cancer treatment using peptide vaccines comprising selecting multiple adequate peptides to be administered, and safety and anti-tumor effects thereof have been verified. Through administration of tailor-made peptide vaccines alone or in combination with anti-cancer drugs, more specifically, excellent clinical effects and safety have been achieved in cases of brain tumor, uterine cervix cancer, prostate cancer, and pancreatic cancer (Terasaki, M. et al., J. Clin. Oncol., 2011, 29 (3): 337-44; Noguchi, M. et al., Cancer Immunol. Immunother., 2010, 59 (7): 1001-9; Yanagimoto, H. et al., Cancer Sci., 2007, 98 (4): 605-11).

The cell-mediated immunity consisting of epitope specific cytotoxic T lymphocytes (hereafter, abbreviated as "CTL"), which are considered to be major effector cells in cancer treatment using peptide vaccines, is HLA-restrictive. Accordingly, development of peptide vaccines for cancer exclusively targeting patients with a particular HLA type, specifically HLA-A2 or HLA-A24 type, has been attempted because of the large number of patients therewith.

However, Japanese people with such two HLA types account for approximately 40% and 60%, respectively (Sette, A. et al., Immunogenetics, 1999, 50 (3-4): 201-12). Disadvantageously, patients with other HLA types cannot gain benefits from peptide vaccines for cancer. In addition, the time of initiation of treatment would be postponed because of HLA typing performed prior to the initiation of treatment, and it would increase the burden on patients. Accordingly, research and development of peptide vaccines for cancer that are applicable to all cancer patients without HLA typing are desired.

Regarding cancer treatment using peptide vaccines, in addition to activations of CTLs as one of the cell-mediated immunity, inductions of production of immunoglobulins known as the humoral immunity is known to be attributable to survival benefit (Noguchi, M. et al., Cancer Biol. Ther., 2011, 10 (12):1266-79).

Summary of the Invention

In one aspect, there is provided a peptide having 4 linked epitopes, wherein the 4 epitope peptides are selected independently from the group consisting of CTL epitope peptides: the peptide as shown in SEQ ID NO: 1 (PEP1); the peptide as shown in SEQ ID NO: 2 (PEP2); the peptide as shown in SEQ ID NO: 4 (PEP4); the peptide as shown in SEQ ID NO: 5 (PEP5); the peptide as shown in SEQ ID NO: 6 (PEP6); the peptide as shown in SEQ ID NO: 7 (PEP7); the peptide as shown in SEQ ID NO: 8 (PEP8); the peptide as shown in SEQ ID NO: 9 (PEP9); the peptide as shown in SEQ ID NO: 10 (PEP10); the peptide as shown in SEQ ID NO: 13 (PEP13); the peptide as shown in SEQ ID NO: 15 (PEP15); the peptide as shown in SEQ ID NO: 17 (PEP17); and the peptide as shown in SEQ ID NO: 18 (PEP18), and are linked via linkers, wherein the peptide having 4 linked epitopes optionally comprise other peptide sequences consisting of hydrophilic amino acids, and wherein the peptide having 4 linked epitopes comprises PEP4 at the C terminus.

In another aspect, there is provided a peptide having 4 linked epitopes, wherein the 4 epitope peptides are selected independently from the group consisting of CTL epitope peptides: the peptide as shown in SEQ ID NO: 1 (PEP 1); the peptide as shown in SEQ ID NO: 2 (PEP2); the peptide as shown in SEQ ID NO: 4 (PEP4); the peptide as shown in SEQ ID NO: 5 (PEP5); the peptide as shown in SEQ ID NO: 6 (PEP6); the peptide as shown in SEQ ID NO: 7 (PEP7); the peptide as shown in SEQ ID NO: 8 (PEP8); the peptide as shown in SEQ ID NO: 9 (PEP9); the peptide as shown in SEQ ID NO: 10 (PEP 10); the peptide as shown in SEQ ID NO: 13 (PEP 13); the peptide as shown in SEQ ID NO: 15 (PEP 15); the peptide as shown in SEQ ID NO: 17 (PEP17); and the peptide as shown in SEQ ID NO: 18 (PEP18), and are linked via linkers, wherein the peptide having 4 linked epitopes optionally comprise other peptide sequences consisting of hydrophilic amino acids, and wherein the peptide having 4 linked epitopes comprises a sequence selected from the following sequences, wherein "-(L)-" represents a linker:

- PEP5-(L)-PEP6-(L)-PEP9-(L)-PEP4;
- PEP9-(L)-PEP5-(L)-PEP6-(L)-PEP4;
- PEP6-(L)-PEP5-(L)-PEP9-(L)-PEP4;
- PEP6-(L)-PEP9-(L)-PEP5-(L)-PEP4;

- PEP9-(L)-PEP6-(L)-PEP5-(L)-PEP4;
- PEP5-(L)-PEP9-(L)-PEP6-(L)-PEP4;
- PEP 1-(L)-PEP7-(L)-PEP8-(L)-PEP2;
- PEP 1-(L)-PEP8-(L)-PEP7-(L)-PEP2;
- PEP7-(L)-PEP 1-(L)-PEP8-(L)-PEP2;
- PEP8-(L)-PEP7-(L)-PEP1-(L)-PEP2;
- PEP8-(L)-PEP 1-(L)-PEP7-(L)-PEP2;
- PEP7-(L)-PEP13-(L)-PEP8-(L)-PEP2;
- PEP8-(L)-PEP13-(L)-PEP7-(L)-PEP2;
- PEP 13-(L)-PEP7-(L)-PEP8-(L)-PEP2;
- PEP8-(L)-PEP7-(L)-PEP13-(L)-PEP2;
- PEP 13-(L)-PEP8-(L)-PEP7-(L)-PEP2;
- PEP 1 3-(L)-PEP 1 5-(L)-PEP 1 8-(L)-PEP10;
- PEP 1 3-(L)-PEP 1 8-(L)-PEP 1 5-(L)-PEP10;
- PEP 1 5-(L)-PEP 1 3-(L)-PEP 1 8-(L)-PEP10;
- PEP 1 5-(L)-PEP 1 8-(L)-PEP 1 3-(L)-PEP10;
- PEP 1 8-(L)-PEP 1 3-(L)-PEP 1 5-(L)-PEP10;
- PEP 1 8-(L)-PEP 1 5-(L)-PEP 1 3-(L)-PEP10;
- 43 - PEP1-(L)-PEP15-(L)-PEP18-(L)-PEP10;
- PEP1-(L)-PEP18-(L)-PEP15-(L)-PEP10;
- PEP15-(L)-PEP1-(L)-PEP18-(L)-PEP10;
- PEP15-(L)-PEP18-(L)-PEP1-(L)-PEP10;
- PEP18-(L)-PEP1-(L)-PEP15-(L)-PEP10;
- PEP18-(L)-PEP15-(L)-PEP1-(L)-PEP10;
- PEP 1 5-(L)-PEP 1 7-(L)-PEP 1 3-(L)-PEP10;
- PEP 1 5-(L)-PEP 1 3-(L)-PEP 1 7-(L)-PEP10;
- PEP 1 7-(L)-PEP 1 5-(L)-PEP 1 3-(L)-PEP10;
- PEP 1 7-(L)-PEP 1 3-(L)-PEP 1 5-(L)-PEP10;
- PEP 1 3-(L)-PEP 1 7-(L)-PEP 1 5-(L)-PEP10;

- PEP 1 3-(L)-PEP 1 5-(L)-PEP 1 7-(L)-PEP10;

- PEP6-(L)-PEP18-(L)-PEP5-(L)-PEP9;

- PEP6-(L)-PEP5-(L)-PEP18-(L)-PEP9;

- PEP 1 -(L)-PEP7-(L)-PEP6-(L)-PEP5; and

- PEP7-(L)-PEP 1-(L)-PEP6-(L)-PEP.

In another aspect, there is provided a combination of CTLs obtained by stimulating peripheral blood lymphocytes using the peptide having 4 linked epitopes as described herein.

In yet another aspect, there is provided a pharmaceutical composition comprising, as an active ingredient, the peptide having 4 linked epitopes as described herein or the combination as described herein.

In yet another aspect, there is provided a pharmaceutical composition comprising two or more peptides selected from among the peptides having 4 linked epitopes as described herein.

In yet another aspect, there is provided the use of the peptide having 4 linked epitopes as described herein, the combination as described herein, or the pharmaceutical composition as described herein for the manufacture of a medicament for treating cancer.

Objects to Be Attained by the Invention

An object of the present invention is to provide a cancer antigen peptide that can be administered as a peptide vaccine for cancer to a wide range of cancer patients and that can strongly induce immunoglobulins, regardless of the HLA types of patients.

CLAIMS

[1] A peptide having 4 linked epitopes, wherein the 4 epitope peptides are selected independently from the group consisting of CTL epitope peptides: the peptide as shown in SEQ ID NO: 1 (PEP1); the peptide as shown in SEQ ID NO: 2 (PEP2); the peptide as shown in SEQ ID NO: 4 (PEP4); the peptide as shown in SEQ ID NO: 5 (PEP5); the peptide as shown in SEQ ID NO: 6 (PEP6); the peptide as shown in SEQ ID NO: 7 (PEP7); the peptide as shown in SEQ ID NO: 8 (PEP8); the peptide as shown in SEQ ID NO: 9 (PEP9); the peptide as shown in SEQ ID NO: 10 (PEP10); the peptide as shown in SEQ ID NO: 13 (PEP13); the peptide as shown in SEQ ID NO: 15 (PEP15); the peptide as shown in SEQ ID NO: 17 (PEP17); and the peptide as shown in SEQ ID NO: 18 (PEP18), and are linked via linkers, wherein the peptide having 4 linked epitopes optionally comprise other peptide sequences consisting of hydrophilic amino acids, and wherein the peptide having 4 linked epitopes comprises PEP4 at the C terminus.

[2] A peptide having 4 linked epitopes, wherein the 4 epitope peptides are selected independently from the group consisting of CTL epitope peptides: the peptide as shown in SEQ ID NO: 1 (PEP 1); the peptide as shown in SEQ ID NO: 2 (PEP2); the peptide as shown in SEQ ID NO: 4 (PEP4); the peptide as shown in SEQ ID NO: 5 (PEP5); the peptide as shown in SEQ ID NO: 6 (PEP6); the peptide as shown in SEQ ID NO: 7 (PEP7); the peptide as shown in SEQ ID NO: 8 (PEP8); the peptide as shown in SEQ ID NO: 9 (PEP9); the peptide as shown in SEQ ID NO: 10 (PEP 10); the peptide as shown in SEQ ID NO: 13 (PEP 13); the peptide as shown in SEQ ID NO: 15 (PEP 15); the peptide as shown in SEQ ID NO: 17 (PEP17); and the peptide as shown in SEQ ID NO: 18 (PEP18), and are linked via linkers, wherein the peptide having 4 linked epitopes optionally - comprise other peptide sequences consisting of hydrophilic amino acids, and wherein the peptide having 4 linked epitopes comprises a sequence selected from the following sequences, wherein "-(L)-" represents a linker:

- PEP5-(L)-PEP6-(L)-PEP9-(L)-PEP4;
- PEP9-(L)-PEP5-(L)-PEP6-(L)-PEP4;

- PEP6-(L)-PEP5-(L)-PEP9-(L)-PEP4;

- PEP6-(L)-PEP9-(L)-PEP5-(L)-PEP4;

- PEP9-(L)-PEP6-(L)-PEP5-(L)-PEP4;

- PEP5-(L)-PEP9-(L)-PEP6-(L)-PEP4;

- PEP 1-(L)-PEP7-(L)-PEP8-(L)-PEP2;

- PEP 1-(L)-PEP8-(L)-PEP7-(L)-PEP2;

- PEP7-(L)-PEP 1-(L)-PEP8-(L)-PEP2;

- PEP8-(L)-PEP7-(L)-PEP1-(L)-PEP2;

- PEP8-(L)-PEP 1-(L)-PEP7-(L)-PEP2;

- PEP7-(L)-PEP13-(L)-PEP8-(L)-PEP2;

- PEP8-(L)-PEP13-(L)-PEP7-(L)-PEP2;

- PEP 13-(L)-PEP7-(L)-PEP8-(L)-PEP2;

- PEP8-(L)-PEP7-(L)-PEP13-(L)-PEP2;

- PEP 13-(L)-PEP8-(L)-PEP7-(L)-PEP2;

- PEP 1 3-(L)-PEP 1 5-(L)-PEP 1 8-(L)-PEP10;

- PEP 1 3-(L)-PEP 1 8-(L)-PEP 1 5-(L)-PEP10;

- PEP 1 5-(L)-PEP 1 3-(L)-PEP 1 8-(L)-PEP10;

- PEP 1 5-(L)-PEP 1 8-(L)-PEP 1 3-(L)-PEP10;

- PEP 1 8-(L)-PEP 1 3-(L)-PEP 1 5-(L)-PEP10;

- PEP 1 8-(L)-PEP 1 5-(L)-PEP 1 3-(L)-PEP10;

- 43 - PEP1-(L)-PEP15-(L)-PEP18-(L)-PEP10;

- PEP1-(L)-PEP18-(L)-PEP15-(L)-PEP10;

- PEP15-(L)-PEP1-(L)-PEP18-(L)-PEP10;

- PEP15-(L)-PEP18-(L)-PEP1-(L)-PEP10;

- PEP18-(L)-PEP1-(L)-PEP15-(L)-PEP10;

- PEP18-(L)-PEP15-(L)-PEP1-(L)-PEP10;

- PEP 1 5-(L)-PEP 1 7-(L)-PEP 1 3-(L)-PEP10;

- PEP 1 5-(L)-PEP 1 3-(L)-PEP 1 7-(L)-PEP10;

- PEP 1 7-(L)-PEP 1 5-(L)-PEP 1 3-(L)-PEP10;

- PEP 1 7-(L)-PEP 1 3-(L)-PEP 1 5-(L)-PEP10;
- PEP 1 3-(L)-PEP 1 7-(L)-PEP 1 5-(L)-PEP10;
- PEP 1 3-(L)-PEP 1 5-(L)-PEP 1 7-(L)-PEP10;
- PEP6-(L)-PEP18-(L)-PEP5-(L)-PEP9;
- PEP6-(L)-PEP5-(L)-PEP18-(L)-PEP9;
- PEP 1 -(L)-PEP7-(L)-PEP6-(L)-PEP5; and
- PEP7-(L)-PEP 1-(L)-PEP6-(L)-PEP.

[3] The peptide having 4 linked epitopes according to claim 1 or claim 2, which comprises 4 non-redundant peptides selected from among PEP1, PEP2, PEP4, PEP5, PEP6, PEP7, PEP8, PEP9, PEP10, PEP13, PEP15, PEP17, and PEP18.

[4] The peptide having 4 linked epitopes according to any one of claims 1 to 3, wherein the linker is an amino acid linker.

[5] The peptide having 4 linked epitopes according to any one of claims 1 to 4, wherein the amino acid linker is an arginine dimer or arginine trimer composed of two or three arginine residues linked to each other.

[6] The peptide having 4 linked epitopes according to any one of claims 1 to 5, wherein the other peptide sequence consisting of hydrophilic amino acids is linked to the N terminus.

[7] The peptide having 4 linked epitopes according to any one of claims 1 to 6, wherein the other peptide sequence consisting of hydrophilic amino acids is composed of an arginine trimer or arginine tetramer composed of three or four arginine residues linked to each other.

[8] The peptide having 4 linked epitopes according to any one of claims 1 to 7, which consists of the amino acid sequence as shown in SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO:

61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, or SEQ ID NO: 66.

[9] A combination of CTLs obtained by stimulating peripheral blood lymphocytes using the peptide having 4 linked epitopes according to any one of claims 1 to 8.

[10] A pharmaceutical composition comprising, as an active ingredient, the peptide having 4 linked epitopes according to any one of claims 1 to 8 or the combination according to claim 9.

[11] A pharmaceutical composition comprising two or more peptides selected from among the peptides having 4 linked epitopes according to any one of claims 1 to 8.

[12] The pharmaceutical composition according to claim 10 or claim 11, which is an immunotherapeutic agent.

[13] Use of the peptide having 4 linked epitopes according to any one of claims 1 to 8, the combination according to claim 9, or the pharmaceutical composition according to any one of claims 10 to 12 for the manufacture of a medicament for treating cancer.