DESCRIPTION

Title of Invention: CONJUGATE OF ANTI-CDH3 HUMANIZED ANTIBODY AND DRUG, AND USE THEREOF

Technical Field

[0001]

The present invention relates to a conjugate of an anti-CDH3 humanized antibody and a drug. The present invention further relates to a medicine comprising the above-described conjugate.

Background Art

[0002]

Cancers are major diseases and leading causes of death, yet the need for effective treatments remains unmet. In recent years, to address the issue of conventional chemotherapy damaging normal cells, there has been active research into cancer treatment using molecularly targeted drugs, i.e., agents designed to target specific molecules expressed exclusively on cancer cells.

[0003]

CDH3 (P-cadherin), a cell membrane surface antigen, was identified as one of the targets. CDH3 is a membrane protein discovered as a molecule involved in calcium-dependent homophilic cell adhesion. Proteins having cadherin repeats consisting of approximately 110 amino acid residues with high mutual homology are referred to as the cadherin superfamily, and CDH3 is a major member of this group.

[0004]

Examples of elevated CDH3 expression in certain types of cancer cells have been reported, and antibody-based cancer therapies are being investigated for cancers where CDH3 expression is higher in tumor tissue than in normal tissue.

[0005]

One effective means of enhancing the anti-cancer activity of an antibody is to conjugate it with a substance (drug) that has potent toxicity. Administering a highly toxic substance singularly to a patient causes damage to normal tissue as well and thus cannot serve as an effective therapeutic means. However, when a drug is linked to an antibody that binds to a cancer cell-specific antigen, it leads to the acquisition of the ability to kill only cancer cells without adversely affecting normal tissue. Such drugs are called antibody-drug conjugates (ADCs). In other words, drugs exhibit no toxicity when conjugated to antibodies. However, once certain types of antibodies are conjugated to cells expressing their target antigens, they are internalized into the cells and degraded within lysosomes. Therefore, after a certain type of antibody conjugated with a drug is internalized into a cell, the antibody is intracellularly degraded, the drug is released, and toxicity is manifested only within specific

cells, which is effective for killing these cells.

[0006]

Patent Documents 1 and 2 describe anti-CDH3 (P-cadherin) antibodies and antibody-drug conjugates thereof. Patent Documents 3 to 6 describe antibody-drug conjugates and synthetic intermediates thereof.

Prior Art Documents

Patent Documents

[0007]

Patent Document 1: WO2013/150623

Patent Document 2: WO2014/126198

Patent Document 3: WO2023/163227

Patent Document 4: WO2023/163234

Patent Document 5: WO2023/234426

Patent Document 6: WO2023/234427

Summary of Invention

Objects to be Solved by the Invention

[0008]

It is an object of the present invention to provide a novel anti-CDH3 humanized antibody-drug conjugate.

Means for Solving the Objects

[0009]

As a result of intensive studies directed towards achieving the above-described objects, the present inventors have succeeded in combining a complementarity-determining region (CDR) sequence defined by an antibody that specifically recognizes CDH3 with various human-derived framework region (FR) sequences, and introducing an appropriate amino acid mutation to improve affinity so as to generate an anti-CDH3 humanized antibody with low immunogenicity, and further using the anti-CDH3 humanized antibody to generate an anti-CDH3 humanized antibody-drug conjugate that more efficiently kills cancer cells expressing CDH3; thus, the present invention has been completed. According to the present invention, the following inventions are provided.

[0010]

<1> An antibody-drug conjugate in which an anti-CDH3 humanized antibody and a chemotherapeutic agent having a linker are linked, wherein

the anti-CDH3 humanized antibody is:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an

amino acid sequence set forth in SEQ ID NO: 2;

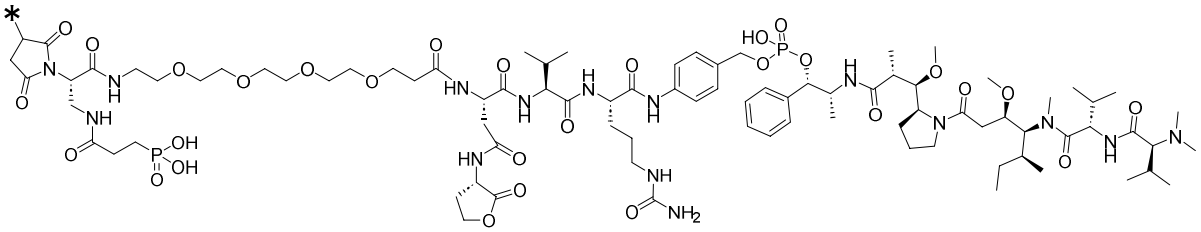
(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4;

(3) a fragment of the anti-CDH3 humanized antibody described in (1) or (2) above, which has CDH3-binding ability; or

(4) an antibody consisting of a partial sequence of the antibody described in (1) or (2) above, which has CDH3-binding ability,

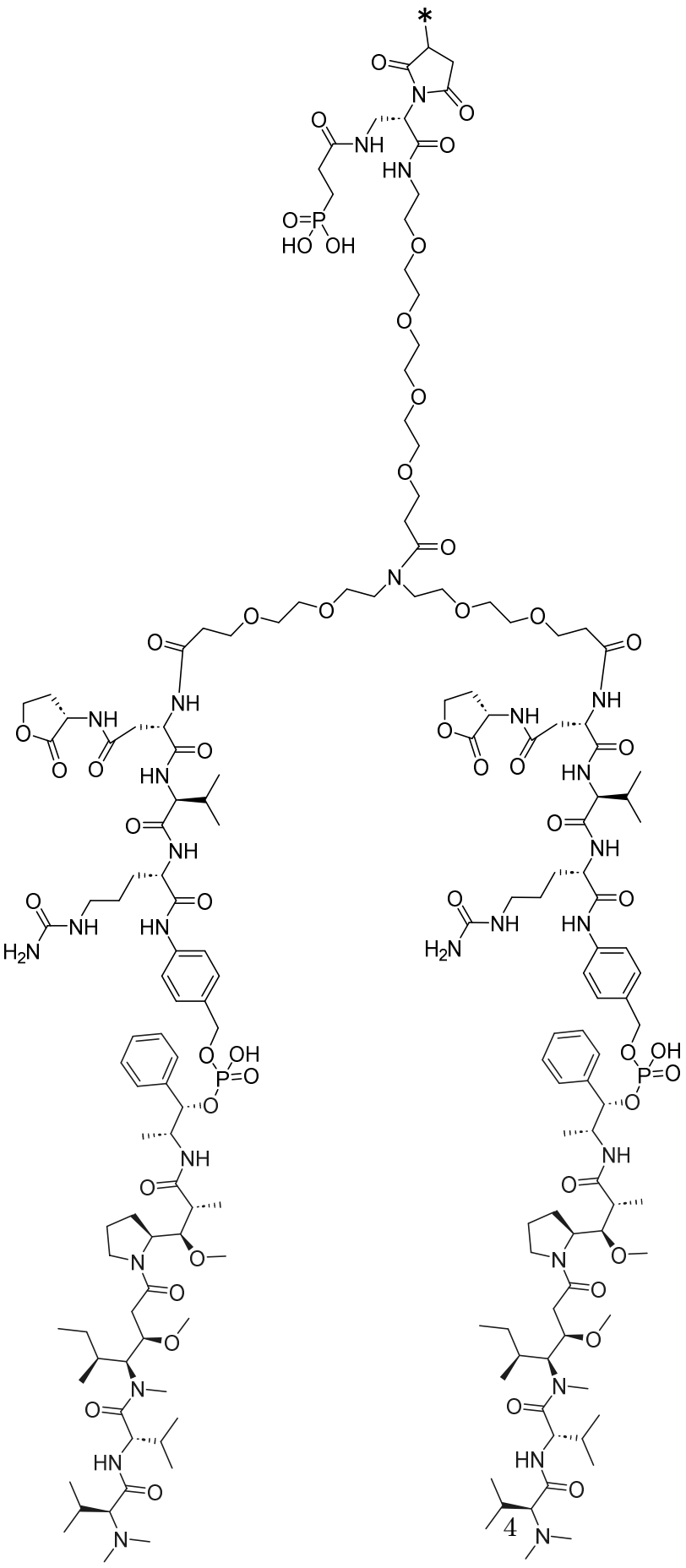
wherein

the chemotherapeutic agent having a linker is given by Formula (x):



(where * indicates a linking point with the antibody); or

Formula (y):



(where * indicates a linking point with the antibody),
pharmacologically acceptable salt thereof, or hydrate thereof.
<2> The antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to <1>, wherein the anti-CDH3 humanized antibody is any of the following antibodies:
(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or
(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.
<3> The antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to <1>, wherein an average of 1 to 10 molecules of the chemotherapeutic agent having a linker are conjugated to one molecule of the humanized anti-CDH3 antibody.
<4> A medicament for treating a disease characterized by overexpression of CDH3, comprising the antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to any one of <1> to <3>.
<5> The medicament according to <4>, wherein the disease characterized by overexpression of CDH3 is a tumor or cancer.
[0011]

The present invention further provides a method for treating a disease characterized by CDH3 overexpression, comprising administering the aforementioned antibody-drug conjugate to a patient.

The present invention further provides the use of the aforementioned antibody-drug conjugate in producing a medicament for treating a disease characterized by overexpression of CDH3.

The present invention further provides the aforementioned antibody-drug conjugate for use in treating a disease characterized by overexpression of CDH3.

Advantageous Effects of Invention

[0012]

The humanized anti-CDH3 antibody is expected to exhibit reduced immunogenicity compared to its original antibody. A humanized antibody is composed of an appropriate combination of the CDR sequences of the original antibody and human-derived FR sequences. In a case in which the antibody fails to demonstrate affinity for the antigen, attempts can be made to restore affinity by introducing further amino acid mutations into the antibody variable regions. An anti-CDH3 humanized antibody that specifically binds to CDH3 can be obtained by combining CDR sequences with appropriate FR sequences and introducing amino acid mutations, as necessary. An

immunoconjugate formed by linking the anti-CDH3 humanized antibody of the present invention obtained in this manner with a chemotherapeutic agent exhibits potent cytotoxic activity against CDH3-expressing cancer cells, compared to an antibody unbound to a chemotherapeutic agent.

By administering the immunoconjugate of the present invention to a patient having CDH3-expressing cancer cells, it is possible to exert a potent anti-cancer effect while also achieving reduced immunogenicity of the immunoconjugate itself. The immunoconjugate of the present invention is useful as an anticancer agent.

Brief Description of Drawings

[0013]

[Figure 1] Figure 1 shows the proportion of viable cells in the cell viability assays for ADC1 and ADC3.

[Figure 2] Figure 2 shows the proportion of viable cells in the cell viability assays for ADC1 and ADC2.

[Figure 3] Figure 3 shows the proportion of viable cells in the cell viability assays for ADC3 and ADC4.

[Figure 4] Figure 4 shows the results of an *in vivo* drug efficacy study on mice for ADC3.

[Figure 5] Figure 5 shows the body weight results in an *in vivo* drug efficacy study on mice for ADC3.

[Figure 6] Figure 6 shows the results of an *in vivo* drug efficacy study on mice for ADC4.

[Figure 7] Figure 7 shows the body weight results in an *in vivo* drug efficacy study on mice for ADC4.

[Figure 8] Figure 8 shows the results of an *in vivo* mouse drug efficacy study for ADC3, ADC5, and ADC7.

[Figure 9] Figure 9 shows the body weight results in an *in vivo* mouse drug efficacy study for ADC3, ADC5, and ADC7.

[Figure 10] Figure 10 shows the results of an *in vivo* pharmacokinetic study on mice.

Embodiments of Carrying out the Invention

[0014]

Next, the present invention will be described in more detail.

The present invention relates to an antibody-drug conjugate in which an anti-CDH3 humanized antibody and a chemotherapeutic agent having a linker are linked, wherein

the anti-CDH3 humanized antibody is:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2;

(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4;

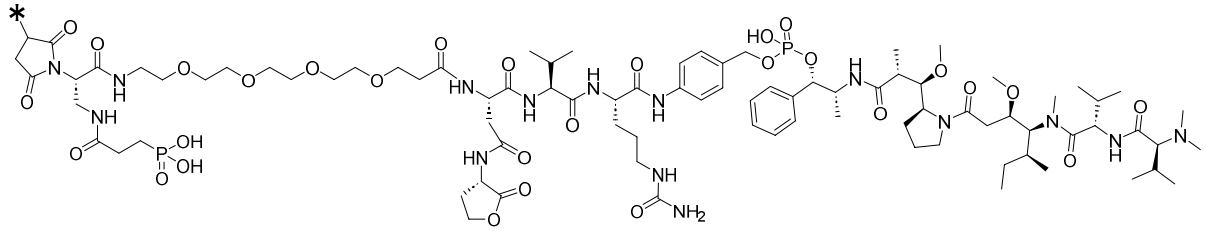
(3) a fragment of the anti-CDH3 humanized antibody described in (1) or (2) above, which has CDH3-binding ability; or

(4) an antibody consisting of a partial sequence of the antibody described in (1) or (2) above, which has CDH3-binding ability,

wherein

the chemotherapeutic agent having a linker is given by

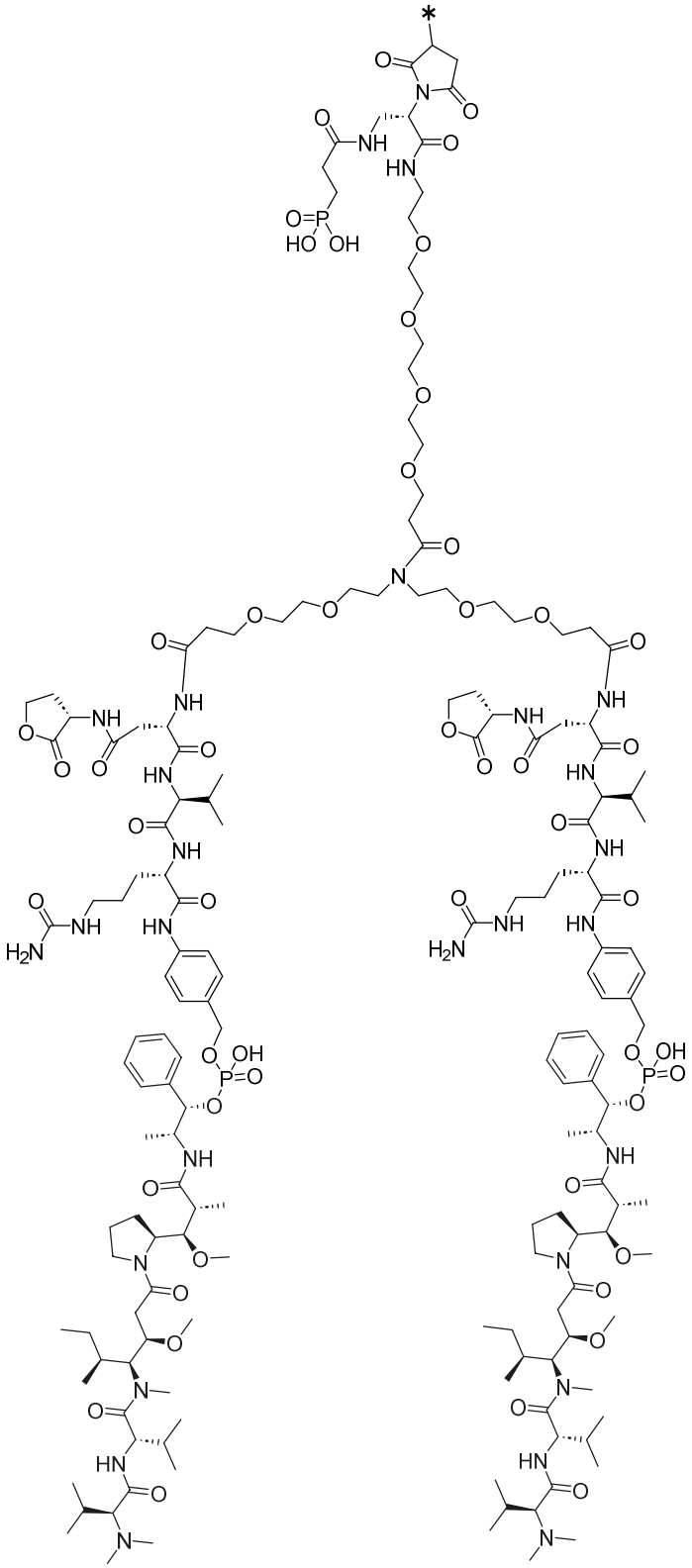
Formula (x):



(where * indicates a linking point with the antibody),

or

Formula (y):



(where * indicates a linking point with the antibody),

pharmacologically acceptable salt thereof, or hydrate thereof.

In the present invention, a compound represented by Formula (x) or Formula (y) above is

used as the chemotherapeutic agent having a linker. Note that, it has been found that when a chemotherapeutic agent having a linker other than that represented by Formula (x) or Formula (y) (for example, an agent in which the chemical structure of a portion of the linker differs, or an agent employing monomethyl auristatin E (MMAE) or eribulin) is used, the therapeutic efficacy is lower than that of the antibody-drug conjugate of the present invention.

[0015]

< Anti-CDH3 Humanized Antibody >

The antibody of the present invention is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2;

(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4.

[0016]

Preferably, the antibody of the present invention is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.

[0017]

The anti-CDH3 humanized antibody of the present invention is provided by combining a CDR sequence defined by an antibody that specifically recognizes CDH3 (preferably human CDH3) with various suitable human-derived FR sequences, and further by introducing an appropriate amino acid mutation to improve affinity.

[0018]

In one embodiment, the antibody of the present invention binds to CDH3 expressed on the cell surface. In one embodiment, the antibody of the present invention binds to an epitope within the CDH3 region. Preferably, the antibody of the present invention binds to CDH3 expressed on the surfaces of human cells, and particularly preferably binds to CDH3 expressed on the surfaces of cancer cells.

[0019]

CDH3 (preferably human CDH3) or a partial peptide thereof can be used as an antigen for

producing the antibody of the present invention. One example is a soluble CDH3 protein corresponding to the CDH3 extracellular region (corresponding to amino acids 108 to 654 of SEQ ID NO: 2 in WO2014/126198), although the invention is not limited thereto.

[0020]

The antibody of the present invention is a humanized monoclonal antibody. In the present invention, hybridomas are obtained through immunization of mice as a source material for generating a humanized monoclonal antibody. Such materials can be obtained by various methods well known in the art. For example, they can be obtained using the method described below, but are not limited to this method.

[0021]

To establish hybridomas producing an antibody that specifically binds to CDH3, CDH3 or a partial peptide thereof is first administered as an antigen to a mouse. The dosage per animal is 0.1 to 100 mg when no adjuvant is used, and 1 to 100 μg when an adjuvant is used. Examples of adjuvants include Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), and aluminum hydroxide adjuvant. Immunization is primarily performed by intravenous, subcutaneous, or intraperitoneal injection. Furthermore, the interval between immunizations is not particularly limited; immunization is performed 1 to 10 times, preferably 2 to 5 times, at intervals of several days to several weeks, preferably 2 to 5 weeks. Then, antibody-producing cells are harvested for 1 to 60 days, preferably 1 to 14 days, after the final immunization. Examples of antibody-producing cells include spleen cells, lymph node cells, and peripheral blood cells, with spleen cells or local lymph node cells being preferred.

[0022]

To obtain a hybridoma, cell fusion is performed between antibody-producing cells and myeloma cells. For myeloma cells, generally available mouse-derived cell lines possessing drug selectivity (such as sensitivity to HAT medium) can be used. Examples thereof include P3X63-Ag.8.U1 (P3U1) and NS-1.

[0023]

Cell fusion can be performed by mixing antibody-producing cells at a concentration of 1×10^6 to 1×10^7 cells/mL and myeloma cells at a concentration of 2×10^5 to 2×10^6 cells/mL in an animal cell culture medium, such as serum-free DMEM or RPMI-1640, and conducting the fusion reaction in the presence of a cell fusion promoter. Polyethylene glycol or a similar compound with an average molecular weight of 1,000 to 6,000 Daltons can be used as a cell fusion promoter. Alternatively, antibody-producing cells and myeloma cells can be fused using a commercially available cell fusion apparatus that utilizes electrical stimulation.

[0024]

Hybridomas can be obtained through culture procedures using a selective medium. After appropriately diluting a cell suspension (e.g., with RPMI-1640 medium containing fetal bovine serum), cells are seeded onto a microtiter plate at a density of approximately 3×10^5 cells/well, selective medium is added to each well, and the cells are cultured while appropriately replacing the selective medium. As a result, cells that begin to grow approximately 14 days after the start of culture in a selective medium can be obtained as hybridomas.

[0025]

Next, the culture supernatant of the proliferating hybridomas is screened for the presence of an antibody of interest. Screening of hybridomas may be performed according to a standard method, which is not particularly limited. For example, a portion of the culture supernatant from wells containing growing hybridomas can be collected, and hybridomas producing antibodies that bind to CDH3 can be screened for using a method such as enzyme immunoassay or radioimmunoassay. Cloning of fused cells is performed using a method such as limiting dilution, thereby enabling the establishment of hybridomas as the final product, i.e., monoclonal-antibody-producing cells.

[0026]

Using the established hybridomas as a starting material, humanization of the antibody derived from the hybridomas can be achieved using a known method. Specifically, a DNA sequence designed to link the CDR sequences of a mouse antibody with the FR sequences of a human antibody is synthesized via PCR from several oligonucleotides prepared to have overlapping terminal regions. A humanized antibody is obtained by ligating the obtained DNA with DNA encoding a human antibody constant region, subsequently incorporating the resulting construct into an expression vector, and introducing the vector into a host for production (such as EP239400 or WO96/02576).

[0027]

CDR sequences represent regions within the variable regions of antibodies that exhibit particularly marked differences and play a crucial role in determining antibody specificity. The amino acid residues belonging to this region are considered to include many residues directly involved in binding to the antigen and in specificity; three such regions exist in each of the light-chain and heavy-chain variable regions.

CDR sequences are defined by sequence comparison according to Kabat et al. (Sequences of proteins of immunological interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) and also by three-dimensional structure according to Chothia et al. (J. Mol. Biol.; 196, p901 (1987)).

[0028]

Regarding the CDRs defined by Kabat, the light chain variable region CDRs are generally considered to be located around residues 24 to 34, 50 to 56, and 89 to 97, and the heavy chain variable

region CDRs around residues 31 to 35, 50 to 65, and 95 to 102; however, not all residues in these regions necessarily participate directly in antigen binding, and it is not always that they correspond exactly to CDR sequences defined based on three-dimensional structures.

[0029]

The human FR sequences to be selected are selected as appropriate for the given situation. FR sequences derived from the human germline, selected based on optimal alignment, were used herein.

[0030]

Specifically, the variable region sequences of the mouse antibody are screened against a library of known, publicly available human variable region sequences. Among them, the human variable region sequence with the highest sequence similarity can be used as the human germline-derived human FR sequence for the humanized antibody (Sims et al., J. Immunol. 151:2296 (1993); Chothia et al., J. Mol. Biol. 196:901 (1987); Tempest et al., Biotechnology 9:266 (1991)).

[0031]

The germline sequence with the highest sequence similarity can be identified by aligning the sequence of the original antibody against databases containing many such registered libraries (e.g., IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg)).

[0032]

The suitability of a selected FR sequence for humanization can be determined based on whether the combination with the respective antibody clone's CDR sequences is appropriate and whether the sequence was able to maintain a suitable three-dimensional structure for the binding-target antigen.

[0033]

A humanized antibody that is expressed by grafting CDR sequences sandwiched between human-derived FR sequences often exhibits reduced affinity when the sequence selection is inappropriate. This indicates that there are residues within the FR sequences that also play an important role in maintaining the structure. It is possible to perform amino acid residue substitutions in response to this phenomenon. For example, based on alignment results, the sequence can be reshaped by substituting residues with those found in a mouse antibody at positions homologous to the human-derived sequence. This may make it possible to improve the reduction in affinity caused by humanization.

[0034]

The position of the amino acid residue to be substituted varies depending on the antibody of interest and, in many cases, is not identified until actual expression takes place. In the following Examples, it was intended to improve affinity by substituting amino acid residues at one or more

positions selected from position 55 of the light chain variable region and positions 49, 71, and 78 of the heavy chain variable region, while positions where substitutions are performed in a relatively large number of documents (e.g., Proc. Natl. Acad. Sci. USA; 89, p. 4285 (1992)); however, the substitution positions, combinations, and types of substituted amino acid residues are arbitrary and not limited to these examples.

[0035]

Hosts for antibody production are often of mammalian origin. Meanwhile, those skilled in the art can appropriately select the specific host cell system best suited for the gene product to be expressed. Examples of common host cell systems include, but are not limited to, CHO-derived cell lines (Chinese hamster ovary cell lines), CV1 (monkey kidney line), COS (a derivative of CV1 expressing SV40 T-antigen), SP2/0 (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), 293 (human kidney), and 293T (a derivative of 293 expressing SV40 T-antigen). Host cell lines can be obtained from various manufacturers, the American Tissue Culture Collection (ATCC), or organizations that publish papers described in the literature.

[0036]

As the host cell system, a CHO-derived cell line deficient in the dgfr gene expression or SP2/0 can preferably be used (Urlaub, G. et al., Somat. Cell. Mol. Genet. 12, 555 (1986) [Non-Patent Document 4]; and Schulman, M. et al., Nature 276, 269 (1978) [Non-Patent Document 5]). Most preferably, the host cell system is a DHFR-deficient CHO cell line.

[0037]

Transfection of the plasmid into host cells can be performed using any technique. Specific methods include, but are not limited to, transfection (including the calcium phosphate method, DEAE method, lipofection, and electroporation), methods for introducing DNA utilizing envelopes such as that of Sendai virus, microinjection, and infection using viral vectors such as retroviruses or adenoviruses (Current Protocols in Molecular Biology, Chapter 9: Introduction of DNA into Mammalian Cells, John Wiley and Sons, Inc.). The most preferable technique is the introduction of the plasmid into a host by electroporation.

[0038]

These antibodies may be monovalent, bivalent, or multivalent antibodies, provided that they have the ability to bind to CDH3. Furthermore, as long as they retain the ability to bind to CDH3, these antibodies may be low-molecular-weight antibodies such as antibody fragments, modified antibodies, or partial sequences of antibodies. Examples of low-molecular-weight antibodies, such as antibody fragments, include Fab, Fab', F(ab')2, Fv, scFv (single-chain Fv), and diabodies. Furthermore, they may be derived from the fusion of an Fc region with an antibody fragment or a low-molecular-weight antibody, such as Fab, Fab', F(ab')2, Fv, scFv (single-chain Fv), or a diabody. To

obtain such antibodies, genes encoding them may be constructed, introduced into expression vectors, and then expressed in suitable host cells.

[0039]

< Chemotherapeutic Agent Having Linker >

As a chemotherapeutic agent having a linker, one represented by Formula (x) or Formula (y) above is used.

In the case of a chemotherapeutic agent having a linker represented by Formula (x), the compound prior to conjugation with an antibody can be synthesized using a method similar to the method in Reference Example 3 described in WO 2023163227, Example 3 described in WO 2023163234, Reference Example A3 described in WO 2023234426, or Example A3 described in WO 2023234427.

In the case of a chemotherapeutic agent having a linker represented by Formula (y), the compound prior to conjugation with an antibody can be synthesized using a method similar to the method in Reference Example 2 described in WO 2023234426 or Example 2 described in WO 2023234427.

[0040]

< Method for Linking Antibody to Chemotherapeutic Agent Having Linker >

The conjugation of an antibody with a chemotherapeutic agent having a linker can be performed by a method in accordance with the descriptions in Patent Documents 3 to 6 (WO 2023/163227, WO 2023/163234, WO 2023/234426, and WO 2023/234427).

[0041]

The number of chemotherapeutic agent molecules bound per molecule of the humanized anti-CDH3 antibody, fragment thereof, or partial sequence thereof is not particularly limited, but preferably 1 to 16 molecules, and more preferably 6 to 16 molecules, of the chemotherapeutic agent are bound. As an example, 1 to 16 molecules of auristatin E may be conjugated to one molecule of an anti-CDH3 humanized antibody, a fragment thereof, or a partial sequence thereof.

Furthermore, the conjugate of the anti-CDH3 humanized antibody of the present invention and a drug can be represented by General Formula (I): A-(B-C)a.

(In the formula, A is the moiety obtained by removing a sulfhydryl group from the reduced antibody, B is the group -S-, C is a chemotherapeutic agent having a linker, and a is the average number of linker-drug units (B-C) attached per antibody molecule, representing a number from 1 to 10 that may include a fraction. Hereinafter, regarding ADC, the average number of drugs conjugated per antibody or the drug-to-antibody ratio is referred to as "DAR"; this is a value ranging from 1 to 20 (which may include decimal fractions) and is expressed herein to one or two decimal places.)

DAR is preferably a number from 2 to 18, and more preferably a number from 3 to 15.

[0042]

< Form of Antibody-Drug Conjugate >

Examples of the "pharmacologically acceptable salts" described herein include alkali metal salts such as lithium, sodium, and potassium; Group 2 metal salts such as magnesium and calcium; salts with aluminum or zinc; salts with amines such as ammonia, choline, diethanolamine, lysine, ethylenediamine, tert-butylamine, tert-octylamine, tris(hydroxymethyl)aminomethane, N-methyl-glucosamine, triethanolamine, and dehydroabietylamine; salts with inorganic acids such as hydrogen chloride, hydrogen bromide, hydrogen iodide, sulfuric acid, nitric acid, and phosphoric acid; salts with organic acids such as formic acid, acetic acid, trifluoroacetic acid, propionic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, maleic acid, lactic acid, malic acid, tartaric acid, citric acid, methanesulfonic acid, ethanesulfonic acid, and benzenesulfonic acid; or salts with acidic amino acids such as aspartic acid and glutamic acid. Furthermore, "pharmacologically acceptable salts" also encompass intramolecular salts.

[0043]

The antibody-drug conjugate of the present invention and a pharmacologically acceptable salt thereof may be obtained as hydrates or solvates, and the present invention encompasses all such forms.

The antibody-drug conjugate of the present invention or a salt thereof may absorb moisture, acquire adsorbed water, or form a hydrate upon exposure to the atmosphere or recrystallization; such antibody-drug conjugates or salts thereof containing water are also encompassed by the present invention.

[0044]

In a case in which the chemotherapeutic agent moiety having a linker of the antibody-drug conjugate of the present invention includes a partial structure having an asymmetric center and optical isomerism can occur, any optical isomer thereof is included in the present invention.

[0045]

The present invention also encompasses compounds labeled with various radioactive or non-radioactive isotopes. One or more of the atoms constituting the antibody-drug conjugate of the present invention may also contain non-natural proportions of atomic isotopes. Examples of atomic isotopes include deuterium (^{2}H), tritium (^{3}H), iodine-125 (^{125}I), and carbon-14 (^{14}C). In addition, the antibody-drug conjugate of the present invention can be radiolabeled with a radioisotope such as tritium (^{3}H), iodine-125 (^{125}I), or carbon-14 (^{14}C). Radiolabeled compounds are useful as therapeutic or prophylactic agents, research reagents (e.g., assay reagents), and diagnostic agents (e.g., *in vivo* imaging agents). All isotopic variants of the antibody-drug conjugate of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

[0046]

< Medicaments and Methods of Use >

According to the present invention, there is provided a medicament for treating a disease characterized by CDH3 overexpression, comprising the antibody-drug conjugate of the present invention.

Diseases characterized by CDH3 overexpression are not particularly limited, provided that CDH3 is expressed in the cells involved; examples include tumors or cancers. Examples thereof include hematopoietic tumors or cancers, such as lymphomas (Hodgkin lymphoma and non-Hodgkin lymphoma) and leukemia, as well as solid tumors or solid cancers. Examples of hematopoietic tumors or cancers include follicular lymphoma, anaplastic large cell lymphoma, mantle cell lymphoma, acute myeloblastic leukemia, chronic myeloid leukemia, chronic lymphocytic leukemia, diffuse large B-cell lymphoma, and multiple myeloma. Examples of solid tumors or solid carcinomas include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, Synovial tumor, mesothelioma, Ewing tumor, leiomyosarcoma, rhabdomyosarcoma, Kaposi sarcoma, colon cancer, colorectal cancer, kidney cancer, pancreatic cancer, bone cancer, breast cancer, ovarian cancer, prostate cancer, esophageal cancer, stomach cancer, oral cavity cancer, nasal cavity cancer, throat cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatocellular carcinoma, biliary tract carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical carcinoma, uterine carcinoma, testicular tumor, small-cell lung carcinoma, bladder cancer, lung cancer, epithelial cancer, thyroid cancer, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, skin cancer, melanoma, neuroblastoma, and retinoblastoma; however, the tumor cells to be treated need only be tumor cells that express CDH3.

[0047]

The antibody-drug conjugate of the present invention can be administered to a mammal, preferably a human.

The substances used in the pharmaceutical composition containing the antibody-drug conjugate of the present invention are optionally selected from formulation additives and other substances commonly used in the art.

[0048]

The antibody-drug conjugate of the present invention can be administered as a pharmaceutical composition containing one or more pharmaceutically acceptable ingredients. For

example, the pharmaceutical composition typically comprises one or more pharmaceutically acceptable carriers. Examples of such carriers include sterilized liquids, such as water, mineral oil, animal oil, and vegetable oils (such as peanut oil, soybean oil, and sesame oil). Water is a typical carrier when the pharmaceutical composition is administered intravenously. Aqueous solutions of sodium chloride, dextrose, and glycerol may also be used as liquid carriers, particularly for injectable solutions. Various pharmacologically acceptable substances known in the art can be used as excipients. The pharmaceutical composition may also contain, if necessary, minor amounts of a wetting agent or emulsifying agent, or a pH buffering agent. Examples of pharmaceutically acceptable carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

[0049]

The antibody-drug conjugate of the present invention can be administered via various routes. A preferred route of administration is parenteral administration; examples of parenteral administration include subcutaneous, intravenous, intramuscular, or intrasternal injection. Intravenous administration is more preferable, and either infusion or bolus injection is possible.

[0050]

A pharmaceutical composition suitable for intravenous administration to humans is formulated according to a standard method. A representative pharmaceutical composition for intravenous administration is a sterile, isotonic aqueous buffer solution. The pharmaceutical composition may optionally contain a local anesthetic (e.g., lignocaine) to alleviate pain at the injection site.

[0051]

The pharmaceutical composition may be a pharmaceutical composition consisting of the antibody-drug conjugate of the present invention, or it may be a pharmaceutical composition containing the antibody-drug conjugate and one or more additional antitumor agents.

[0052]

The antibody-drug conjugate of the present invention can also be administered in combination with other antitumor agents, thereby enhancing the antitumor effects. Other antitumor agents used for such purposes may be administered concomitantly with, separately from, or sequentially to the antibody-drug conjugate, and the administration intervals for each may be varied. Examples of such antitumor agents include abraxane, carboplatin, cisplatin, gemcitabine, irinotecan (CPT-11), paclitaxel, pemetrexed, sorafenib, vinblastine, and drugs described in WO 2003/038043, as well as LH-RH analogs (such as leuprorelin and goserelin), estramustine phosphate, estrogen antagonists (such as tamoxifen and raloxifene), and aromatase inhibitors (such as anastrozole, letrozole, and exemestane); however, the agents are not limited to these, provided that they have antitumor activity. Such pharmaceutical compositions can be formulated as lyophilized preparations or liquid

preparations. When formulating the pharmaceutical composition as a freeze-dried preparation, suitable pharmaceutical additives used in the art may be added. This also applies to liquid formulations.

[0053]

Although the composition and concentration of the pharmaceutical composition vary depending on the method of administration, they may also vary based on the affinity of the antibody-drug conjugate contained in the composition for CDH3 and the antitumor activity of the conjugated antitumor drug. When administering the antibody-drug conjugate of the present invention to humans, for example, a dose of approximately 0.001 to 100 mg/kg may be administered either once or a plurality of times at intervals ranging from once every 1 day to once every 180 days.

[0054]

The present invention will be described in further detail by the following examples; however, these examples are intended for illustrative purposes only, and the scope of the invention is not limited by them. All prior art documents cited herein are incorporated herein by reference.

Examples

[0055]

Reference Example 1: Method for Producing Humanized Antibody

(1) Production of Anti-CDH3 Mouse Antibody

An anti-CDH3 mouse antibody was prepared as described in Examples 1 to 9 of Japanese Patent No. 6377601, and the sequences of the variable regions were obtained through sequence analysis of the anti-CDH3 mouse antibody.

[0056]

Sequence analysis was performed using ApE and GENETYX (GENETYX CORPORATION). CLUSTALW was used for gene alignment. HPRD, NCBI BLAST, UCSC (University of California, Santa Cruz) Blat, and UniProt were used to obtain and verify gene and protein information.

[0057]

Among the variable regions of the mouse-hybridoma-derived anti-CDH3 mouse antibody sequenced as described above, the amino acid sequences corresponding to the CDR sequences of T004 are shown below.

SLTSYGVH : SEQ ID NO: 9 (CDR-H1)

GVIWSGGSTD : SEQ ID NO: 10 (CDR-H2)

ARNSNNGFAY: SEQ ID NO: 11 (CDR-H3)

NIYSNLA: SEQ ID NO: 12 (CDR-L1)

LLVYAAKN : SEQ ID NO: 13 (CDR-L2)

QHFYDTPWT: SEQ ID NO: 14 (CDR-L3)
In addition, the amino acid sequences corresponding to the CDR sequences of T004b are shown below.
GYSFTAYN: SEQ ID NO: 15 (CDR-H1)
IDPYSGII: SEQ ID NO: 16 (CDR-H2)
ARRGYYDGGFDY: SEQ ID NO: 17 (CDR-H3)
QDITNY: SEQ ID NO: 18 (CDR-L1)
YTS: SEQ ID NO: 19 (CDR-L2)
QQDSKHPRT: SEQ ID NO: 20 (CDR-L3)
Note that CDR-H1, CDR-H2, and CDR-H3 represent the CDR sequences constituting the heavy chain, and CDR-L1, CDR-L2, and CDR-L3 represent the CDR sequences constituting the light chain. The indicated CDR amino acid sequences were obtained by searching the nucleotide sequences of the anti-CDH3 mouse antibody variable regions using IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg).
[0058]
(2) Construction of Transient Expression Vector for Anti-CDH3 Antibody

For chimerization, regarding the light chain, a gene was designed by linking the gene for the light chain variable region of the anti-CDH3 mouse antibody to a chimeric light chain expression vector encoding human Ck. Regarding the heavy chain, the design involved linking the gene encoding the heavy chain variable region of the anti-CDH3 mouse antibody to a vector encoding the human Cg1 region.
[0059]

For humanization, mouse-derived CDR sequences were combined with human-derived FR sequences, and a human Fc region was further linked, thereby constructing a stable production cell line using a humanized antibody expression vector. More specifically, the procedure was as follows.
[0060]

The regions corresponding to the mouse FR sequences were replaced with human-derived FR sequences, and the full-length sequence was artificially synthesized by GenScript. Human germline FR sequences were used for the amino acid sequences of the regions corresponding to the FR sequences. The germline FR sequences were designed by inputting the nucleotide sequences of the cloned anti-CDH3 mouse antibody into IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg) and selecting the sequences with the highest similarity. In addition, amino acid sequences were substituted (reshaped) to address the reduced affinity.
[0061]

The amino acid sequences of the heavy-chain or light-chain variable regions of the

humanized anti-CDH3 antibody used in this study are shown below.

Note that for T004 and T004b, human germline FR sequences that are most similar to the FR sequences of the respective parental mouse antibodies were used. In addition, T004 and T004b incorporated the amino acid sequence substitutions as indicated.

[0062]

Antibody No. T004-VH

QVQLVESGGGVVQPGRSLRLSCAASGFSLTSYGVHWVRQAPGKGLEWVGVIWSGGSTD YADSVKGRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARNSNNGFAYWGQGTLVTVSS (SEQ ID NO: 1)

(R71K and L78V substitutions are present relative to the selected germline FR sequence.)

[0063]

Antibody No. T004-VL

DIQLTQSPSSLSASVGDRVTITCRASQNIYSNLAWYQQKPGKAPKLLVYAAKNLASGVPSR FSGSGSGTDFTLTISSLQPEDFATYYCQHFYDTPWTFGQGTKVEIK (SEQ ID NO: 2)

(E55A substitution is present relative to the selected germline FR sequence.)

[0064]

Antibody No. T004b-VH

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT YAEKFQGRVTITVDKSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVS S (SEQ ID NO: 3)

(A72V and T74K substitutions are present relative to the selected germline FR sequence.)

[0065]

Antibody No. T004b-VL

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSR FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIK (SEQ ID NO: 4)

(E55H substitution is present relative to the selected germline FR sequence.)

[0066]

The artificially synthesized genes designed to possess these sequences were inserted into pCXN vectors: the heavy-chain variable region gene was inserted into a pCXN vector containing a human IgG1-derived constant region gene, and the light-chain variable region gene was inserted into a pCXN vector containing a human κ-chain-derived constant region gene.

[0067]

The full-length amino acid sequence of the anti-CDH3 humanized antibody obtained by allowing the genes to be expressed is shown below. Here, the anti-CDH3 humanized antibody T004 consists of the H chain T004-H and the L chain T004-L, and the anti-CDH3 humanized antibody

T004b consists of the H chain T004b-H and the L chain T004b-L.

Antibody No. T004-H

QVQLVESGGGVVQPGRSLRLSCAASGFSLTSYGVHWVRQAPGKGLEWVGVIWSGGSTD
YADSVKGRFTISKDNSKNTVYLQMNSLRAEDTAVYYCARNSNNGFAYWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 5)

[0068]

Antibody No. T004-L

DIQLTQSPSSLSASVGDRVTITCRASQNIYSNLAWYQQKPGKAPKLLVYAAKNLASGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQHFYDTPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 6)

[0069]

Antibody No. T004b-H

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT
YAEKFQGRVTITVDKSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 7)

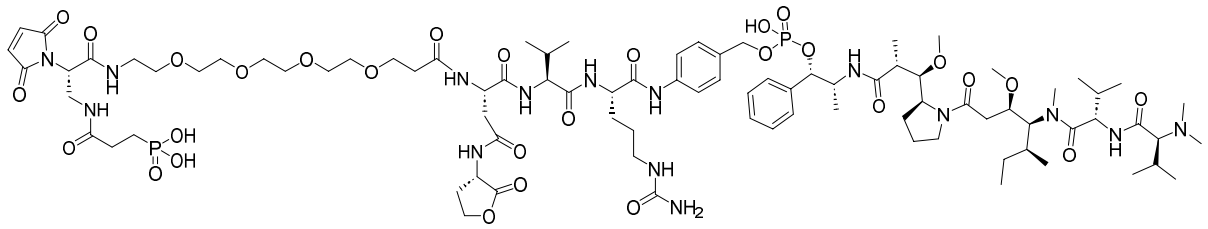
[0070]

Antibody No. T004b-L

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 8)

[0071]

< Synthesis Example 1 > PGAP-1



((6S,9S,12S,31S)-1-amino-6-((4-(((((1S,2R)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-2-((S)-2-(dimethylamino)-3-methylbutanamide)-N,3-dimethylbutanamide)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamide)-1-phenylpropoxy)(hydroxy)phosphoryl)oxy)methyl)phenyl)carbamoyl)-31-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-isopropyl-1,8,11,14,30,34-hexaoxo-12-(2-oxo-2-(((S)-2-oxotetrahydrofuran-3-yl)amino)ethyl)-17,20,23,26-tetraoxa-2,7,10,13,29,33-hexaazahexatriacontan-36-yl)phosphonic acid

[0072]

The compound of Synthesis Example 1 can be synthesized using a method similar to the method in Reference Example 3 described in WO 2023163227, Example 3 described in WO 2023163234, Reference Example A3 described in WO 2023234426, or Example A3 described in WO 2023234427.

The MS data for Synthesis Example 1 are shown below.

MS (DUIS) m/z 1921 $(M+H)^+$

MS (DUIS) m/z 1919 $(M-H)^-$

(Analysis conditions)

Instrument: LCMS-2050 System (Shimadzu Corporation)

Column: ACQUITY UPLC BEH C18 Column, 130 Å, 1.7 μm, 2.1 mm × 50 mm (Waters)

Eluent: Solution A: 0.1% aqueous formic acid; Solution B: 0.1% formic acid in acetonitrile.

Gradient (B%): 25 (0 min) → 95 (0.75 min) → 95 (1.5 min)

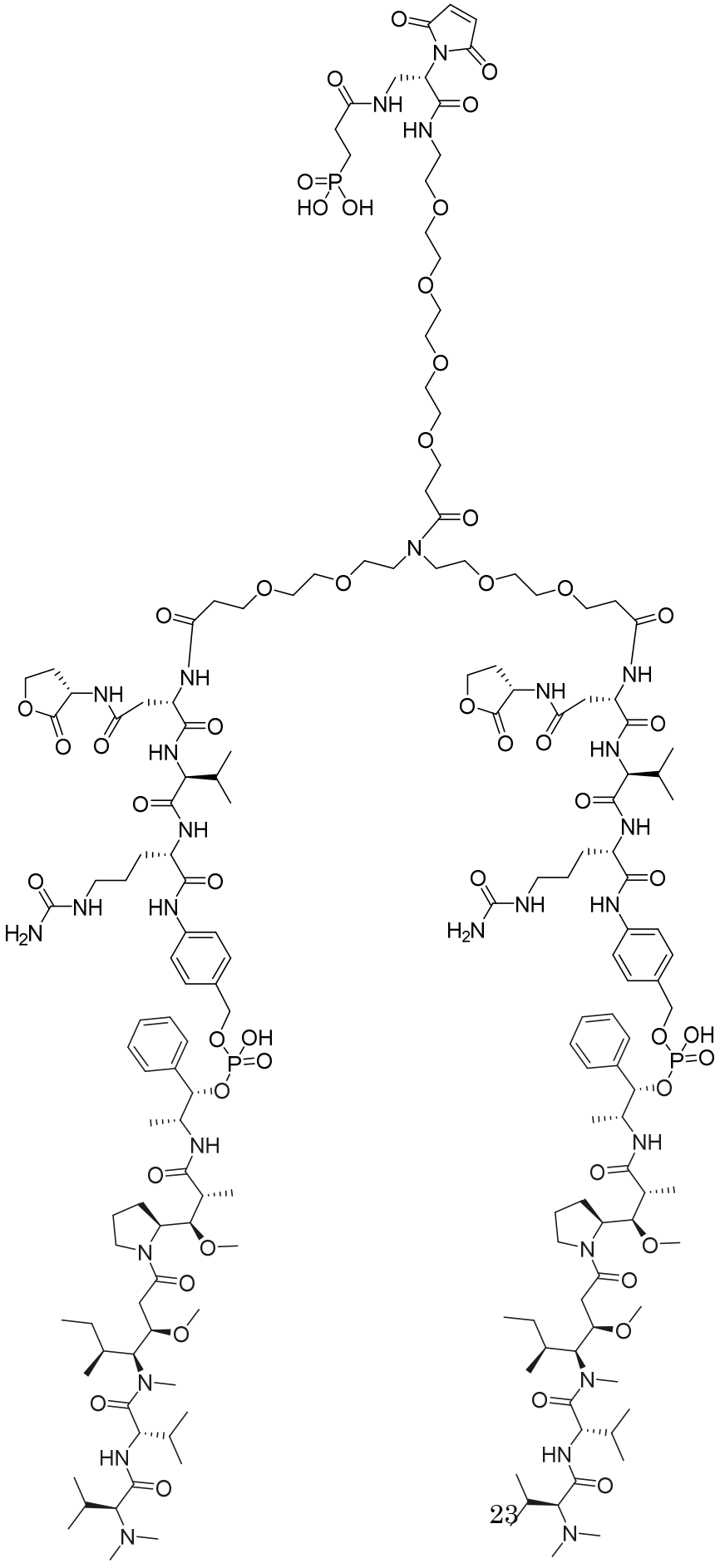
Temperature: 40°C

Flow rate: 0.8 mL/min

Injection volume: 5 μL

[0073]

< Synthesis Example 2 > PGAP-2



[0074]

((6S, 9S, 12S, 41S)-1-amino-23-((6S, 9S, 12S)-1-amino-6-((4-(((((1S, 2R)-2-((2R, 3R)-3-(S)-1-((3R,4S,5S)-4-((S)-2-((S)-2-(dimethylamino)-3-methylbutanamido)-N,3-dimethylbutanamido)-3-methoxy-5-methylheptanoyl)pyrrolidine-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)(hydroxy)phosphoryl)oxy)methyl)phenyl)carbamoyl)-9-isopropyl-1,8,11,14-tetraoxo-12-(2-oxo-2-(((S)-2-oxotetrahydrofuran-3-yl)amino)ethyl)-17,20-dioxa-2,7,10,13-tetraazadocosan-22-yl)-6-((4-(((((1S,2R)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-2-((S)-2-(dimethylamino)-3-methylbutanamide)-N,3-dimethylbutanamide)-3-methoxy-5-methylheptanoyl)pyrrolidine-2-yl)-3-methoxy-2-methylpropanamide)-1-phenylpropoxy)(hydroxy)phosphoryl)oxy)methyl)phenyl)carbamoyl)-41-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-9-isopropyl-1,8,11,14,24,40,44-heptaoxo-12-(2-oxo-2-(((S)-2-oxotetrahydrofuran-3-yl)amino)ethyl)-17,20,27,30,33,36-hexaoxa-2,7,10,13,23,39,43-heptaazahexatetracontan-46-yl)phosphonic acid

[0075]

The compound of Synthesis Example 2 can be synthesized using a method similar to the method in Reference Example 2 described in WO 2023234426 or Example 2 described in WO 2023234427.

The MS data for Synthesis Example 2 are shown below.

MS (DUIS) m/z 1797 $(M+2H)^{2+}$

MS (DUIS) m/z 1795 $(M-2H)^{2-}$

(Analysis conditions)

Instrument: LCMS-2050 System (Shimadzu Corporation)

Column: ACQUITY UPLC BEH C18 Column, 130 Å, 1.7 μm, 2.1 mm × 50 mm (Waters)

Eluent: Solution A: 0.1% aqueous formic acid; Solution B: 0.1% formic acid in acetonitrile.

Gradient (B%): 25 (0 min) → 95 (0.75 min) → 95 (1.5 min)

Temperature: 40°C

Flow rate: 0.8 mL/min

Injection volume: 5 μL

[0076]

< Synthesis of Antibody-Drug Conjugate >

The antibody-drug conjugate of the present invention was synthesized by the following method. The antibody-drug conjugates to be obtained or obtained in the following examples are represented by General Formula (I).

General Formula (I): A-(B-C)a

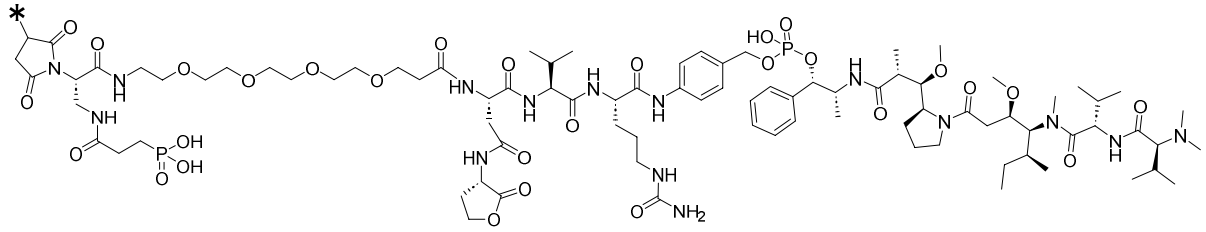
(In the formula, A is the moiety obtained by removing a sulfhydryl group from the reduced antibody, B is the group -S-, C is a chemotherapeutic agent having a linker, and a is the average number of linker-drug units (B-C) attached per antibody molecule, representing a number from 1 to 10 that may include a fraction. Hereinafter, regarding ADC, the average number of drugs conjugated per antibody or the drug-to-antibody ratio is referred to as "DAR"; this is a value ranging from 1 to 20 (which may include decimal fractions) and is expressed herein to one or two decimal places.)

[0077]

< Example 1: T004-PGAP-1 (ADC1) >

Synthesis of an antibody-drug conjugate of General Formula (I), where A is the moiety obtained by removing a sulfhydryl group from the reduced antibody T004, B is -S-, and C is the following Formula (x).

Formula (x):



(In the formula, * indicates a linking point with B.)

[0078]

< 1-1 > Buffer exchange for T004:

EDTA (manufactured by Thermo Fisher Scientific) was added to phosphate-buffered saline (hereinafter referred to as PBS; manufactured by Thermo Fisher Scientific) to a concentration of 10 mM (hereinafter referred to as PBS/EDTA). To each NAP-10 column (manufactured by Cytiva) pre-equilibrated with PBS/EDTA, 1 mL of the stock solution of T004 obtained in Reference Example 1 was applied; the sample was then eluted with 1.5 mL of PBS/EDTA, and the fraction containing T004 (1.5 mL) was collected, thereby obtaining a T004 solution. The absorbance at 280 nm of the T004 solution was measured using a microplate reader (FlexStation 3 manufactured by Molecular Devices) to determine the antibody concentration (Antibody concentration: 3.4 mg/mL).

[0079]

< 1-2 > Reduction of T004:

The T004 solution obtained in < 1-1 > above was diluted with PBS/EDTA, and the concentration thereof was adjusted to approximately 2.5 mg/mL. A 0.99 mL aliquot of this solution was placed in a 1.5 mL microtube, and 10 μL of a PBS/EDTA solution of TCEP hydrochloride (manufactured by FUJIFILM Wako Pure Chemical Corporation) was added (such that the amount of

TCEP was 6.9 equivalents per antibody molecule). A reduction reaction targeting the disulfide bonds between the peptide chains constituting antibody T004 was performed by incubating at 37°C for 2 hours, yielding a reduced T004 solution.

[0080]

< 1-3 > Conjugate formation between reduced T004 and PGAP-1 (Synthesis Example 1):

To 900 μL of the reduced T004 solution obtained in < 1-2 > above, 100 μL of a dimethyl sulfoxide solution containing PGAP-1 obtained in Synthesis Example 1 was added (such that the amount of PGAP-1 was 15 equivalents per antibody molecule), and the mixture was reacted for 1 hour under ice-cooling, thereby obtaining an antibody-drug conjugate (T004-PGAP-1) solution.

[0081]

< 1-4 > Preparation of reference reduced T004:

To 900 μL of the reduced T004 solution, 100 μL of dimethyl sulfoxide was added, and the mixture was allowed to stand on ice for 1 hour, thereby obtaining a reference reduced T004 solution.

[0082]

< 1-5 > Purification of antibody-drug conjugate:

To each pre-conditioned Protein A column (MonoSpin 96WP ProA manufactured by GL Sciences Inc.), 200 μL of the antibody-drug conjugate solution obtained in 1-3 above was added, and the conjugate was allowed to adsorb to the column. After washing the column with the wash buffer supplied with the column, the antibody-drug conjugate was eluted using the elution buffer supplied with the column.

The antibody-drug conjugate solution (1 mL) eluted from the Protein A column was applied to a NAP-10 column pre-equilibrated with PBS/EDTA and eluted with 1.5 mL of PBS/EDTA, and the fraction containing the antibody-drug conjugate (1.5 mL) was collected, thereby obtaining the purified antibody-drug conjugate. The absorbance at 280 nm of the purified antibody-drug conjugate was measured using a microplate reader (Absorbance: 1.19).

[0083]

< 1-5a > Purification of antibody-drug conjugate for Test Example

The same procedure as in < 1-5 > was performed using EDTA-free PBS (Absorbance: 1.22). The purified antibody-drug conjugate obtained in this procedure was used in the Test Example.

[0084]

< 1-6 > Purification of reference reduced T004:

To each pre-conditioned Protein A column (MonoSpin 96WP ProA manufactured by GL Sciences Inc.), 200 μL of the reference reduced T004 solution obtained in < 1-4 > above was added, and the conjugate was allowed to adsorb to the column. After washing the column with the wash buffer supplied with the column, reduced T004 was eluted using the elution buffer supplied with the

column; the eluate was then applied to a NAP-10 column pre-equilibrated with PBS/EDTA and eluted with 1.5 mL of PBS/EDTA, and the fraction (1.5 mL) containing reduced T004 was collected, thereby obtaining a reference solution of purified and reduced T004. The absorbance at 280 nm of the purified and reduced T004 solution was measured using a microplate reader to determine the antibody concentration (Antibody concentration: 0.85 mg/mL).

[0085]

< 1-7 > DAR calculation using Ellman's assay

The purified antibody-drug conjugate solution (eluted with PBS/EDTA) and the reference purified reduced T004 solution were placed in an amount of 480 μL into separate microcentrifuge tubes, 20 μL of a 10 mM ethanol solution of 5,5'-dithiobis (2-nitrobenzoic acid) (manufactured by FUJIFILM Wako Pure Chemical Corporation) was added thereto, and the mixtures were allowed to react at room temperature for 15 minutes. The absorbance of the obtained reaction solution at 412 nm was measured using a microplate reader. The concentrations of free thiol groups in the purified antibody-drug conjugate solution and the purified reduced T004 solution were determined using a standard curve obtained from an L-Cysteine solution of a known concentration (manufactured by FUJIFILM Wako Pure Chemical Corporation) (free thiol group concentration of purified antibody-drug conjugate solution: 1.8 μM; free thiol group concentration of purified reduced T004 solution: 52 μM).

[0086]

(Calculation of DAR: When there is one chemotherapeutic agent present at C in General Formula (I) (a = DAR))

The number of thiol groups per molecule of reduced T004, $N_{SH, Ab}$, and the number of thiol groups per molecule of the antibody-drug conjugate, $N_{SH, ADC}$, can be determined using the following equations.

$N_{SH, Ab} = C_{SH, Ab}/C_{Ab}$ Equation (1)

$N_{SH, ADC}=C_{SH, ADC}/C_{ADC}$ Equation (2)

(Here, $C_{SH, Ab}$ represents the thiol group concentration in the reduced T004 solution, C_{Ab} represents the antibody concentration in the reduced T004 solution, $C_{SH, ADC}$ represents the thiol group concentration in the antibody-drug conjugate solution, and C_{ADC} represents the antibody concentration in the antibody-drug conjugate solution. Note that, since it is theoretically inconceivable for $N_{SH, Ab}$ to exceed 8, when the calculation using Equation (1) results in a value greater than 8 for $N_{SH, Ab}$, it is regarded as 8.)

The value obtained by subtracting $N_{SH, ADC}$ from $N_{SH, Ab}$ can be considered the DAR of the antibody-drug conjugate. Specifically, the DAR of the antibody-drug conjugate can be calculated using the following equation.

$DAR = N_{SH, Ab} - N_{SH, ADC}$ Equation (3)

Since the total absorbance at a given wavelength is equal to the sum of all absorbing chemical species present in the system, and assuming there is no change in the molar extinction coefficients of the antibody and the drug before and after conjugation, the following equation holds for the absorbance of the antibody-drug conjugate.

$A_{ADC, 280} = C_{ADC}(\varepsilon_{Ab, 280} + DAR \times \varepsilon_{D, 280})$ Equation (4)

(Here, $A_{ADC, 280}$ represents the absorbance at 280 nm of the antibody-drug conjugate solution, C_{ADC} represents the antibody concentration of the antibody-drug conjugate, $\varepsilon_{Ab, 280}$ represents the molar extinction coefficient of T004 at 280 nm, DAR represents the DAR of antibody-drug conjugate, $\varepsilon_{D, 280}$ represents the molar extinction coefficient of PGAP-1 at 280 nm.)

From Equations (1), (2), (3), and (4), the following equation holds for DAR.

$DAR = (N_{SH, Ab}A_{ADC, 280} - C_{SH, ADC}\varepsilon_{Ab, 280})/(A_{ADC, 280} + C_{SH, ADC}\varepsilon_{D, 280})$ Equation (5)

As a result of calculation using Equation (5), the calculated DAR value of the antibody-drug conjugate of Example 1 was 7.7.

[0087]

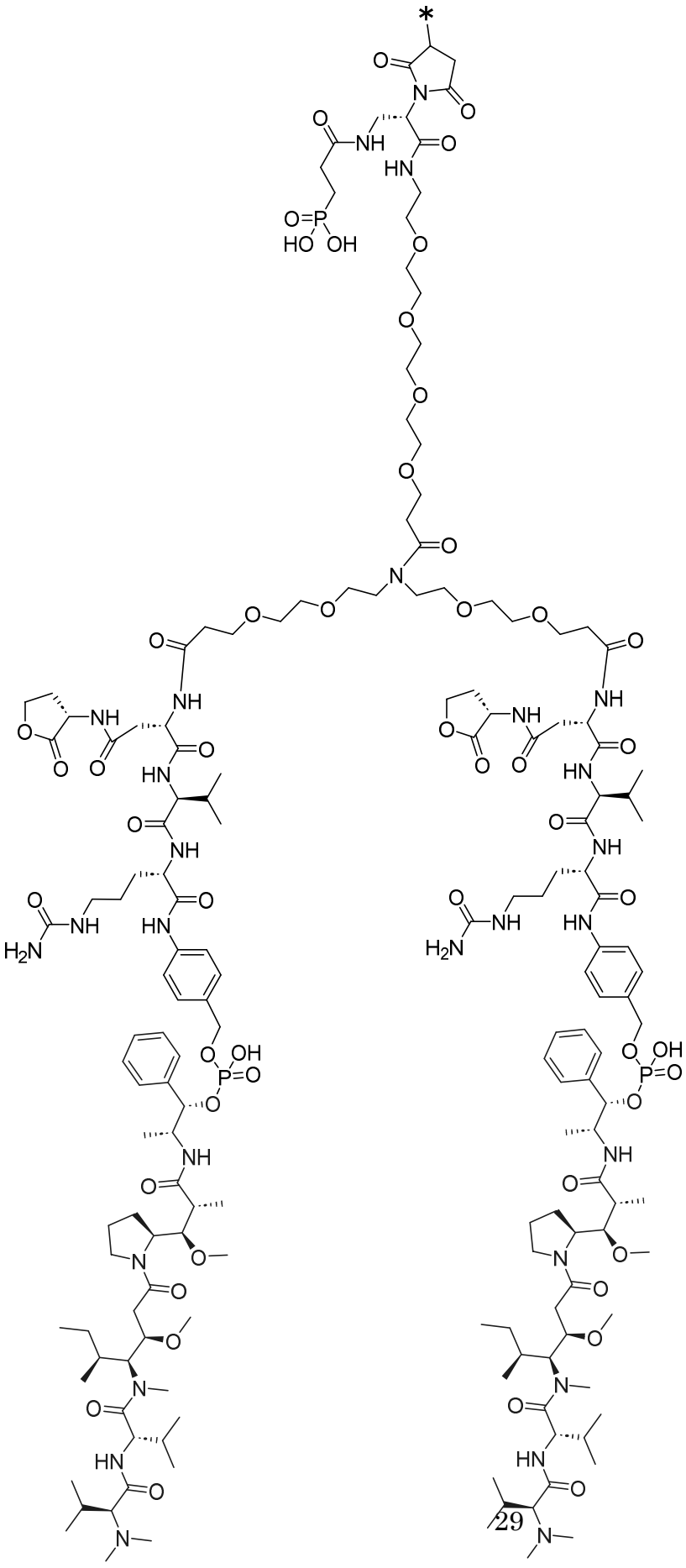
Furthermore, the DAR of the antibody-drug conjugate eluted with PBS, obtained in 1-5a, can be considered equal to the DAR of the antibody-drug conjugate eluted with PBS/EDTA; thus, the antibody concentration of the antibody-drug conjugate solution eluted with PBS can be determined using Equation (4) (Antibody-drug conjugate concentration: 0.83 mg/mL; this concentration is listed in Table 1).

[0088]

< Example 2: T004-PGAP-2 (ADC2) >

Synthesis of an antibody-drug conjugate of General Formula (I), where A is the moiety obtained by removing a sulfhydryl group from the reduced antibody T004, B is -S-, and C is the following Formula (y).

Formula (y):



(In the formula, * indicates a linking point with B.)

[0089]

The antibody-drug conjugate of Example 2 was obtained by performing the same reaction and the treatment procedures as in Example 1, using T004 and PGAP-2 (Synthesis Example 2) instead of PGAP-1. In Example 2, two chemotherapeutic agents are present at C of General Formula (I); in this case, DAR = 2a, and the DAR is the value obtained by doubling the value derived from Equation (5).

[0090]

Example 3: T004b-PGAP-1 (ADC3) and Example 4: T004b-PGAP-2 (ADC4)

Using T004b instead of T004, and PGAP-1 (Synthesis Example 1) or PGAP-2 (Synthesis Example 2), the same reaction and the treatment procedures as in Example 1 or Example 2 were performed, thereby obtaining the antibody-drug conjugates of Example 3 and Example 4, respectively. In Example 4, two chemotherapeutic agents are present at C of General Formula (I), and the DAR is the value obtained by doubling the value derived from Equation (5).

[0091]

The results of Examples 1 to 4 are summarized in Table 1.

[Table 1]

Example No.	The compound used to form the C-moiety	Obtained conjugate (I)				
		A	B	C	DAR Calculated value	Concentration (mg/mL)
1	PGAP-1 (Synthesis Example 1)	T004’	-S-	Formula (x)	7.7	0.83
2	PGAP-2 (Synthesis Example 2)	T004’	-S-	Formula (y)	14.8	0.82
3a	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	7.3	0.81
3b	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	7.6	0.80
4a	PGAP-2	T004b’	-S-	Formula (y)	14.2	0.79

	(Synthesis Example 2)					
4b	PGAP-2 (Synthesis Example 2)	T004b’	-S-	Formula (y)	14.6	0.78

(In the table, T004' represents the moiety obtained by removing a sulfhydryl group from the reduced antibody T004, and T004b' represents the moiety obtained by removing a sulfhydryl group from the reduced antibody T004b. In Examples 3 and 4, the synthesis was performed twice in each case, and the DAR was calculated for each.)

[0092]

< Example 5: T004b-PGAP-1-DAR4 (ADC5) >

Synthesis of an antibody-drug conjugate of General Formula (I), where A is the moiety obtained by removing a sulfhydryl group from the reduced antibody T004b, B is -S-, and C is (x) described in Example 1

[0093]

< 5-1 > Buffer exchange for T004b:

EDTA (manufactured by Thermo Fisher Scientific) was added to phosphate-buffered saline (hereinafter referred to as PBS; manufactured by Thermo Fisher Scientific) to a concentration of 10 mM (hereinafter referred to as PBS/EDTA). To each NAP-10 column (manufactured by Cytiva) pre-equilibrated with PBS/EDTA, 1 mL of the stock solution of T004b obtained in Reference Example 1 was applied; the sample was then eluted with 1.5 mL of PBS/EDTA, and the fraction containing T004b (1.5 mL) was collected, thereby obtaining a T004b solution. The absorbance at 280 nm of the T004b solution was measured using a microplate reader (FlexStation 3 manufactured by Molecular Devices) to determine the antibody concentration (Antibody concentration: 3.64 mg/mL).

[0094]

< 5-2 > Reduction of T004b:

The T004b solution obtained in < 1-1 > above was diluted with PBS/EDTA, and the concentration thereof was adjusted to approximately 2.5 mg/mL. A 0.99 mL aliquot of this solution was placed in a 1.5 mL microtube, and 10 μL of a PBS/EDTA solution of TCEP hydrochloride (manufactured by FUJIFILM Wako Pure Chemical Corporation) was added (such that the amount of TCEP was 3.45 equivalents per antibody molecule). A reduction reaction targeting the disulfide bonds between the peptide chains constituting antibody T004b was performed by incubating at 37°C for 2 hours, yielding a reduced T004b solution.

[0095]

< 5-3 > Conjugate formation between reduced T004b and PGAP-1 (Synthesis Example 1):

To 900 μL of the reduced T004b solution obtained in < 1-2 > above, 100 μL of a dimethyl sulfoxide solution containing PGAP-1 obtained in Synthesis Example 1 was added (such that the amount of PGAP-1 was 15 equivalents per antibody molecule), and the mixture was reacted for 1 hour under ice-cooling, thereby obtaining an antibody-drug conjugate (T004b-PGAP-1) solution.

[0096]

< 5-4 > Purification of antibody-drug conjugate:

To each pre-conditioned Protein A column (MonoSpin 96WP ProA manufactured by GL Sciences Inc.), 200 μL of the antibody-drug conjugate solution obtained in 1-3 above was added, and the conjugate was allowed to adsorb to the column. After washing the column with the wash buffer supplied with the column, the antibody-drug conjugate was eluted using the elution buffer supplied with the column.

The antibody-drug conjugate solution (1 mL) eluted from the Protein A column was applied to a NAP-10 column pre-equilibrated with PBS and eluted with 1.5 mL of PBS such that the fraction containing the antibody-drug conjugate (1.5 mL) was collected, thereby obtaining the purified antibody-drug conjugate. The absorbance at 280 nm of the purified antibody-drug conjugate was measured using a microplate reader (Absorbance: 1.33).

[0097]

< 5-5 > Drug-to-antibody ratio (DAR) measurement by HIC analysis:

The DAR profile of the antibody-drug conjugate solution eluted with PBS was measured using a high-performance liquid chromatography (HPLC) system (LC-20AD manufactured by Shimadzu Corporation) equipped with a hydrophobic chromatography column (TSKgel Butyl-NPR, 4.6 × 35 mm, manufactured by Tosoh Corporation). Mobile phase A consists of 0.03 M phosphate buffer and 1.5 M ammonium sulfate (pH 7.0), while mobile phase B consists of 90% v/v 0.03 M phosphate buffer (pH 7.0) and 10% v/v isopropanol. For sample preparation for analysis, the antibody-drug conjugate solution was diluted two-fold with a mixture of 50% v/v mobile phase A and 50% v/v mobile phase B, thereby preparing an analysis sample. When the antibody-drug conjugate solution was at a high concentration, a sample was prepared by dilution with PBS to approximately 1 mg/mL. A 10 μL aliquot of the analysis sample was injected into the column at a flow rate of 0.8 mL/min, and absorbance at 280 nm was monitored for 35 minutes using the gradient mode. The gradient was set to mobile phase A at 75% (0.0 min) – 35% (30.0 min) – 5% (30.1 min) – 5% (35.0 min). The average DAR was calculated by summing the products of the area percentage and the DAR value for each DAR, determined using software (LabSolutions manufactured by Shimadzu Corporation).

(Average DAR of antibody-drug conjugate: 3.79)

[0098]

< 5-6 > Calculation of Antibody-Drug Conjugate Solution Concentration

Since the total absorbance at a given wavelength is equal to the sum of all absorbing chemical species present in the system, and assuming there is no change in the molar extinction coefficients of the antibody and the drug before and after conjugation, the following equation holds for the absorbance of the antibody-drug conjugate.

$A_{ADC,\,280} = C_{ADC}(\varepsilon_{Ab,\,280} + DAR \times \varepsilon_{D,\,280})$ Equation (1)

(Here, $A_{ADC,\,280}$ represents the absorbance at 280 nm of the antibody-drug conjugate solution, C_{ADC} represents the antibody concentration of the antibody-drug conjugate, $\varepsilon_{Ab,\,280}$ represents the molar extinction coefficient of T004b at 280 nm, DAR represents the DAR of antibody-drug conjugate, $\varepsilon_{D,\,280}$ represents the molar extinction coefficient of PGAP-1 at 280 nm.)

C_{ADC} can be determined using Equation (1) (Antibody-drug conjugate C_{ADC}: 0.95 mg/mL).

[0099]

Example 6

The T004b solution obtained in the same manner as in Example < 5-1 > was diluted with PBS/EDTA to adjust the concentration to approximately 2.5 mg/mL. A 0.99 mL aliquot of this solution was placed in a 1.5 mL microtube, and 10 μL of a PBS/EDTA solution of TCEP hydrochloride (manufactured by FUJIFILM Wako Pure Chemical Corporation) was added (such that the amount of TCEP was 3.45 equivalents per antibody molecule). The mixture was incubated at 4°C for 6 hours to reduce the disulfide bonds in the hinge region of antibody T004b, yielding a reduced T004b solution. Thereafter, the same reaction and the treatment procedures as in Example 5 were performed, thereby obtaining the antibody-drug conjugate of Example 6 (ADC6).

[0100]

Example 7

The T004b solution obtained in the same manner as in Example < 5-1 > was diluted with PBS/EDTA to adjust the concentration to approximately 2.5 mg/mL. A 0.99 mL aliquot of this solution was placed in a 1.5 mL microtube, and 10 μL of a PBS/EDTA solution of TCEP hydrochloride (manufactured by FUJIFILM Wako Pure Chemical Corporation) was added (such that the amount of TCEP was 3.8 equivalents per antibody molecule). The mixture was incubated at 4°C for 6 hours to reduce the disulfide bonds in the hinge region of antibody T004b, yielding a reduced T004b solution. Thereafter, the same reaction and the treatment procedures as in Example 5 were performed, thereby obtaining the antibody-drug conjugate of Example 7 (ADC7).

[0101]

The results of Examples 5 to 7 are summarized in Table 2.

[0102]

[Table 2]

Example No.	The compound used to form the C-moiety	Obtained conjugate (I)				
		A	B	C	Average DAR	Concentration (mg/mL)
5a	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	3.79	0.95
5b	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	4.54	0.91
6a	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	3.59	0.95
6b	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	3.70	0.92
7a	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	3.57	0.88
7b	PGAP-1 (Synthesis Example 1)	T004b’	-S-	Formula (x)	3.82	0.90

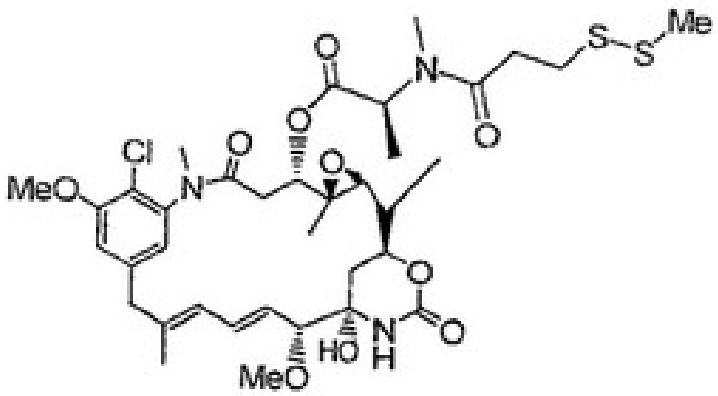
(In the table, T004b' represents the moiety obtained by removing a sulfhydryl group from the reduced antibody T004b. In Examples 5, 6 and 7, the synthesis was performed twice, and the DAR was calculated for each.)

[0103]

< Example 8: Preparation of Drug-Conjugate Antibody for Comparison (Control ADC) >

< 8-1 > Synthesis of drug

DM1-SMe was prepared as previously described in U.S. Patent Nos. 5,208,020 and 6,333,410 (B1).



[0104]

< 8-2 > Reduction treatment of conjugated drug

A mixture was prepared by combining 0.78 mg of DM1-SMe dissolved in 300 μL of ethanol, 180 μL of 50 mM potassium phosphate buffer (pH 7.5), and 20 μL of TCEP Solution (Bond-Breaker; Thermo Fisher Scientific), and was then reacted at room temperature for at least 30 minutes under a nitrogen atmosphere, thereby reducing the drug.

The reduced drug was purified by HPLC, subjected to solvent removal, and then dissolved in dimethylacetamide to a concentration of 10 mg/mL.

[0105]

< 8-3 > Preparation of maleimidated antibody

To 1 mg/mL T004, a 30-fold molar excess of Sulfo-SMCC (Pierce) was added, and the mixture was reacted at 30°C for 1 hour.

To remove the excess cross-linking agent, desalting was performed using a desalting column (Zeba Spin Column, Thermo Fisher Scientific) equilibrated with 50 mM potassium phosphate, 50 mM NaCl, and 2 mM EDTA (pH 6.5).

[0106]

< 8-4 > Modification of antibody with drug

Maleimide-modified T004 (1 mg/mL) was reacted overnight at room temperature with the reduced drug in an amount equivalent to 1.7 times the number of conjugated maleimide groups, in a buffer containing 50 mM potassium phosphate, 50 mM NaCl, and 2 mM EDTA (pH 6.5). Subsequently, the excess of the drug was removed by gel filtration.

[0107]

< 8-5 > Quantification of antibody-drug conjugation level

The number of drug conjugated per antibody was determined by measuring absorbance at 252 nm and 280 nm. In the quantification method, the extinction coefficients εAb_{280} = 223,000$M^{-1}cm^{-1}$, εAb_{252} = 82,510$M^{-1}cm^{-1}$, $\varepsilon DM1_{280}$ = 5,180$M^{-1}cm^{-1}$, and $\varepsilon DM1_{252}$ = 26,160$M^{-1}cm^{-1}$described in

the Non-Patent Document: (Widdison, W. C., Wilhelm, S. D., Cavanagh, E. E., et al. (2006) Semisynthetic maytansine analogues for the targeted treatment of cancer. J.Med.Chem.,49,4392-4408) were used.

[0108]

< Example 9: Cytotoxicity Assay >

The therapeutic efficacy of the ADC was evaluated using human CDH3-expressing cell lines HCC1954, BxPC3, and KLM1. First, these cell lines were seeded into 384-well plates (Greiner) at a density of 1,000 cells/well and cultured overnight at 37°C and 5% CO_2. On the following day, ADC1, ADC2, ADC3, and ADC4, which had been serially diluted in advance, were added to each well. After incubation for 4 days, cell viability was evaluated using CellTiter-Glo (Promega). The viability of each well was calculated by setting the luminescence value of wells containing cells cultured in the medium alone as 100% viability. The treatment concentration required to inhibit cell proliferation or survival by 50% (IC_{50}) was calculated using XLfit 5.2 (IDBS), and pIC50 ($Log(-IC_{50})$) was used for comparison.

[0109]

Table 3 summarizes the pIC_{50} results for various cell lines (HCC1954, BxPC3, KLM1) with ADCs 1 to 4. In the cell lines used, both ADC1 and ADC3, which employed the antibodies T004 and T004b, respectively, exhibited strong antiproliferative activity (Figure 1). Strong cell proliferation inhibitory effects were also observed with ADC1 and ADC2 (in which the antibody was T004) and with ADC3 and ADC4 (in which the antibody was T004b) (Figures 2 and 3).

[0110]

[Table 3]

	HCC1954	BxPC3	KLM1
ADC1	5.4	4.5	3.4
ADC2	5.6	4.8	4.2
ADC3	5.4	4.3	3.1
ADC4	5.6	4.7	3.9
Control ADC	5.9	3.3	2.6

[0111]

< Example 10: *In Vivo* Mouse Efficacy Study >

Cells of a human pancreatic cancer cell line, BxPC3, were cultured and expanded until the cell count reached approximately twice the amount required for transplantation.

On the day of transplantation, the cells were washed with PBS(-), detached using TrypLE Express (Thermo Fisher Scientific), and centrifuged at 1,000 rpm for 5 minutes, thereby collecting the pellet. The pellet was suspended in antibiotic- and serum-free RPMI-1640 medium and Matrigel

(Corning, 356237) to a concentration of 5×10^6 cells/mL, and 100 μL of the suspension was transplanted subcutaneously into the right flank of each mouse.

For the *in vivo* study, each group consisted of five mice, and each test solution was administered via the tail vein. The administration solutions contained ADC3 and ADC4 at 0.5, 1, and 2 mg/kg, respectively, and the control ADC at 2 mg/kg. A single dose was administered when the mean tumor volume reached approximately 150 mm^3.

[0112]

The results are shown in Figures 4 to 7. Both ADC3 and ADC4 demonstrated high therapeutic efficacy at the lowest dose of 0.5 mg/kg. There was no effect on body weight.

[0113]

Another *in vivo* study was conducted to compare the therapeutic efficacy in mice of ADCs 3, 5, and 7 with different drug-to-antibody ratios (DARs). As shown in Figure 8, mice administered with ADC5 and ADC7 (0.5 mg/kg), which had an average DAR of 4, exhibited significant therapeutic efficacy comparable to that of ADC3 (0.5 mg/kg), which had an average DAR of 8. In addition, no weight loss was observed in mice administered with the ADCs (Figure 9).

[0114]

< Example 11: Mouse Pharmacokinetic Study >

ADC3, ADC5, and ADC7 were administered at a single oral dose of 3 mg/kg to ICR mice; blood samples were collected over time (at 5 minutes, Day 3, Day 7, and Day 14), and plasma antibody levels (TAb) were measured by ELISA.

A solution of anti-T004b antibody (2.5 μg/mL; an antibody solution prepared in-house) in DPBS (GIBCO, 14190144) was added in an amount of 100 μL to each well of a 96-well polystyrene plate coated with MaxiSorp (trademark) (Thermo Scientific, 442404). The plate was incubated at 4°C overnight. After incubation, the plate was washed three times with PBS containing 0.05% Tween-20 (PBS-T). Next, the wells were blocked with a 100% Block Ace (KAC, UKB500) solution at room temperature for at least 1 hour. After blocking, the plate was washed three times with PBS-T. To generate a calibration curve, a standard solution was prepared by performing a 2-fold serial dilution in seven steps of the antibody or ADC solution in TBS-T (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween; prepared in-house), starting from 10 ng/mL. A plasma sample was prepared by performing a 2-fold serial dilution in four steps starting from an arbitrary dilution factor. The plasma sample and the standard solution (100 μL) were added to the wells of the ELISA plate and incubated at room temperature for 1 hour. After incubation, the sample was removed, and the plate was washed three times with PBS-T. A solution of HRP-F(ab')2 fragment anti-human IgG Fcγ (Jackson ImmunoResearch Laboratories, Inc.) was diluted 1:10,000 in TBS-T, and 100 μL was added to each well. The plate was incubated at room temperature for 1 hour. After incubation, the sample was

removed, and the plate was washed three times with PBS-T. A solution of TMB Soluble Reagent (ScyTek Laboratories) was added to each well (100 μL). The plate was incubated at room temperature for 10 to 20 minutes, and the reaction was quenched with 100 μL of TMB Stop Buffer (ScyTek Laboratories). The plate was read at 450 nm and 620 nm using a Multiskan FC absorbance microplate reader (Thermo Fisher Scientific). The concentration at each dilution factor was calculated from the calibration curve, and the mean value was determined.

The results are shown in Figure 10. ADC3, ADC5, and ADC7 exhibited nearly identical pharmacokinetics, which were also the same as those of T004b, the unconjugated antibody.

CLAIMS

[Claim 1]

An antibody-drug conjugate in which an anti-CDH3 humanized antibody and a chemotherapeutic agent having a linker are linked, wherein

the anti-CDH3 humanized antibody is:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2;

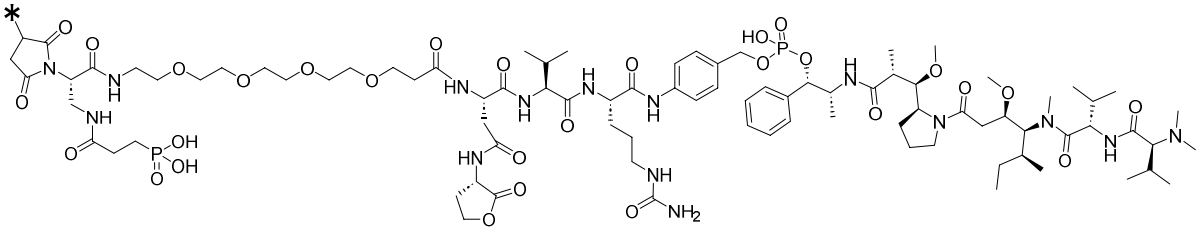
(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4;

(3) a fragment of the anti-CDH3 humanized antibody described in (1) or (2) above, which has CDH3-binding ability; or

(4) an antibody consisting of a partial sequence of the antibody described in (1) or (2) above, which has CDH3-binding ability,

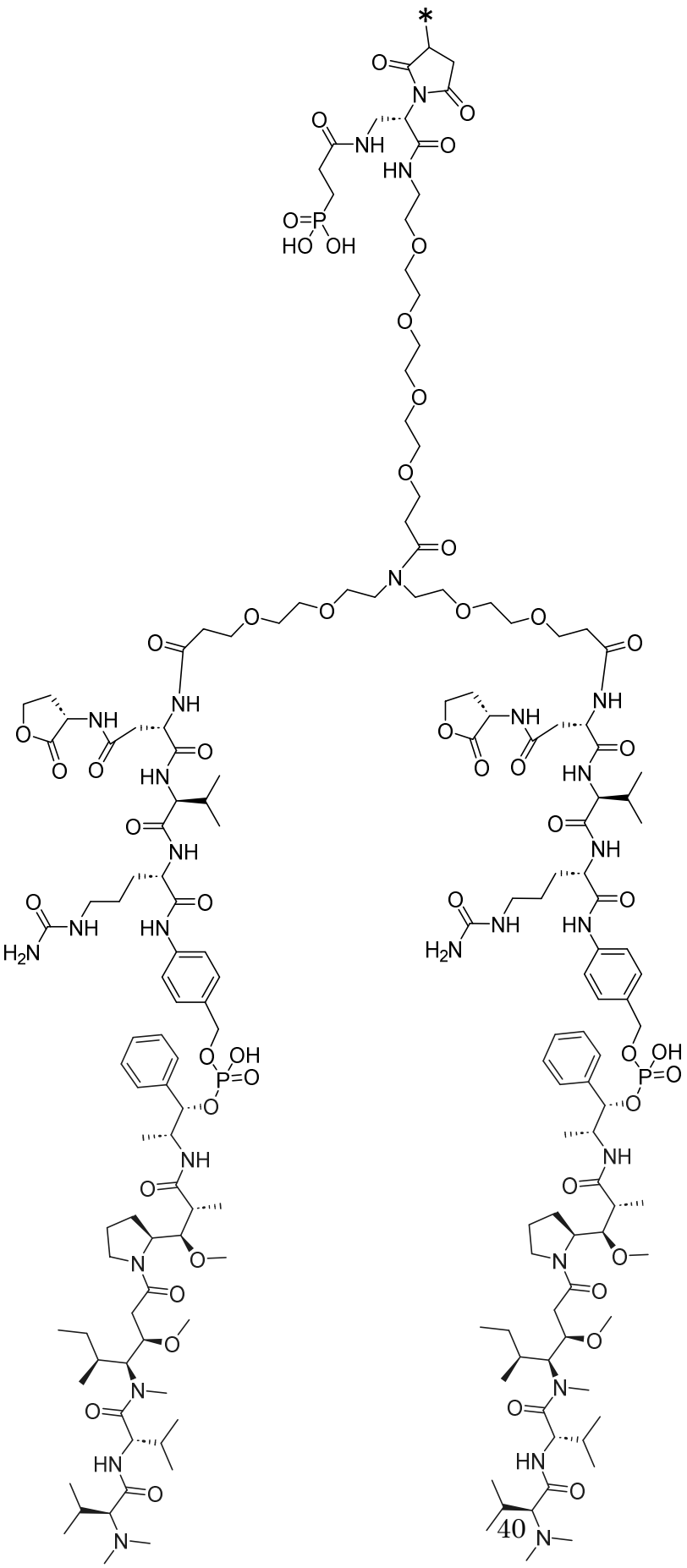
wherein

the chemotherapeutic agent having a linker is given by Formula (x):



(where * indicates a linking point with the antibody); or

Formula (y):



(where * indicates a linking point with the antibody),
pharmacologically acceptable salt thereof, or hydrate thereof.
[Claim 2]
The antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to claim 1, wherein the anti-CDH3 humanized antibody is any of the following antibodies:
(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or
(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.
[Claim 3]
The antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to claim 1, wherein an average of 1 to 10 molecules of the chemotherapeutic agent having a linker are conjugated to one molecule of the humanized anti-CDH3 antibody.
[Claim 4]
A medicament for treating a disease characterized by overexpression of CDH3, comprising the antibody-drug conjugate, pharmacologically acceptable salt thereof, or hydrate thereof according to any one of claims 1 to 3.
[Claim 5]
The medicament according to claim 4, wherein the disease characterized by overexpression of CDH3 is a tumor or cancer.