

Fig. 31 is a graph showing the results of the blood toxicity (leukocyte count) in tumor-bearing mice after administration of a ^{225}Ac -labeled antibody prepared using ^{225}Ac -labeled DOTAGA-DBCO.

5 Fig. 32 is a graph showing the results of the blood toxicity (platelet count) in tumor-bearing mice after administration of a ^{225}Ac -labeled antibody prepared using ^{225}Ac -labeled DOTAGA-DBCO.

Fig. 33 is a graph showing the results of the
10 hepatotoxicity (ALT, AST) in tumor-bearing mice after administration of a ^{225}Ac -labeled antibody prepared using ^{225}Ac -labeled DOTAGA-DBCO.

Fig. 34 is a graph showing the results of the kidney toxicity (BUN) in tumor-bearing mice after administration of a
15 ^{225}Ac -labeled antibody prepared using ^{225}Ac -labeled DOTAGA-DBCO.

Fig. 35 shows the results of PET-CT imaging over time using a [^{89}Zr]Random-DFO-anti-MUC5AC humanized antibody.

Fig. 36 is a graph showing the results of VOI (volume of interest, three-dimensional ROI) analysis of the tumor (-●-),
20 heart (blood) (...■...) and liver (...Δ...) at each time point in PET images of a [^{89}Zr]Random-DFO-anti-MUC5AC humanized antibody.
[Description of Embodiments]

[0012]

Unless otherwise specified, the terms used in the present
25 specification can be used in the meaning generally used in the art.

[0013]

(1) Conjugate 1

The present invention provides a conjugate of a chelating
30 agent chelated with a radionuclide (hereinafter to be also referred to as the chelating agent of the present invention), and an antibody, wherein the aforementioned radionuclide is a metal nuclide emitting α particle, and the aforementioned antibody is a humanized antibody specifically binding to MUC5AC

preferably not more than 5, most preferably not more than 3,
amino acids are deleted, substituted, or added, is also used as
the heavy chain variable region of the humanized antibody to be
used in the present invention as long as it can bind to MUC5AC
5 when combined with the light chain variable region in the
present invention.

[0026]

The light chain variable region of the humanized antibody
to be used in the present invention is not limited to the amino
10 acid sequence shown in SEQ ID NO: 5 to SEQ ID NO: 8 and also
includes variants maintaining functions. That is, a mutated
light chain variable region consisting of an amino acid
sequence having not less than 90%, preferably not less than 95%,
further preferably not less than 98%, most preferably not less
15 than 99%, sequence identity with the amino acid sequence shown
in SEQ ID NO: 5 to SEQ ID NO: 8 is also used as the light chain
variable region of the humanized antibody to be used in the
present invention as long as it can bind to MUC5AC when
combined with the heavy chain variable region in the present
20 invention.

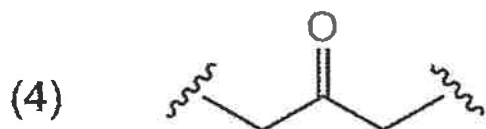
[0027]

In addition, as the light chain variable region of the
humanized antibody to be used in the present invention, a
mutated light chain variable region consisting of the amino
25 acid sequence shown in SEQ ID NO: 5 to SEQ ID NO: 8, wherein
not more than 10, preferably not more than 8, further
preferably not more than 5, most preferably not more than 3,
amino acids are deleted, substituted, or added, is also
encompassed in the light chain variable region of the humanized
30 antibody to be used in the present invention as long as it can
bind to MUC5AC when combined with the heavy chain variable
region in the present invention.

[0028]

The humanized antibody to be used in the present
35 invention can be produced by a method generally performed in

amino acid having a thiol group in the side chain, and may be the same or different. Examples of the amino acid having a thiol group in the side chain include cysteine and homocysteine. Such amino acid residues are preferably bonded by a disulfide
5 bond, or a sulfide group is preferably bonded thereto via a linker shown by the following formula (4). In the formula (4), the wavy line indicates the binding part with the sulfide group.
[0046]



10 [0047]

Instead of the aforementioned combination of Xaa1 and Xaa3, one of Xaa1 and Xaa3 may be an amino acid residue derived from an amino acid having a thiol group in the side chain, and the other may be an amino acid residue derived from an amino
15 acid having a haloacetyl group in the side chain. These are bonded via a thioether bond. The terminal of the haloacetyl group is substituted with a halogen such as iodine or the like, and the halogen is eliminated by a reaction with the thiol group in the other side chain, whereby a thioether bond is
20 formed.

[0048]

Specific examples of the amino acid sequence of the antibody-modification peptide represented by the formula (i) include the peptides described in WO 2016/186206, WO
25 2017/217347 and WO 2018/230257, and these can also be used.
[0049]

Among these, the amino acid sequence of the antibody-modification peptide preferably has any one of the following sequences (1) - (14), more preferably the following sequence
30 (1), (2), (13) or (14). In the following amino acid sequences (1) - (14), (Xaa2) is a lysine residue, a cysteine residue, an aspartic acid residue, a glutamic acid residue, a 2-

buffer), tetramethylammonium acetate buffer and the like, and the like can be used.

[0075]

While the amount of the solvent is not particularly
5 limited, from the aspect of practicality in the production step, the lower limit at the start of step (A) is not less than 0.01 mL, preferably not less than 0.1 mL, more preferably not less than 1.0 mL, further preferably not less than 10 mL, further more preferably not less than 100 mL, and upper limit is
10 preferably not more than 1000 mL, more preferably not more than 100 mL, further preferably not more than 10 mL, further more preferably not more than 1.0 mL. For example, it is within the range of not less than 0.01 mL and not more than 100 mL.

[0076]

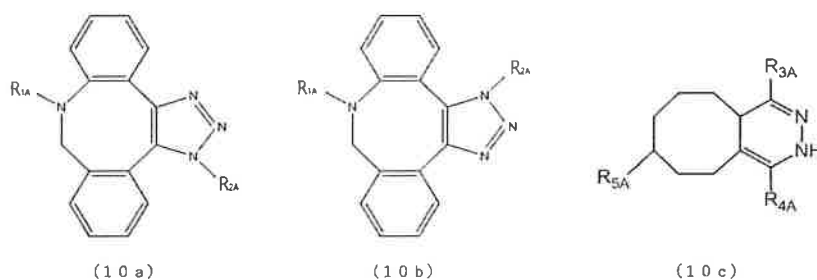
15 As the concentration of the chelating agent in the reaction mixture of the complex formation reaction, from the aspect of the yield of the desired chelating agent, the lower limit at the start of step (A) is each independently preferably not less than 0.001 $\mu\text{mol/L}$, more preferably not less than 0.01
20 $\mu\text{mol/L}$, further preferably not less than 0.1 $\mu\text{mol/L}$, more preferably not less than 1 $\mu\text{mol/L}$, and the upper limit is preferably not more than 1000 $\mu\text{mol/L}$, more preferably not more than 100 $\mu\text{mol/L}$, further preferably not more than 10 $\mu\text{mol/L}$. For example, it is within the range of not less than 1 $\mu\text{mol/L}$
25 and not more than 100 $\mu\text{mol/L}$.

[0077]

The temperature of the complex formation reaction may be, for example, room temperature (25°C) or under heating conditions. To simultaneously achieve suppression of
30 decomposition of the chelating agent and improvement of complex formation efficiency, the lower limit is preferably not less than 20°C, more preferably not less than 30°C, further preferably not less than 35°C, further more preferably not less than 37°C, particularly preferably not less than 45°C. The
35 upper limit is preferably not more than 150°C, more preferably

contained at any ratio.

[0108]



[0109]

5 In the formula (10a) and the formula (10b), R_{1A} is a binding site with a chelate linker, and R_{2A} is a binding site with an antibody-modification linker. In the formula (10c), one of R_{3A} and R_{4A} is a hydrogen atom, a methyl group, a phenyl group or a pyridyl group, and the other is a binding site with
10 a chelate linker, and R_{5A} is a binding site with an antibody-modification linker.

[0110]

(1-5) Radiopharmaceutical

The conjugates produced by the methods of the
15 aforementioned (1-4) may also be used as they are or after purification for the preparation of radiopharmaceuticals containing the conjugate as the active ingredient. A radiopharmaceutical refers to a composition containing the conjugate of the present invention, namely, an anti-MUC5AC
20 humanized antibody labeled with a radionuclide (metal nuclide emitting α particle) or a derivative thereof, and in a form suitable for administration to the body of a subject. A radiopharmaceutical can be produced, for example, by dissolving the conjugate of the present invention produced by the
25 aforementioned method in a solvent mainly composed of water and substantially isotonic with a living body. In this case, the radiopharmaceutical is preferably in the form of an aqueous

[0179]

Table 11

Comparison of tumor weight

	antibody control group	²²⁵ Ac-labeled monovalent antibody administration group
administered radioactivity amount (kBq)	-	25
tumor weight (g)	2.48±0.45	0.56±0.20*

mean±standard deviation, n=5 significance level: *P<0.05 vs
5 antibody control group

[0180]

The results of the changes in body weight over time are shown in Fig. 11. In the ²²⁵Ac-labeled monovalent antibody
10 administration group, a decrease in the body weight was found at an early stage of administration; however, it was not below 0.9 in a relative ratio.

[0181]

A dissection was performed on the last day of the
15 observation period, and liver, kidney and spleen were collected and weighed. The comparison results of the organ are shown in the following Table. It was found that only the weight of spleen is statistically significantly low in the ²²⁵Ac-labeled monovalent antibody administration group as compared with the
20 antibody control group.

in each tissue at each time point among the ^{89}Zr -labeled antibodies.

[0226]

Example 14: Biodistribution experiment of ^{89}Zr -labeled anti-MUC5AC humanized antibodies prepared using ^{89}Zr -labeled DOTA-Bn-DBCO and DOTAGA-DBCO

For confirmation of more detailed pharmacokinetics, ^{89}Zr -labeled antibodies prepared using ^{89}Zr -labeled DOTA-Bn-DBCO and DOTAGA-DBCO were administered to tumor-bearing mice, and biodistribution experiment was performed 20, 68, 188 hr after administration. The ^{89}Zr -labeled antibodies were produced according to Example 10 as in Example 13.

[0227]

For the biodistribution experiment, the ^{89}Zr -labeled antibodies were administered from the tail vein to tumor-bearing mice prepared by a method similar to that in Example 4-2. The radiochemical purity and animal information of the administered the ^{89}Zr -labeled anti-MUC5AC humanized antibodies are shown in Table 24. The radioactivity amount of about 5 MBq was administered to all groups.

[0228]

[Table 24]

Administered radioactivity amount and animal information (on administration of the ^{89}Zr -labeled antibodies)

name of antibody	^{89}Zr -labeled anti-MUC5AC humanized antibody					
	DOTA-Bn			DOTAGA		
time point (hr)	20	68	188	20	68	188
tumor volume (mm ³)	409 ±78	441 ±150	484 ±149	482 ±124	513 ±206	505 ±155
body weight (g)	21.5 ±1.2	22.0 ±0.5	22.7 ±1.4	21.2 ±1.1	22.5 ±0.7	22.4 ±1.2
radiochemical purity (%)	97%			95%		

(mean±standard deviation, n=4)

[0229]