. The cancer antigen peptide used in the pharmaceutical composition of the present invention can be one species, or can be two or more species. Whether a cancer antigen WT1 peptide exerts a therapeutic effect on angiogenic diseases in a subject or not depends on whether the cancer antigen peptide corresponds to an HLA type of the subject or not. Currently, with respect to many cancer antigen peptides, it is known that what cancer antigen peptide is compatible with a certain HLA type, and thus a cancer antigen peptide used in the present invention can be selected in accordance with an HLA type of a subject. Thus, two or more species of cancer antigen peptides can be used in a pharmaceutical composition of the present invention to adapt broader ranges

of subjects. Various angiogenic diseases are also known. Examples of the angiogenic disease include, but are not limited to, various types of cancers, multiple myeloma, articular rheumatism, psoriasis, intraocular angiogenic diseases such as wet-type age-related macular degeneration, atherosclerotic plaque, vascular malformation, vascular agglutination, ovarian hypertrophy syndrome, polycystic ovary, granuloma, angioma, a hypertrophic scar, keloid, scleroderma, and verruca.

[0014]

A cancer antigen peptide can be administered to a subject, or added to a sample such as blood taken from a subject to obtain antigen presentation cells presenting said cancer antigen peptide. The antigen presentation cells presenting a cancer antigen stimulate lymphocytes to induce or activate killer T cells or helper T cells. An effect of the cancer antigen peptide is derived from the antigen presentation cells, killer T cells, or helper T cells induced or activated as mentioned above. Accordingly, in another aspect, the present invention relates to a pharmaceutical composition containing antigen presentation cells, killer T cells, or helper T cells induced or activated with a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of angiogenic disease. The antigen presentation cells, killer T cells, or helper T cells used in the above-mentioned pharmaceutical composition can be induced or activated with any cancer antigen peptide, for example, induced or activated with a WT1 peptide.

[0015]

Typical types of angiogenic disease include intraocular angiogenic

diseases. Further, widely used species of cancer antigen peptide include a WT1 peptide. Accordingly, in a further aspect, the present invention relates to a pharmaceutical composition containing a WT1 peptide or variant WT1 peptide for treatment of intraocular angiogenic diseases.

[0016]

As used herein, a WT1 peptide refers to a partial peptide of a cancer antigenic protein WT1 encoded by WT1 gene (Wilms' tumor gene 1). An amino acid sequence of the cancer antigenic protein WT1 is represented by SEQ ID NO: 40. Thus, a WT1 peptide is a peptide consisting of a successive amino acid sequence included in the cancer antigenic protein WT1. A WT1 peptide is well-known to a person skilled in the art, and there are many reports specifically concerning cancer immunotherapy. A WT1 peptide can be produced by publicly known methods such as a genetic engineering procedure or a chemical synthesis method. Unless otherwise indicated, a WT1 peptide used herein refers to a peptide derived from a human cancer antigenic protein WT1.

[0017]

A WT1 peptide used in the present invention can be a variant WT1 peptide consisting of an amino acid in which one or several amino acids are replaced, deleted, or added in the amino acid sequence of a WT1 peptide. The variant WT1 peptide can be a WT1 peptide having an artificially modified amino acid sequence (also called as a modified WT1 peptide), a WT1 peptide obtained by natural mutation, or a WT1 peptide having difference in an amino acid sequence due to animal species which is the origin of the WT1 peptide. As used herein, the variant WT1 peptide has a similar therapeutic

effect on intraocular angiogenic diseases to that of a natural type WT1 peptide. Methods of replacing, deleting, or adding in an amino acid sequence known to a person skilled in the art include genetic engineering procedure and chemical methods, and thus a person skilled in the art can easily obtain a desired amino acid sequence. The term one or several refers to, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably 1, 2, 3, 4, or 5, more preferably 1, 2, or 3. Furthermore preferably, the term one or several refers to 1 or 2.

[0018]

The variant WT1 peptides include peptides having a modification of an amino acid residue in a WT1 peptide. Types of modification and methods for modification of an amino acid residue in a peptide are known to a person skilled in the art. As specific examples for modification, various substances such as an amino acid, a peptide, or their analogs can be bound to N-terminus and/or C-terminus of a WT1 peptide. The above-mentioned various substances can be substances for controlling solubility of a WT1 peptide of the present invention, increasing its stability (e.g., anti-protease activity), delivering a WT1 peptide of the present invention specifically to predetermined tissues/organs, or enhancing uptake efficiency of antigen presentation cells. On the other hand, the above-mentioned various substances can be made such that the various substances can be eliminated in vivo to provide a WT1 peptide.

[0019]

As used herein, unless otherwise indicated, the term a WT1 peptide includes a variant WT1 peptide.

[0020]

Length of a WT1 peptide used in the present invention is not specifically limited, however a WT1 peptide consisting of about 7 to about 30 amino acids is preferred. A preferred WT1 peptide has regularity of a sequence (motif) of an antigen peptide, which is bound to and presented by an HLA molecule, and has a binding ability to an HLA molecule. A binding ability to an HLA molecule can be analyzed by methods known in the art. Such methods include, for example, computer-based methods such as Rankpep, BIMAS, and SYFPEITHI, and a competitive binding assay with a known WT1 peptide having binding ability to an HLA molecule. Further, a preferred WT1 peptide activates killer T cells or helper T cells.

[0021]

Many WT1 peptides activating killer T cells (herein called as a killer WT1 peptide) are publicly known. Generally, a preferred killer WT1 peptide has an amino acid sequence consisting of 7 to 12 amino acids. A more preferred killer WT1 peptide has an amino acid sequence consisting of 9 amino acids. Examples of killer WT1 peptides which can be used in the present invention include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1), Arg Tyr Pro Ser Cys Gln Lys Lys Phe (SEQ ID NO: 2), Arg Tyr Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 3), Ala Tyr Leu Pro Ala Val Pro Ser Leu (SEQ ID NO: 4), Asn Tyr Met Asn Leu Gly Ala Thr Leu (SEQ ID NO: 5), Asp Gln Leu Lys Arg His Gln Arg Arg (SEQ ID NO: 6), Val Thr Phe Asp Gly Thr Pro Ser Tyr (SEQ ID NO: 7), Gln Gly Ser Leu Gly Glu Gln Gln Tyr (SEQ ID NO: 8), Cys Met Thr Trp Asn Gln Met Asn Leu

(SEQ ID NO: 9), Leu Ser His Leu Gln Met His Ser Arg (SEQ ID NO: 10), Phe Ser Arg Ser Asp Gln Leu Lys Arg (SEQ ID NO: 11), Ser Asp Gln Leu Lys Arg His Gln Arg (SEQ ID NO: 12), Thr Ser Glu Lys Pro Phe Ser Cys Arg (SEQ ID NO: 13), Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID NO: 14), Ser Ala Ser Glu Thr Ser Glu Lys Arg (SEQ ID NO: 15), Ser His Leu Gln Met His Ser Arg Lys (SEQ ID NO: 16), Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID NO: 17), Ser Leu Gly Glu Gln Gln Tyr Ser Val (SEQ ID NO: 18), Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19), Phe Leu Gly Glu Gln Gln Tyr Ser Val (SEQ ID NO: 20), Ser Met Gly Glu Gln Gln Tyr Ser Val (SEQ ID NO: 21), Ser Leu Met Glu Gln Gln Tyr Ser Val (SEQ ID NO: 22), Phe Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 23), Arg Leu Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 24), Arg Met Met Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 25), or Arg Met Phe Pro Asn Ala Pro Tyr Val (SEQ ID NO: 26), and variant WT1 peptides of the above-mentioned peptides. Other examples of killer WT1 peptides include, but are not limited to, those disclosed in International Publication No. WO 2000/018795 (incorporated herein by reference in its entirety).

[0022]

Many WT1 peptides activating helper T cells (herein called as a helper WT1 peptide) are publicly known. Generally, a preferred helper WT1 peptide has an amino acid sequence consisting of 10 to 25 amino acids. A more preferred helper WT1 peptide has an amino acid sequence consisting of 13 to 20 amino acids. Examples of helper WT1 peptides which can be used in the present invention include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO:

27), Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe Ser Gly Gln Phe Thr Gly (SEQ ID NO: 28), Pro Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly (SEQ ID NO: 29), Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 30), Phe Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg (SEQ ID NO: 31), or Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32), and variant WT1 peptides of the above-mentioned peptides.

[0023]

A WT1 peptide used in a pharmaceutical composition of the present invention can be one species, or two or more species. Further, a WT1 peptide used in the pharmaceutical composition of the present invention can be a killer WT1 peptide or helper WT1 peptide, or a killer WT1 peptide and a helper WT1 peptide can be mixed for use in the pharmaceutical composition.

[0024]

In the present invention, a dimer of WT1 peptides can be used. The dimer of WT1 peptides can be obtained by forming a disulfide bond between two WT1 peptides having cysteine residues.

[0025]

A WT1 peptide used in a pharmaceutical composition of the present invention can be one species, or can be two or more species. Whether a WT1 peptide exerts a therapeutic effect on intraocular angiogenic diseases in a subject or not depends on whether the WT1 peptide corresponds to HLA type of the subject or not. Currently, with respect to many WT1 peptides, it is known that what WT1 peptide is compatible with a certain HLA type, and thus a WT1 peptide used in the present invention can be selected in

accordance with an HLA type of a subject. Further, two or more species of WT1 peptides can be used in a pharmaceutical composition of the present invention to adapt broader ranges of subjects.

[0026]

For example, when a subject is HLA-A*0201-positive, examples of preferred WT1 peptides used in a pharmaceutical composition of the present invention include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Asp Leu Asn Ala Leu Leu Pro Ala Val (SEQ ID NO: 33), Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1), or Ser Leu Gly Glu Gln Gln Tyr Ser Val (SEQ ID NO: 18), and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0027]

For example, when a subject is HLA-A*2601-positive, examples include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Val Thr Phe Asp Gly Thr Pro Ser Tyr (SEQ ID NO: 7), Gln Gly Ser Leu Gly Glu Gln Gln Tyr (SEQ ID NO: 8), or Asp Gln Leu Lys Arg His Gln Arg Arg (SEQ ID NO: 34), and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0028]

For example, when a subject is HLA-A*3303-positive, examples include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Leu Ser His Leu Gln Met His Ser Arg (SEQ

ID NO: 10), Phe Ser Arg Ser Asp Gln Leu Lys Arg (SEQ ID NO: 11), Ser Asp Gln Leu Lys Arg His Gln Arg (SEQ ID NO: 12), or Thr Ser Glu Lys Pro Phe Ser Cys Arg (SEQ ID NO: 13), and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0029]

For example, when a subject is HLA-A*1101-positive, examples include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Ala Ala Gly Ser Ser Ser Ser Val Lys (SEQ ID NO: 35), Pro Ile Leu Cys Gly Ala Gln Tyr Arg (SEQ ID NO: 14), Arg Ser Ala Ser Glu Thr Ser Glu Lys (SEQ ID NO: 36), Ser Ala Ser Glu Thr Ser Glu Lys Arg (SEQ ID NO: 15), Ser His Leu Gln Met His Ser Arg Lys (SEQ ID NO: 16), Thr Gly Val Lys Pro Phe Gln Cys Lys (SEQ ID NO: 17), Lys Thr Cys Gln Arg Lys Phe Ser Arg (SEQ ID NO: 37), Ser Cys Arg Trp Pro Ser Cys Gln Lys (SEQ ID NO: 38), or Asn Met His Gln Arg Asn Met Thr Lys (SEQ ID NO: 39), and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0030]

For example, when a subject is HLA-A*2402-positive, examples include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 9), or Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19), a dimer of peptides comprising or consisting of amino acid sequence of SEQ ID NO: 9, a cystine form of peptides comprising or consisting of amino acid

sequence of SEQ ID NO: 9, and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0031]

For example, when a subject is HLA-DRB1-positive, HLA-DRB3-positive, HLA-DRB4-positive, HLA-DRB5-positive, HLA-DPB1-positive, or HLA-DQB1-positive, examples include, but are not limited to, peptides comprising or consisting of the following amino acid sequences: Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO: 27), Glu Gln Cys Leu Ser Ala Phe Thr Val His Phe Ser Gly Gln Phe Thr Gly (SEQ ID NO: 28), Pro Asn His Ser Phe Lys His Glu Asp Pro Met Gly Gln Gln Gly (SEQ ID NO: 29), Asn Leu Tyr Gln Met Thr Ser Gln Leu Glu Cys Met Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 30), Phe Arg Gly Ile Gln Asp Val Arg Arg Val Pro Gly Val Ala Pro Thr Leu Val Arg (SEQ ID NO: 31), or Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32), and peptides comprising or consisting of an amino acid sequence in which one or several amino acids are replaced, deleted, or added in the amino acid sequence mentioned above.

[0032]

As an active ingredient in a pharmaceutical composition of the present invention, a polynucleotide encoding a WT1 peptide can be used. A base sequence of the polynucleotide can be determined based on the amino acid sequence of the WT1 peptide. The polynucleotide can be manufactured by, for example, publicly known DNA synthesis method or RNA synthesis method, such as a chemical synthesis method, PCR method, and the like.

[0033]

The pharmaceutical composition of the present invention, and a drug used for any of treatment and prevention or for both treatment and prevention of angiogenic disease can be used in combination. Examples of the drug used for any of treatment and prevention or both treatment and prevention of angiogenic disease include, but are not limited to, bevacizumab, cetuximab, panitumumab, and the like. Examples of the drug used for any of treatment and prevention or both treatment and prevention of intraocular angiogenic diseases include, but are not limited to, vascular endothelial growth factor inhibitors, such as aflibercept, pegaptanib sodium, ranibizumab, and the like.

[0034]

Intraocular angiogenic diseases which can be treated with a pharmaceutical composition of the present invention include, but are not limited to, wet-type age-related macular degeneration, myopic macular degeneration, angioid streaks, central serous chorioretinopathy, various types of retinal pigment epitheliopathy, choroidal atrophy, choroideremia, choroidal osteoma, diabetic retinopathy, retinopathy of prematurity, rubeotic glaucoma, and corneal neovascularization.

[0035]

Routes of administration for a pharmaceutical composition of the present invention are not specifically limited, however examples of preferred route of administration include intracutaneous administration, subcutaneous administration, percutaneous administration, and transmucosal administration, such as eye drops, nasal spray, and sublingual.

[0036]

Dosage forms of a pharmaceutical composition of the present invention are not specifically limited, and examples of dosage form include a liquid medicine for injection, an eye drop liquid medicine, a liquid medicine for nasal spray, a lotion, creams, a patch, sublingual tablet, and a troche. These dosage forms can be manufactured and administered by methods well-known to a person skilled in the art.

[0037]

When a pharmaceutical composition of the present invention is used, the dose of a WT1 peptide can be appropriately changed based on consideration of types of WT1 peptides, route of administration, dosage form, types of disease, severity of diseases, state of health of a patient, and the like. In general, the dose of a WT1 peptide for adult is 0.1 μg/kg to 1 mg/kg daily. Further, types of WT1 peptides, route of administration, and dosage form can be appropriately changed. The pharmaceutical composition of the present invention can include a suitable adjuvant such as aluminum hydroxide in addition to a pharmaceutically acceptable carrier and a diluent. On the other hand, the pharmaceutical composition of the present invention can contain a WT1 peptide encapsulated in liposomes.

[0038]

In another aspect, the present invention relates to use of a WT1 peptide for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases. In a further aspect, the present invention relates to use of a WT1 peptide for the manufacture of a medicament for any of treatment and prevention or for both treatment and

prevention of intraocular angiogenic diseases. In still another aspect, the present invention relates to a method for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases characterized by administrating a WT1 peptide to a subject in need of any of treatment and prevention or both treatment and prevention of intraocular angiogenic diseases. The above description also applies to these aspects.

[0039]

In further aspect, the present invention relates to a pharmaceutical composition containing antigen presentation cells, killer T cells, or helper T cells induced or activated with a WT1 peptide for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases. Further, the present invention relates to use of antigen presentation cells, killer T cells, or helper T cells induced or activated with a WT1 peptide for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases. Moreover, the present invention relates to use of antigen presentation cells, killer T cells, or helper T cells induced or activated with a WT1 peptide for the manufacture of a medicament for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases. In still another aspect, the present invention relates to a method for any of treatment and prevention or for both treatment and prevention of intraocular angiogenic diseases characterized by administering antigen presentation cells, killer T cells, or helper T cells induced or activated with a WT1 peptide to a subject in need of any of treatment and prevention or both treatment and prevention of intraocular angiogenic diseases. The above description also applies to these

aspects.

[0040]

Methods for inducing or activating antigen presentation cells, killer T cells or helper T cells are known to a person skilled in the art. In vivo, a WT1 peptide can be administered to a subject to induce or activate killer T cells and helper T cells. In vitro, for example, a sample containing lymphocytes derived from a subject can be reacted with a WT1 peptide-HLA molecule complex to obtain killer T cells. Further, for example, peripheral blood mononuclear cells derived from a subject can be cultured in the presence of a WT1 peptide to induce WT1-specific CTL from said peripheral blood mononuclear cells. Moreover, for example, immature antigen presentation cells derived from a subject can be cultured in the presence of a WT1 peptide to induce antigen presentation cells which present a WT1 peptide via an HLA molecule. The immature antigen presentation cells refer to cells which can mature into antigen presentation cells, and examples of the immature antigen presentation cells include immature dendritic cells. Further, for example, a WT1 peptide can be added to antigen presentation cells to activate helper T cells. Antigen presentation cells, killer T cells, or helper T cells used in a pharmaceutical composition of the present invention can be induced or activated with any WT1 peptide. Thus induced or activated antigen presentation cells, killer T cells, or helper T cells can be administered to a subject, preferably to a subject from which these cells were obtained to carry out treatment and prevention of intraocular angiogenic diseases.

[0041]

The above description of treatment and/or prevention is mainly with respect to intraocular angiogenic diseases, however, the above description also applies to other angiogenic diseases.

[0042]

The present invention is described below specifically and in more detail with reference to examples, but the examples should not be construed to limit the invention in any way.

EXAMPLE

Example 1

[0043]

Demonstration of a therapeutic effect of a WT1 peptide vaccine using a mouse model of laser-induced choroid coat angiogenesis

1. Experimental Method

1-1. Test substance

Two species of WT1 peptides: $WT1_{126}$ (Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1)) and $WT1_{35}$ (Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO: 27)), were used as test substances.

[0044]

1-2. Animal used for experiment

Male C57BL/6J mice (provided by CLEA Japan, Inc.) were used. At the beginning of the experiment, the mice were 8 weeks old (body weight: about 20 to 25 g). The mice were free-fed with laboratory chow

(manufactured by Oriental Yeast Co., Ltd.) and tap water. Six mice were used for each administration group.

[0045]

1-3. Dose and method of administration

As a WT1 peptide, $WT1_{126}$ or $WT1_{35}$ was dissolved in sterilized PBS so that the concentration of the solution was 22.7 mg/mL. The resultant was diluted to 0.227 or 2.27 mg/mL with sterilized PBS. The resultant WT1 solution and Montanide ISA51 VG were mixed so that the volume ratio was 1:1.27 to prepare emulsions of 0.1 μl/μL, 1 μl/μL, and 10 μl/μL. Each of the emulsions was intracutaneously administered at 10 μL/body. Administered doses were 1 μg/body, 10 μg/body, and 100 μg/body. Timing of administrations were on 15, 22, 29, and 36 days after photocoagulation.

[0046]

Specifically, with respect to the mice of administration groups (groups 1 to 4) administered with the test substance (WT1 peptide), the administration was carried out through the following procedure.

(1) Grouping was carried out on the basis of area of fluorescence leakage on 14 days after the photocoagulation.

(2) Body weight measurement and general symptom observation were carried out 15, 22, 29, and 36 days after photocoagulation.

(3) Abdomen was shaved with hair clippers, and then intracutaneous administration was carried out using a 30G injection needle.

[0047]

Constitutions of the experiment groups (6 animals/group) are summarized in Table 1.

Table 1

Group number	Test substance	Route of administration
1	PBS	Intracutaneous
2	1 μg/body $WT1_{126}$ or $WT1_{35}$	
3	10 μg/body $WT1_{126}$ or $WT1_{35}$	
4	100 μg/body $WT1_{126}$ or $WT1_{35}$	

[0048]

1-4. Photocoagulation and ocular fundus imaging

Procedure used was as follows.

(1) Body weight measurement and general symptom observation were carried out.

(2) Dilatation of pupils was carried out with Mydrin-P (manufactured by Santen Pharmaceutical Co., Ltd.), and then general anesthesia was performed by intramuscular administration at 1 mL/kg of a mixture of Ketalar injection and Celactal injection (7:1).

(3) Photocoagulation was carried out using Multi Color Laser Photocoagulator (red) (manufactured by NIDEK CO., LTD.) under the following coagulation conditions: spot size of 50 μm, output of 60 mW, and coagulation time of 0.1 second.

(4) A cover glass for tissue observation was used as a contact lens, and then 4 photocoagulations were carried out, provided that sporadic large retinal blood vessels in posterior ocular fundus were excluded.

(5) Ocular fundus imaging was carried out using Micron III (manufactured

by Phoenix Research Labs) promptly after the photocoagulation.

(6) Eye drops of Hyalein ophthalmic solution were applied to both eyes.

[0049]

1-5. Fluorescence ocular fundus angiography

Procedure used was as follows.

(1) Body weight measurement and general symptom observation were carried out 14, 21, 28, 35, and 42 days after photocoagulation.

(2) Dilatation of pupils was carried out with Mydrin-P, and then general anesthesia was performed by intramuscular administration at 1 mL/kg of a mixture of Ketalar injection (manufactured by DAIICHI SANKYO COMPANY, LIMITED) and Celactal injection (manufactured by Bayer) (7:1).

(3) Fluorescite (Manufactured by Alcon, Inc.) was injected at 0.1 mL/kg via the tail vein, and then fluorescence ocular fundus angiography was carried out using Micron III.

(4) An area of fluorescence leakage was calculated using an image analysis software.

[0050]

1-6. Summary of observations, measurements, inspection items, etc.

Fig. 1 summarizes the description mentioned above.

[0051]

1-7. Data analysis

Microsoft EXCEL was used for data analysis, and the obtained results are shown as mean +/- standard deviation. EXSUS version 8.0.0 (SAS ver.9.3, CAC EXICARE Corporation) was used for a statistical analysis, and Dunnett type multigroup comparison (group 1 vs groups 2, 3, and 4) was

selected to carry out a multiple comparison test. A significance level of < 5% was used for all tests.

[0052]

2. Results of Experiments

Measurement results of angiogenesis by fluorescence ocular fundus angiography at 2 weeks after and at 6 weeks after the photocoagulation are shown in Fig. 2. The angiogenesis of the ocular fundus of the mice, which were administered with a WT1 peptide ($WT1_{126}$), at 6 weeks after the photocoagulation was significantly suppressed as compared to that of the mice in PBS administration group.

[0053]

Fluorescence ocular fundus angiography was carried out at 2 weeks, 3 weeks, 4 weeks, and 6 weeks after the photocoagulation to analyze time-dependent change of choroidal neovascular area. Fig. 3 shows data of mice administered with $WT1_{126}$, and Fig. 4 shows data of mice administered with $WT1_{35}$. Decrease in choroidal neovascular area with administration of a WT1 peptide was observed in mice with administration of either WT1 peptide.

In $WT1_{126}$ administration group, choroidal neovascular area was decreased with any applied dose of 1 μg/body, 10 μg/body, and 100 μg/body as compared to that of PBS administration group. Specifically, in the group administered with 100 μg/body of $WT1_{126}$, the decreasing effect was significant, that is, choroidal neovascular area at 6 weeks after the photocoagulation was reduced to about 50% of choroidal neovascular area at 2 weeks after the photocoagulation. On the other hand, in PBS

administration group, choroidal neovascular area at 6 weeks after the photocoagulation was increased to about 230% of choroidal neovascular area at 2 weeks after the photocoagulation.

Also in $WT1_{35}$ administration group, decreasing trend in choroidal neovascular area was also observed with any applied dose of 1 μg/body, 10 μg/body, and 100 μg/body. Choroidal neovascular area at 6 weeks after the photocoagulation was reduced to about 50% of choroidal neovascular area at 2 weeks after the photocoagulation.

Example 2

[0054]

Two species of WT1 peptides: $WT1_{235m}$ (Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19)) and $WT1_{332}$ (Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32)) were used as test substances, and following setting of administration groups were provided to conduct an experiment in the same method and procedure as Example 1. The $WT1_{235m}$ was a peptide in which methionine in position 2 of $WT1_{235}$ (SEQ ID NO: 9) was replaced with tyrosine.

Table 2

Group number	Test substance	Dose of administration	Volume of administration	Number of administration	Mouse strain Number of experiment
1	PBS	-	10	1	C57BL6

			μL/body	(intracutaneously)	12 mice
2	$WT1_{235m}$	200 μg/body	10 μL/body	4 (intracutaneously)	CBF-1 12 mice
3	$WT1_{332}$	200 μg/body	10 μL/body	4 (intracutaneously)	CBF-1 12 mice

[0055]

Fluorescence ocular fundus angiography was carried out at 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks after the photocoagulation to analyze time-dependent change of choroidal neovascular area. Results are shown in Fig. 5. Decrease in choroidal neovascular area with administration of a WT1 peptide was observed in mice with administration of either WT1 peptide of $WT1_{235m}$ or $WT1_{332}$ as compared to that of PBS administration group. In $WT1_{332}$ administration group, choroidal neovascular area at 6 weeks after the photocoagulation was reduced to about 70% of choroidal neovascular area at 2 weeks after the photocoagulation. In $WT1_{235m}$ administration group, increase in choroidal neovascular area was suppressed, that is, choroidal neovascular area at 6 weeks after the photocoagulation was the same as that at 2 weeks after the photocoagulation.

Example 3

[0056]

Time-dependent change of choroidal neovascular area was analyzed when WT1 peptides ($WT1_{126}$, $WT1_{235m}$, and $WT1_{332}$) and Aflibercept were used in combination. Settings of administration groups were provided as follows (combination use groups: group 3, 4, and 5). Intravitreal administration of Aflibercept to the right eye was carried on the same day of laser application.

Table 3

Group number	Administered substance	Dose of administration	Volume of administration	Number of administration	Mouse strain Number of experiment
1	PBS	-	10 μL/body	1	C57BL6 12 mice
2	Aflibercept	40 μg/eye	1 μL/eye	1	C57BL6 12 mice
3	Aflibercept	40 μg/eye	1 μL/eye	1	C57BL6 12 mice
	$WT1_{126}$	100 μg/body	10 μL/body	4	
4	Aflibercept	40 μg/eye	1 μL/eye	1	CBF-1 12 mice
	$WT1_{235m}$	200 μg/body	10 μL/body	4	
5	Aflibercept	40 μg/eye	1 μL/eye	1	CBF-1 12 mice
	$WT1_{332}$	200 μg/body	10 μL/body	4	

[0057]

Results are shown in Fig. 6. Decrease in choroidal neovascular area or suppression of increase in choroidal neovascular area was observed when a WT1 peptide and Aflibercept were used in combination, and thus a combined effect was confirmed.

[0058]

According to the above-described results of experiments, it is confirmed that a WT1 peptide, which is a cancer antigen peptide, is effective in treatment for angiogenic disease.

INDUSTRIAL APPLICABILITY

[0059]

The present invention provides a pharmaceutical composition containing a cancer antigen peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease, and thus can be used in specifically pharmaceutical field.

CLAIMS

1. A pharmaceutical composition comprising a WT1 peptide or variant WT1 peptide for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease.

2. A pharmaceutical composition comprising antigen presentation cells, killer T cells, or helper T cells induced or activated with a WT1 peptide or variant WT1 peptide for any of treatment and prevention, or for both treatment and prevention of angiogenic disease.

3. The pharmaceutical composition according to any one of claim 1 or 2, wherein the WT1 peptide or variant WT1 peptide has a binding ability to an HLA molecule.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the WT1 peptide or variant WT1 peptide activates killer T cells or helper T cells.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein the WT1 peptide has a length of 7 to 30 amino acids.

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein WT1 peptide comprises the following amino acid sequence:

(a) any of the amino acid sequence represented by SEQ ID

NO: 1 to SEQ ID NO: 39, or

(b) an amino acid sequence, wherein one or several amino acids are replaced, deleted, or added in the amino acid sequence according to the above-described (a).

7. The pharmaceutical composition according to any one of claims 1 to 6, wherein the WT1 peptide comprises the following amino acid sequence:

(a) Arg Met Phe Pro Asn Ala Pro Tyr Leu (SEQ ID NO: 1),

(b) Cys Tyr Thr Trp Asn Gln Met Asn Leu (SEQ ID NO: 19),

(c) Ala Pro Val Leu Asp Phe Ala Pro Pro Gly Ala Ser Ala Tyr Gly Ser Leu Gly (SEQ ID NO: 27),

(d) Lys Arg Tyr Phe Lys Leu Ser His Leu Gln Met His Ser Arg Lys His (SEQ ID NO: 32), or

(e) an amino acid sequence, wherein one or several amino acids are replaced, deleted, or added in any of the amino acid sequences according to (a) to (e).

8. The pharmaceutical composition according to any one of claims 1 to 7, wherein the angiogenic disease is an intraocular angiogenic disease.

9. The pharmaceutical composition according to claim 8, wherein the intraocular angiogenic disease is selected from the group consisting of wet-type age-related macular degeneration, myopic macular degeneration, angioid streaks, central serous chorioretinopathy, various types of retinal pigment epitheliopathy, choroidal atrophy, choroideremia, choroidal osteoma,

diabetic retinopathy, retinopathy of prematurity, rubeotic glaucoma, and corneal neovascularization.

10. The pharmaceutical composition according to any one of claims 1 to 9, which is administered by intracutaneous administration, subcutaneous administration, percutaneous administration, or transmucosal administration.

11. The pharmaceutical composition according to any one of claims 1 to 10, which is used in combination with a drug for any of treatment and prevention, or for both treatment and prevention of an angiogenic disease.

Fig. 1

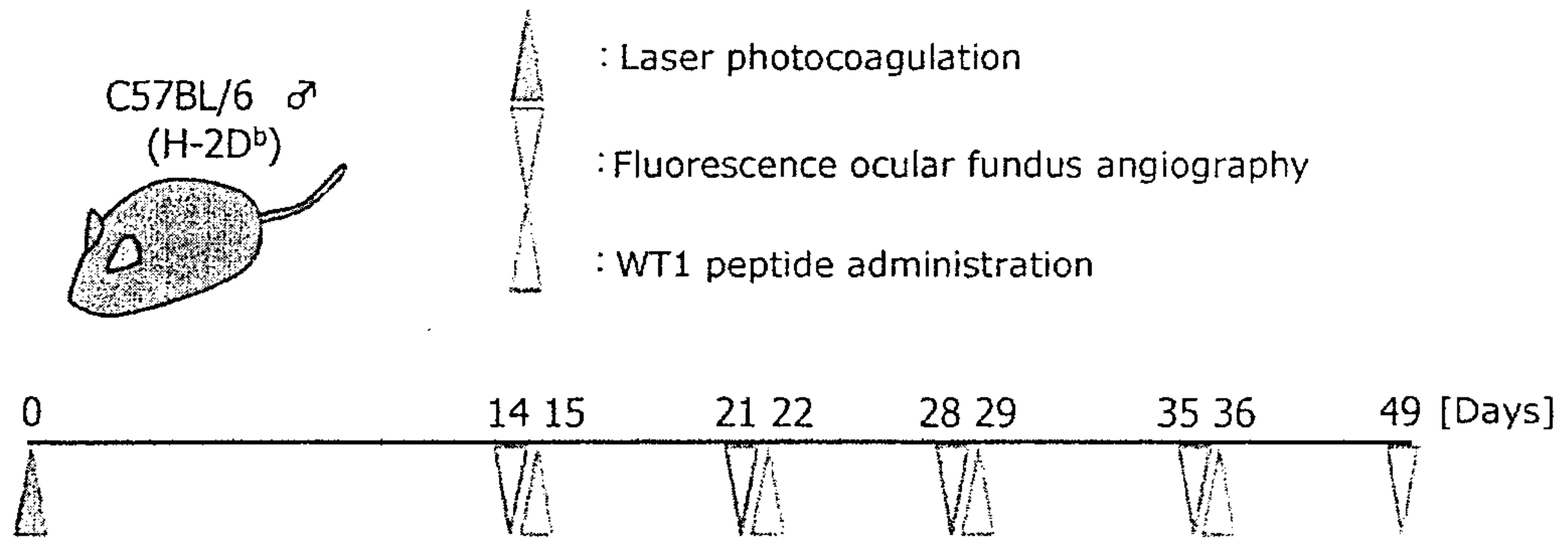

C57BL/6 ♂
(H-2D^b)
: Laser photocoagulation
: Fluorescence ocular fundus angiography
: WT1 peptide administration
0
14 15
21 22
28 29
35 36
49 [Days]

Fig. 2

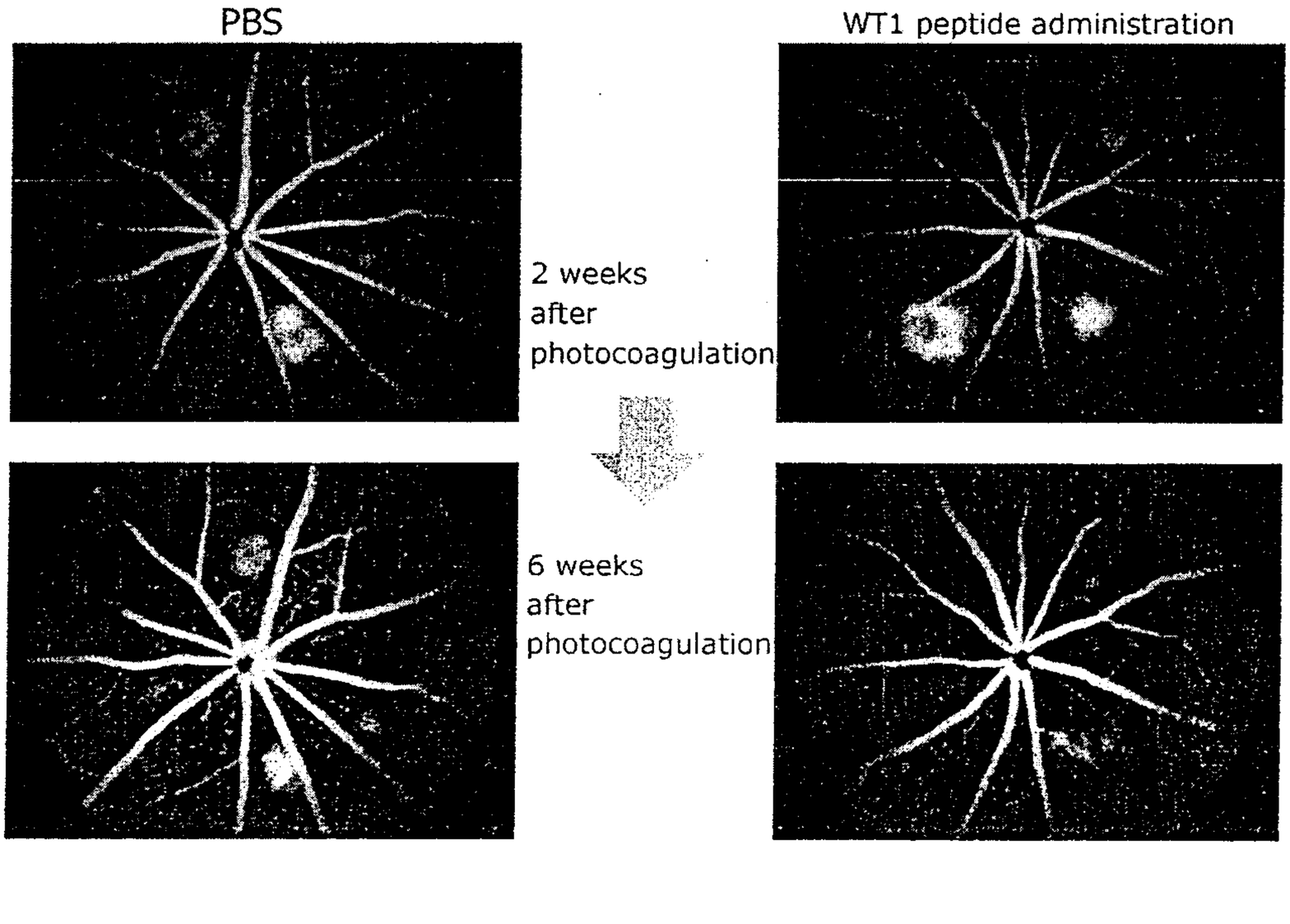

PBS
WT1 peptide administration
2 weeks after photocoagulation
6 weeks after photocoagulation

Fig. 3

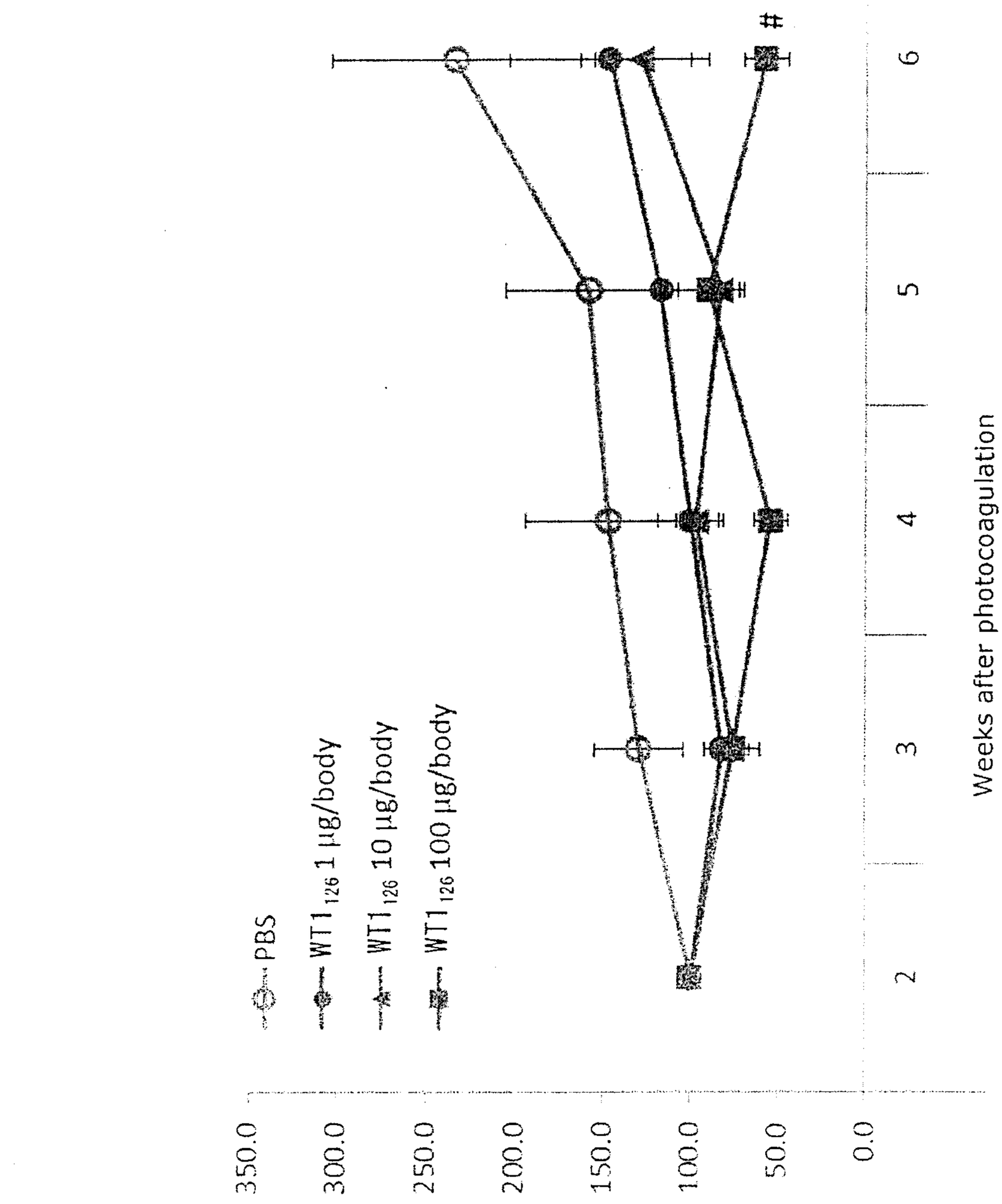

#
PBS
WT1 126 1 µg/body
WT1 126 10 µg/body
WT1 126 100 µg/body
2
3
4
5
6
Weeks after photocoagulation
#: p<0.05 vs PBS group (Dunnett's multiple comparison test).
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Change in chorioidal neovascularization area (%)

Fig. 4

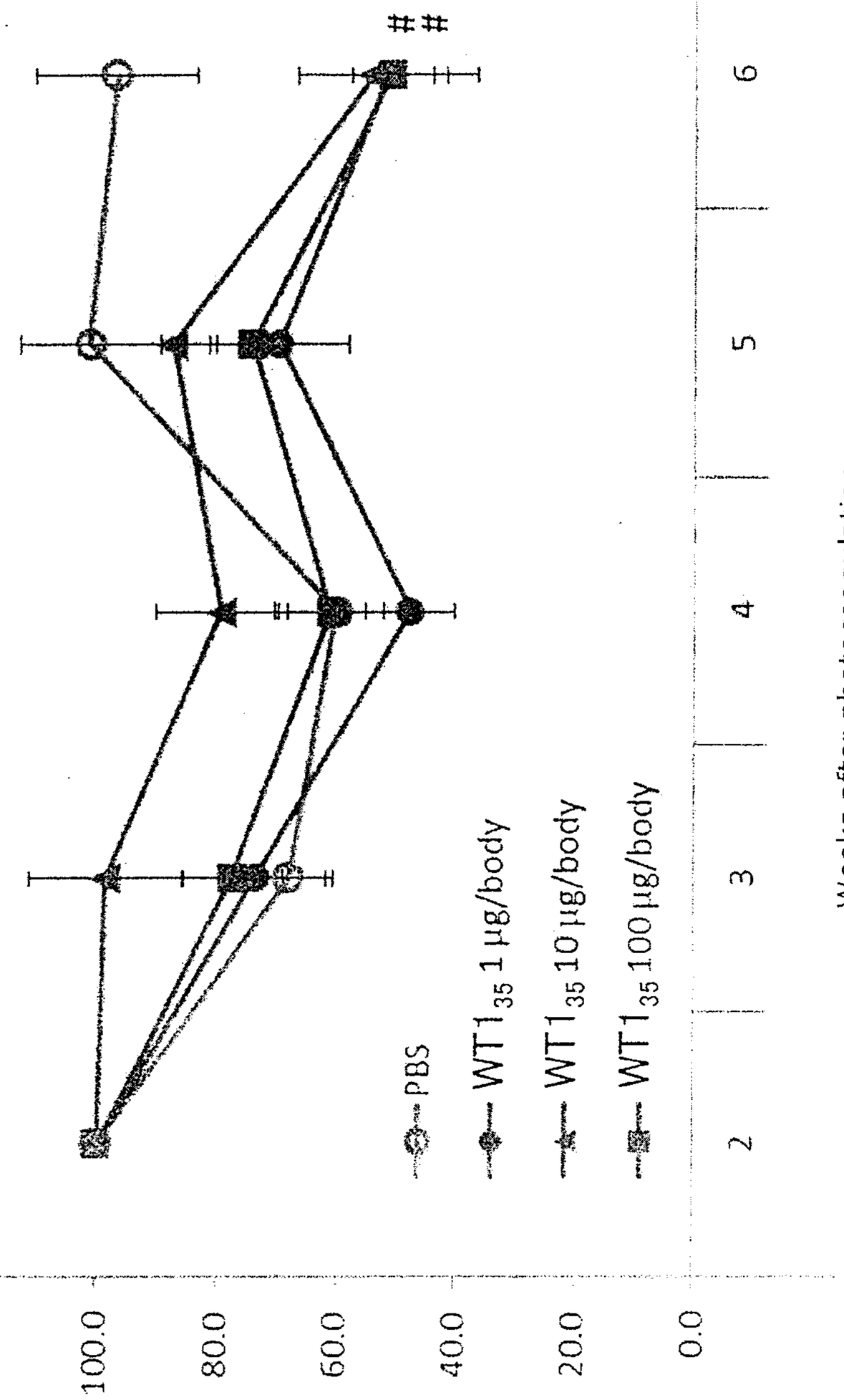

##
PBS
WT1 35 1 μg/body
WT1 35 10 μg/body
WT1 35 100 μg/body
2
3
4
5
6
Weeks after photocoagulation
#: p<0.05 vs PBS group (Dunnett's multiple comparison test).
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Change in choroidal neovascularization area (%)

Fig. 5

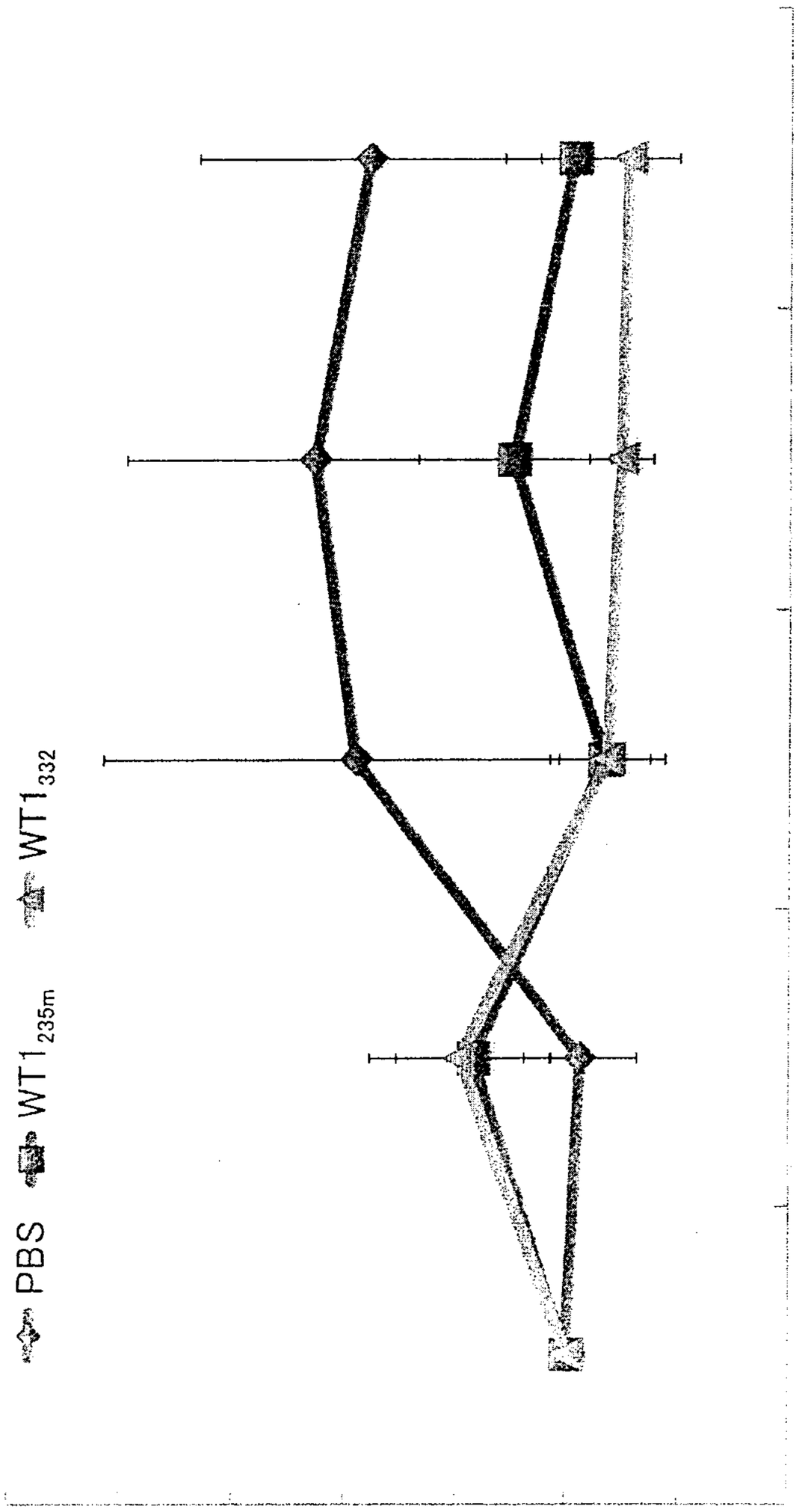
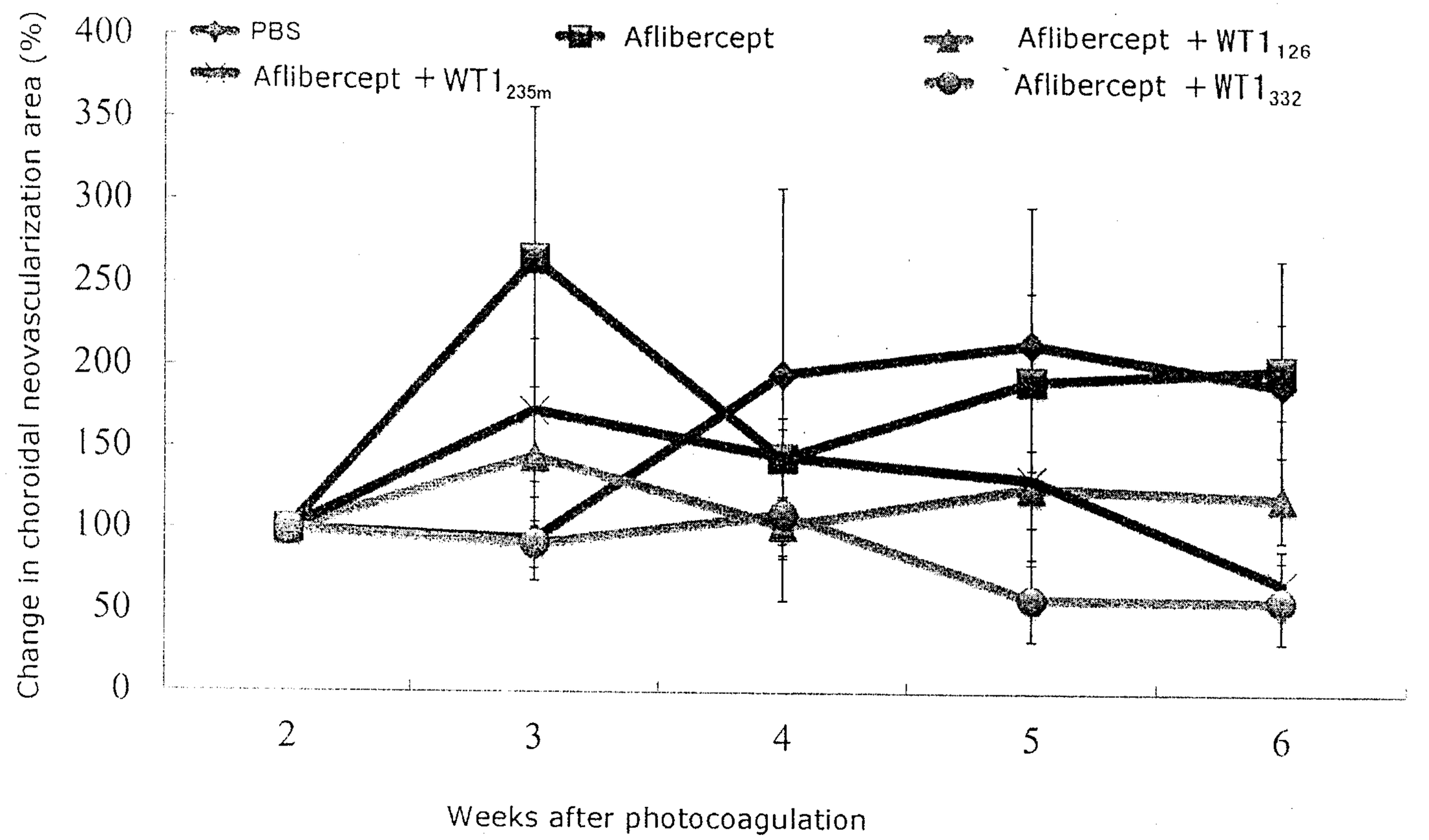
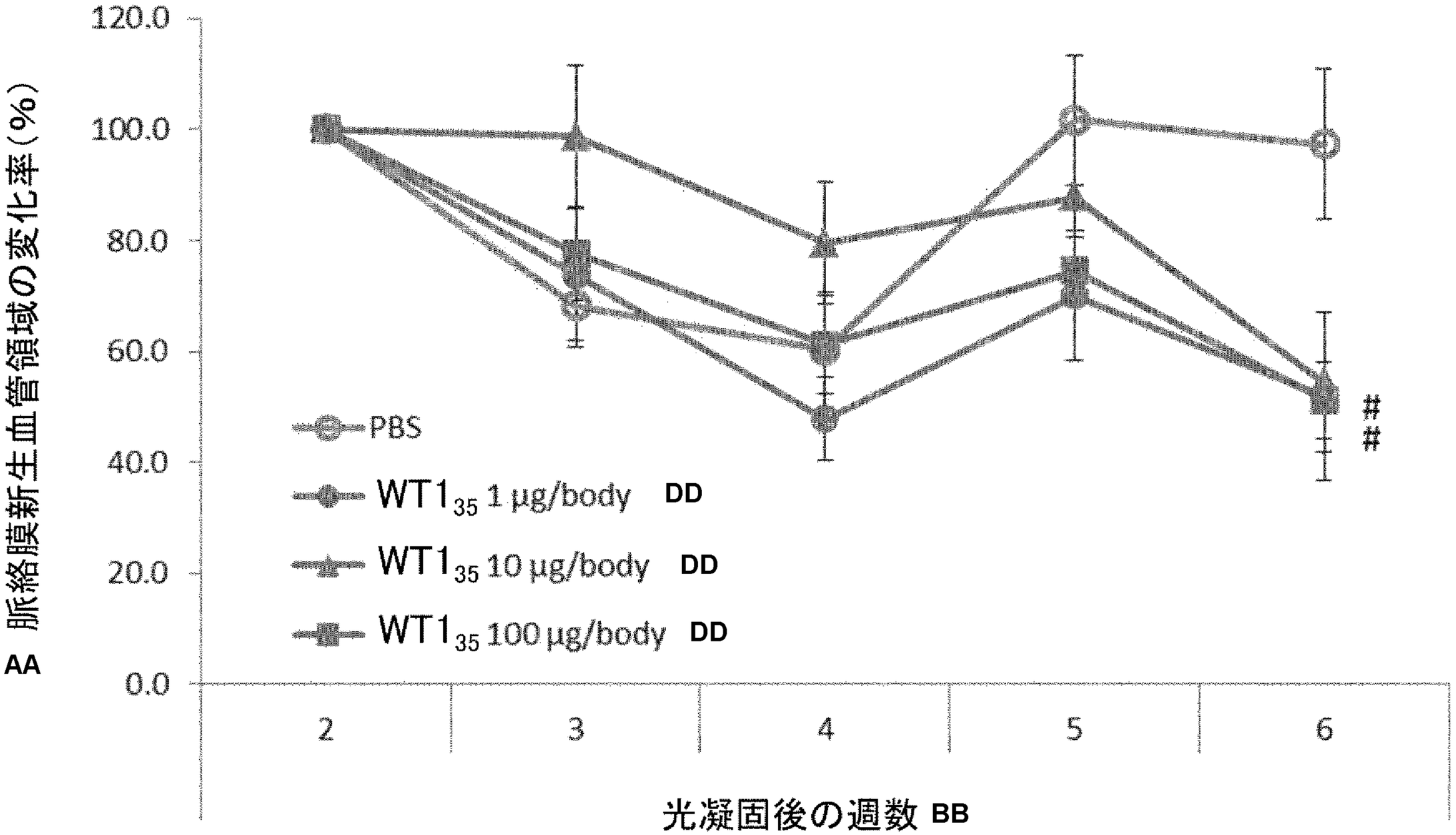

PBS
WT1 235m
WT1 332
Weeks after photocoagulation
2
3
4
5
6
n=7-10 ±S.E.
Mean
Change in chorioidal neovascularization area (%)
350
300
250
200
150
100
50
0

Fig. 6



n=7-11
Mean±S.E.

[図4]



#: p<0.05 vs PBS group (Dunnett's multiple comparison test). CC

FIG. 4:
AA Change (%) in choroidal neovascularization area
BB Time (weeks) after photocoagulation
CC p<0.05 vs PBS group (Dunnett's multiple comparison test)
DD body