Claims

[1] A water-in-oil (w/o) emulsion formulation containing 3 types of peptides having 4 linked CTL epitopes each comprising 4 CTL epitope peptides linked via linkers in an aqueous phase.

[2] The water-in-oil emulsion formulation according to claim 1, which contains 3 types of peptides having 4 linked CTL epitopes and an oil adjuvant.

[3] The water-in-oil emulsion formulation according to claim 1 or 2, which is obtained by mixing an aqueous phase comprising peptides having 4 linked CTL epitopes and an oil phase comprising an oil adjuvant and emulsifying the mixture, wherein the aqueous phase comprising peptides having 4 linked CTL epitopes is a solution comprising 3 types of peptides having 4 linked CTL epitopes: a peptide represented by Formula (I); a peptide represented by Formula (II); and a peptide represented by Formula (III):

A-(L)-B-(L)-C-(L)-PEP2 (I);

D-(L)-E-(L)-F-(L)-PEP4 (II); and

G-(L)-H-(L)-I-(L)-PEP10 (III),

in Formulae (I), (II), and (III),

(L) represents a linker;

PEP2 represents a CTL epitope peptide represented by SEQ ID NO: 2;

PEP4 represents a CTL epitope peptide represented by SEQ ID NO: 4;

PEP10 represents a CTL epitope peptide represented by SEQ ID NO: 10;

A, B, C, D, E, F, G, H, and I are each independently selected from the group consisting of a CTL epitope peptide represented by SEQ ID NO: 1 (PEP1), a CTL epitope peptide represented by SEQ ID NO: 5 (PEP5), a CTL epitope peptide represented by SEQ ID NO: 6 (PEP6), a CTL epitope peptide represented by SEQ ID NO: 7 (PEP7), a CTL epitope peptide represented by SEQ ID NO: 8 (PEP8), a CTL epitope peptide represented by SEQ ID NO: 9 (PEP9), a CTL epitope peptide represented by SEQ ID NO: 13 (PEP13), a CTL epitope peptide represented by SEQ ID NO: 15 (PEP15), and a CTL epitope peptide represented by SEQ ID NO: 18 (PEP18), except for a combination of A representing PEP7 and B representing PEP8; and

the peptides having 4 linked CTL epitopes represented by Formulae (I), (II), and (III) may each have a peptide sequence consisting of hydrophilic amino acids.

[4] The emulsion formulation according to claim 3, wherein, in Formulae (I), (II), and (III), (L) represents a linker; A, B, and C are each independently selected from the group consisting of PEP7, PEP8, and PEP13, except for a combination of A representing PEP7 and B representing PEP8; D, E, and F are each independently selected from the group consisting of PEP5, PEP6, and PEP9; and G, H, and I are each independently selected from the group consisting of PEP1, PEP15, and PEP18, and the peptide represented by Formula (I) may have a peptide sequence consisting of hydrophilic amino acids.

[5] The emulsion formulation according to claim 3 or 4, wherein the peptide represented by Formula (I) is selected from the group consisting of:

·PEP7-(L)-PEP13-(L)-PEP8-(L)-PEP2;

·PEP8-(L)-PEP7-(L)-PEP13-(L)-PEP2;

·PEP8-(L)-PEP13-(L)-PEP7-(L)-PEP2;

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

·PEP13-(L)-PEP8-(L)-PEP7-(L)-PEP2,

the peptide represented by Formula (II) is selected from the group consisting of:

·PEP5-(L)-PEP6-(L)-PEP9-(L)-PEP4;

·PEP5-(L)-PEP9-(L)-PEP6-(L)-PEP4;

·PEP6-(L)-PEP5-(L)-PEP9-(L)-PEP4;

·PEP6-(L)-PEP9-(L)-PEP5-(L)-PEP4;

·PEP9-(L)-PEP5-(L)-PEP6-(L)-PEP4; and

·PEP9-(L)-PEP6-(L)-PEP5-(L)-PEP4, and

the peptide represented by Formula (III) is selected from the group consisting of:

·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; and

·PEP18-(L)-PEP15-(L)-PEP1-(L)-PEP10, and

the peptide represented by Formula (I) may have a peptide sequence consisting of hydrophilic amino acids at the N terminus.

[6] The emulsion formulation according to any one of claims 3 to 5, wherein, in Formulae (I), (II), and (III), (L) represents an arginine dimer; and a peptide sequence consisting of hydrophilic amino acids is an arginine tetramer.

[7] The emulsion formulation according to any one of claims 3 to 6, wherein the 3 types of peptides having 4 linked CTL epitopes represented by Formulae (I), (II), and (III) are RRRR-PEP7-RR-PEP13-RR-PEP8-RR-PEP2 (SEQ ID NO: 19), PEP5-RR-PEP9-RR-PEP6-RR-PEP4 (SEQ ID NO: 25), and PEP15-RR-PEP18-RR-PEP1-RR-PEP10 (SEQ ID NO: 33).

[8] A method for preparing the emulsion formulation according to any one of claims 1 to 7 comprising the steps (i) and (ii):

(i) a step of injecting a part of an aqueous phase comprising peptides having 4 linked CTL epitopes into an oil phase comprising an oil adjuvant to prepare a mixture; and

(ii) a step of allowing the mixture to reciprocate between syringes connected via a syringe connector,

wherein the step (i) and the step (ii) are repeated to obtain an emulsion formulation comprising the total amount of the oil phase and the aqueous phase.

[9] The method according to claim 8, wherein the amount of a part of the aqueous phase is 1/10 to 1/2 fold (v/v) relative to the amount of the oil phase comprising an oil adjuvant.

[10] An apparatus used for preparing a water-in-oil emulsion formulation containing peptides, which is composed of:

a second syringe 2;

a syringe connector 5 connected to the second syringe 2;

a three-way stopcock 4 connected to the syringe connector 5; and

a first syringe 1 and a third syringe 3 further connected to the three-way stopcock 4,

wherein the apparatus enables production of a water-in-oil emulsion formulation by adding an aqueous phase filled in the third syringe 3 to the oil phase filled in the first syringe 1 or the second syringe 2 and allowing the resulting mixture to reciprocate between the first syringe 1 and the second syringe 2 via the syringe connector 5.

[11] The apparatus according to claim 10, wherein the volumes of the first, the second, and the third syringes are each 3 ml or less.

[12] The apparatus according to claim 10 or 11, wherein an inner diameter of the syringe connector 5 through which the mixture passes is within a range from 0.8 to 1.7 mm.

[13] A kit used for preparing an w/o emulsion containing peptides having 4 linked CTL epitopes, which comprises peptides having 4 linked CTL epitopes each comprising 4 CTL epitope peptides linked via linkers, an oil adjuvant, and the apparatus according to any one of claims 10 to 12.

~~[14] A method for treatment of tumor of a patient comprising administering the water-in-oil (w/o) emulsion formulation according to any one of claims 1 to 7 to a patient.~~

[~~15~~14] The water-in-oil (w/o) emulsion formulation according to any one of claims 1 to 7 for use in the treatment of tumor.

[~~16~~15] Use of a solution of peptides having 4 linked CTL epitopes in the manufacture of the water-in-oil (w/o) emulsion formulation according to any one of claims 1 to 7.

[~~17~~16] The apparatus according to any one of claims 10 to 12 or the kit according to claim 13, which is used for preparing the water-in-oil (w/o) emulsion formulation for the treatment of tumor at the time of use.

[~~18~~17] The apparatus according to any one of claims 10 to 12 or the kit according to claim 13, wherein the water-in-oil (w/o) emulsion formulation is the formulation according to any one of claims 1 to 7.

~~[19] A method for treatment of tumor of a patient comprising:~~

~~(a) preparing the emulsion formulation according to any one of claims 1 to 7 by performing the step (i) and the step (ii):~~

~~(i) a step of injecting a part of an aqueous phase comprising peptides having 4 linked CTL epitopes into an oil phase comprising an oil adjuvant to prepare a mixture; and~~

~~(ii) a step of allowing the mixture to reciprocate between syringes connected via a syringe connector, and~~

~~repeating the step (i) and the step (ii) to obtain the emulsion formulation comprising the total amount of the oil phase and the aqueous phase; and~~

~~(b) administering the resulting emulsion formulation to a patient having tumor.~~