Description

Title of invention:

COMBINATION OF ANTIBODY-DRUG CONJUGATE AND IMMUNE CHECKPOINT INHIBITOR

Technical Field

[0001]

The present invention relates to a pharmaceutical composition and a therapeutic method wherein a specific antibody-drug conjugate and an immune checkpoint inhibitor are administered in combination, and a pharmaceutical composition and a therapeutic method for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect wherein a specific antibody-drug conjugate is included.

Background Art

[0002]

An antibody-drug conjugate (ADC) having a drug with cytotoxicity conjugated to an antibody, whose antigen is expressed on the surface of cancer cells and which also binds to an antigen capable of cellular internalization, and therefore can deliver the drug selectively to cancer cells, is thus expected to cause accumulation of the drug within cancer cells and to kill the cancer cells (Non-Patent Literatures 1 to 5).

[0003]

As one of such antibody-drug conjugates, an antibody-drug conjugate including an antibody and exatecan, which is a topoisomerase I inhibitor, as components is known (Patent Literatures 1 to 7). Among these, anti-HER2 antibody-drug conjugates (Non-Patent Literatures 6, 7), which exert a particularly superior antitumor effect and safety, are currently under clinical studies.

[0004]

Immune checkpoint inhibitors are agents that inhibit the immune suppression system and activate antitumor immunity (Non-Patent Literatures 8 to 10). Known examples of immune checkpoint inhibitors include nivolumab (Patent Literature 8) and pembrolizumab (Patent Literature 9) each of which is an anti-PD-1 antibody; atezolizumab (Patent Literature 10), durvalumab (Patent Literature 11), and avelumab (Patent Literature 12), each of which is an anti-PD-L1 antibody; and ipilimumab (Patent Literature 13) and tremelimumab (Patent Literature 14), each of which is an anti-CTLA-4 antibody.

[0005]

As a case in which an antibody-drug conjugate and an immune checkpoint inhibitor are administered in combination, a study on the use of trastuzumab emtansine (T-DM1) and an anti-CTLA-4/PD-1 antibody in combination is known (Non-Patent Literature 11).

Citation List

Patent Literatures

[0006]

Patent Literature 1: International Publication No. WO 2014/057687

Patent Literature 2: International Publication No. WO 2014/061277

Patent Literature 3: International Publication No. WO 2015/098099

Patent Literature 4: International Publication No. WO 2015/115091

Patent Literature 5: International Publication No. WO 2015/146132

Patent Literature 6: International Publication No. WO 2015/155976

Patent Literature 7: International Publication No. WO 2015/155998

Patent Literature 8: International Publication No. WO 2006/121168

Patent Literature 9: International Publication No. WO 2008/156712

Patent Literature 10: International Publication No. WO 2010/077634

Patent Literature 11: International Publication No. WO 2011/066389

Patent Literature 12: International Publication No. WO 2013/079174

Patent Literature 13: International Publication No. WO 2001/014424

Patent Literature 14: International Publication No. WO 2000/037504

Non-Patent Literatures

[0007]

Non-Patent Literature 1: Ducry, L., et al., Bioconjugate Chem. (2010) 21, 5-13.

Non-Patent Literature 2: Alley, S. C., et al., Current Opinion in Chemical Biology (2010) 14, 529-537.

Non-Patent Literature 3: Damle N. K. Expert Opin. Biol. Ther. (2004) 4, 1445-1452.

Non-Patent Literature 4: Senter P. D., et al., Nature Biotechnology (2012) 30, 631-637.

Non-Patent Literature 5: Howard A. et al., J Clin Oncol 29: 398-405.

Non-Patent Literature 6: Ogitani Y. et al., Clinical Cancer Research (2016) 22(20), 5097-5108.

Non-Patent Literature 7: Ogitani Y. et al., Cancer Science (2016) 107, 1039-1046.

Non-Patent Literature 8: Menon S. et al., Cancers (2016) 8, 106.

Non-Patent Literature 9: Pardoll DM., Nat Rev Cancer (2012) 12, 252-264.

Non-Patent Literature 10: Wolchok JD., Cell (2015) 162, 937.
Non-Patent Literature 11: Muller P. et al., Science Translational Medicine (2015) 7(315), 315ra188.

Summary of Invention

Technical Problem

[0008]

An object of the present invention is to provide a pharmaceutical composition and a therapeutic method which exert a particularly superior antitumor effect and safety through administering an antibody-drug conjugate and an immune checkpoint inhibitor in combination. Another object of the present invention is to provide a pharmaceutical composition and a therapeutic method for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect wherein a specific antibody-drug conjugate is included.

Solution to Problem

[0009]

The present inventors found that an excellent antitumor effect is exerted through administering a specific antibody-drug conjugate and an immune checkpoint inhibitor in combination; and further found that the antibody-drug conjugate has an antitumor immunity-activating effect.

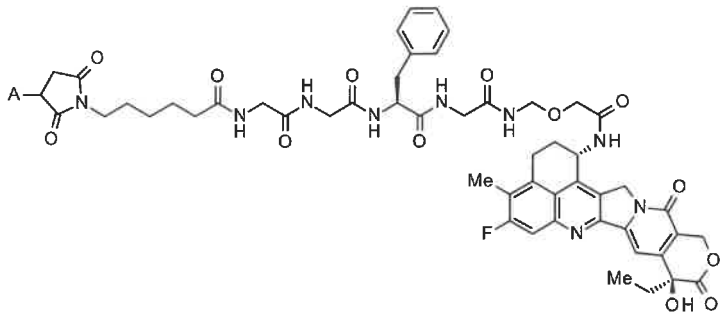
[0010]

Specifically, the present invention relates to the following.

[1] A pharmaceutical composition wherein an antibody-drug conjugate and an immune checkpoint inhibitor are administered in combination, and the antibody-drug conjugate is an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0011]

[Formula 1]



[0012]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[2] The pharmaceutical composition according to [1], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[3] The pharmaceutical composition according to [2], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[4] The pharmaceutical composition according to [2] or [3], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[5] The pharmaceutical composition according to [2] or [3], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[6] The pharmaceutical composition according to any one of [1] to [5], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[7] The pharmaceutical composition according to any one of [1] to [5], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[8] The pharmaceutical composition according to any one of [1] to [5], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[9] The pharmaceutical composition according to any one of [1] to [8], wherein the immune checkpoint inhibitor is

an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[10] The pharmaceutical composition according to [9], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

[11] The pharmaceutical composition according to [9], wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[12] The pharmaceutical composition according to [9], wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[13] The pharmaceutical composition according to any one of [1] to [12], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active components in different formulations, and are administered simultaneously or at different times.

[14] The pharmaceutical composition according to any one of [1] to [12], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered.

[15] The pharmaceutical composition according to any one of [1] to [14], wherein the composition is for treating cancer.

[16] The pharmaceutical composition according to [15], wherein the cancer is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer,

pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[17] The pharmaceutical composition according to [16], wherein the cancer is colorectal cancer.

[18] The pharmaceutical composition according to [16], wherein the cancer is breast cancer.

[19] The pharmaceutical composition according to any one of [1] to [18], wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[20] The pharmaceutical composition according to any one of [1] to [19], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[21] The pharmaceutical composition according to any one of [1] to [20], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[22] The pharmaceutical composition according to [21], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[23] The pharmaceutical composition according to [21], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[24] The pharmaceutical composition according to any one of [1] to [23], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

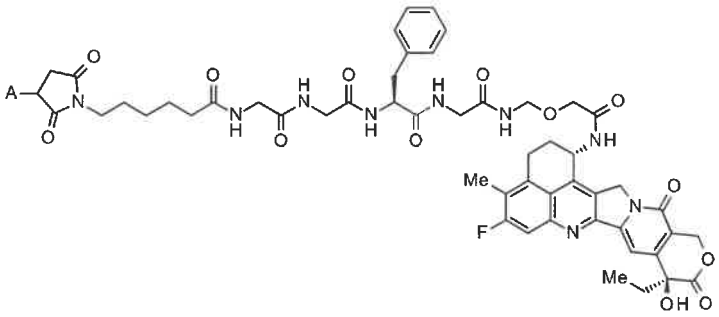
(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[25] The pharmaceutical composition according to any one of [1] to [24], wherein the immune checkpoint inhibitor deactivates an immunosuppression signal generated through elevation of the expression level of PD-L1 on cancer cells promoted by the antibody-drug conjugate, and thereby the antibody-drug conjugate exhibits a higher antitumor effect.

[26] A pharmaceutical composition for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein the pharmaceutical composition contains an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0013]

[Formula 2]



[0014]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[27] The pharmaceutical composition according to [26], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[28] The pharmaceutical composition according to [26] or [27], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[29] The pharmaceutical composition according to [28], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[30] The pharmaceutical composition according to [28], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[31] The pharmaceutical composition according to any one of [26] to [30], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[32] The pharmaceutical composition according to any one of [26] to [31], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[33] The pharmaceutical composition according to [32], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[34] The pharmaceutical composition according to [32] or [33], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid

sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[35] The pharmaceutical composition according to [32] or [33], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[36] The pharmaceutical composition according to any one of [26] to [35], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[37] The pharmaceutical composition according to any one of [26] to [35], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[38] The pharmaceutical composition according to any one of [26] to [35], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[39] The pharmaceutical composition according to any one of [26] to [38], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder

cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

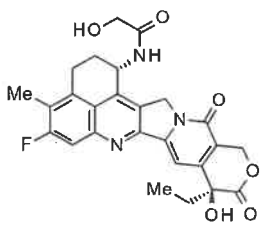
[40] The pharmaceutical composition according to [39], wherein the disease is colorectal cancer.

[41] The pharmaceutical composition according to [39], wherein the disease is breast cancer.

[42] A pharmaceutical composition for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein the pharmaceutical composition releases the compound represented by the following formula:

[0015]

[Formula 3]



[0016]

in a tumor.

[43] The pharmaceutical composition according to [42], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[44] The pharmaceutical composition according to [42] or [43], wherein the compound has a promoting effect on the formation of immune memory against tumor.

[45] The pharmaceutical composition according to any one of [42] to [44], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

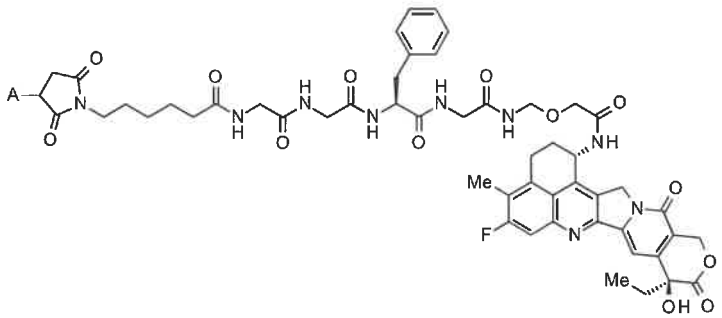
[46] The pharmaceutical composition according to any one of [42] to [45], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma,

peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[47] A therapeutic method wherein an antibody-drug conjugate and an immune checkpoint inhibitor are administered in combination, and the antibody-drug conjugate is an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0017]

[Formula 4]



[0018]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[48] The therapeutic method according to [47], wherein the antibody in the antibody-drug conjugate is an anti-

HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[49] The therapeutic method according to [48], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[50] The therapeutic method according to [48] or [49], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[51] The therapeutic method according to [48] or [49], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[52] The therapeutic method according to any one of [47] to [51], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[53] The therapeutic method according to any one of [47] to [51], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[54] The therapeutic method according to any one of [47] to [51], wherein the average number of units of the drug-

linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[55] The therapeutic method according to any one of [47] to [54], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[56] The therapeutic method according to [55], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

[57] The therapeutic method according to [55], wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[58] The therapeutic method according to [55], wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[59] The therapeutic method according to any one of [47] to [58], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active components in different formulations, and are administered simultaneously or at different times.

[60] The therapeutic method according to any one of [47] to [58], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered.

[61] The therapeutic method according to any one of [47] to [60], wherein the therapeutic method is for treating cancer.

[62] The therapeutic method according to [61], wherein the cancer is at least one selected from the group

consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[63] The therapeutic method according to [62], wherein the cancer is colorectal cancer.

[64] The therapeutic method according to [62], wherein the cancer is breast cancer.

[65] The therapeutic method according to any one of [47] to [64], wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[66] The pharmaceutical composition according to any one of [47] to [65], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[67] The therapeutic method according to any one of [47] to [66], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[68] The therapeutic method according to [67], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[69] The therapeutic method according to [67], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[70] The therapeutic method according to any one of [47] to [69], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

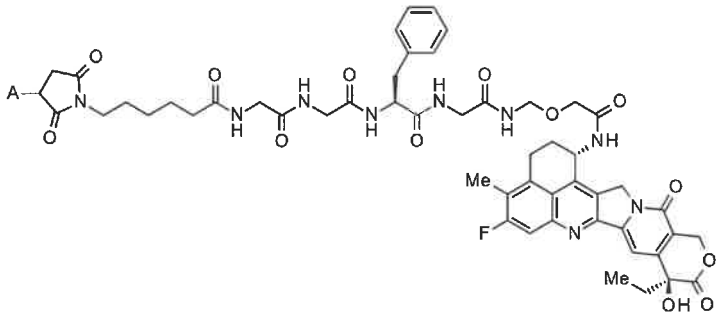
(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[71] The therapeutic method according to any one of [47] to [70], wherein the immune checkpoint inhibitor deactivates an immunosuppression signal generated through elevation of the expression level of PD-L1 on cancer cells promoted by the antibody-drug conjugate, and thereby the antibody-drug conjugate exhibits a higher antitumor effect.

[72] A therapeutic method for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect wherein an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0019]

[Formula 5]



[0020]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond, is administered.

[73] The therapeutic method according to [72], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[74] The therapeutic method according to [72] or [73], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.
[75] The therapeutic method according to [74], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.
[76] The therapeutic method according to [74], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.
[77] The therapeutic method according to any one of [72] to [76], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[78] The therapeutic method according to any one of [72] to [77], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.
[79] The therapeutic method according to [78], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.
[80] The therapeutic method according to [78] or [79], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence

consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[81] The therapeutic method according to [78] or [79], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[82] The therapeutic method according to any one of [72] to [81], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[83] The therapeutic method according to any one of [72] to [81], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[84] The therapeutic method according to any one of [72] to [81], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[85] The therapeutic method according to any one of [72] to [84], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma,

gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

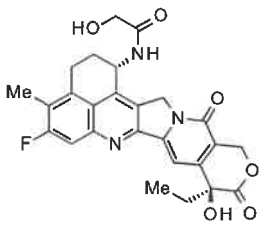
[86] The therapeutic method according to [85], wherein the disease is colorectal cancer.

[87] The therapeutic method according to [85], wherein the disease is breast cancer.

[88] A therapeutic method for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein the therapeutic method releases the compound represented by the following formula:

[0021]

[Formula 6]



[0022]

in a tumor.

[89] The therapeutic method according to [88], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[90] The therapeutic method according to [88] or [89], wherein the compound has a promoting effect on the formation of immune memory against tumor.

[91] The therapeutic method according to any one of [88] to [90], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

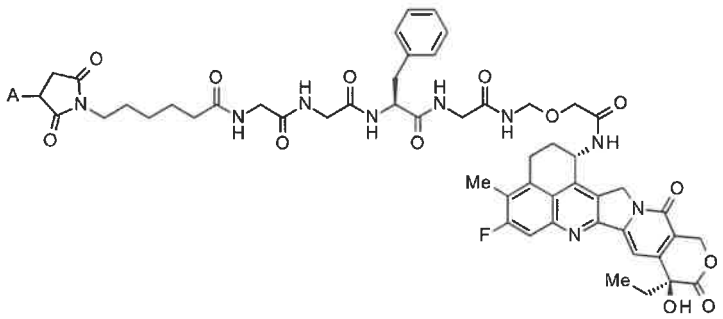
[92] The therapeutic method according to any one of [88] to [91], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial

cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[93] An antibody-drug conjugate for treating a disease through being administered in combination with an immune checkpoint inhibitor, wherein a drug-linker represented by the following formula:

[0023]

[Formula 7]



[0024]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond in the antibody-drug conjugate.

[94] The antibody-drug conjugate according to [93], wherein the antibody in the antibody-drug conjugate is an

anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[95] The antibody-drug conjugate according to [94], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[96] The antibody-drug conjugate according to [94] or [95], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[97] The antibody-drug conjugate according to [94] or [95], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[98] The antibody-drug conjugate according to any one of [93] to [97], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[99] The antibody-drug conjugate according to any one of [93] to [97], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[100] The antibody-drug conjugate according to any one of [93] to [97], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[101] The antibody-drug conjugate according to any one of [93] to [100], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[102] The antibody-drug conjugate according to [101], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

[103] The antibody-drug conjugate according to [101], wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[104] The antibody-drug conjugate according to [101], wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[105] The antibody-drug conjugate according to any one of [93] to [104], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active components in different formulations, and are administered simultaneously or at different times.

[106] The antibody-drug conjugate according to any one of [93] to [104], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered.

[107] The antibody-drug conjugate according to any one of [93] to [106], wherein the antibody-drug conjugate is for treating cancer.

[108] The antibody-drug conjugate according to [107], wherein the cancer is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[109] The antibody-drug conjugate according to [108], wherein the cancer is colorectal cancer.

[110] The antibody-drug conjugate according to [108], wherein the cancer is breast cancer.

[111] The antibody-drug conjugate according to any one of [93] to [110], wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[112] The antibody-drug conjugate according to any one of [93] to [111], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[113] The antibody-drug conjugate according to any one of [93] to [112], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[114] The antibody-drug conjugate according to [113], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[115] The antibody-drug conjugate according to [113], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[116] The antibody-drug conjugate according to any one of [93] to [115], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

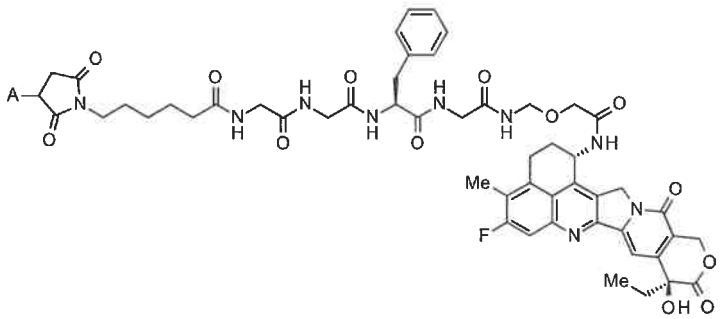
(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[117] The antibody-drug conjugate according to any one of [93] to [116], wherein the immune checkpoint inhibitor deactivates an immunosuppression signal generated through elevation of the expression level of PD-L1 on cancer cells promoted by the antibody-drug conjugate, and thereby the antibody-drug conjugate exhibits a higher antitumor effect.

[118] An antibody-drug conjugate for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein a drug-linker represented by the following formula:

[0025]

[Formula 8]



[0026]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[119] The antibody-drug conjugate according to [118], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[120] The antibody-drug conjugate according to [118] or [119], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[121] The antibody-drug conjugate according to [120], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[122] The antibody-drug conjugate according to [120], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[123] The antibody-drug conjugate according to any one of [118] to [122], for use in treatment of a disease that can be ameliorated through at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[124] The antibody-drug conjugate according to any one of [118] to [123], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[125] The antibody-drug conjugate according to [124], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[126] The antibody-drug conjugate according to [124] or [125], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[127] The antibody-drug conjugate according to [124] or [125], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[128] The antibody-drug conjugate according to any one of [118] to [127], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[129] The antibody-drug conjugate according to any one of [118] to [127], wherein the average number of units of

the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[130] The antibody-drug conjugate according to any one of [118] to [127], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[131] The antibody-drug conjugate according to any one of [118] to [130], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

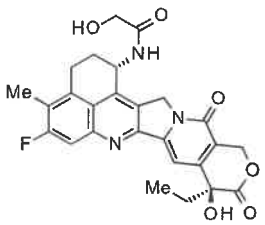
[132] The antibody-drug conjugate according to [131], wherein the disease is colorectal cancer.

[133] The antibody-drug conjugate according to [131], wherein the disease is breast cancer.

[134] A compound for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein the compound is represented by the following formula:

[0027]

[Formula 9]



[0028]

and released in a tumor.

[135] The compound according to [134], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[136] The compound according to [134] or [135], wherein the compound has a promoting effect on the formation of immune memory against tumor.

[137] The compound according to any one of [134] to [136], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

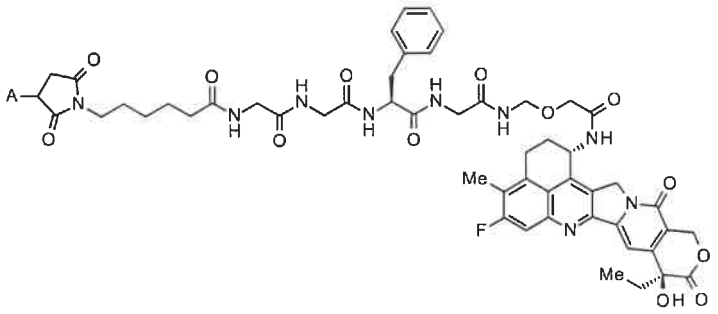
(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[138] The compound according to any one of [134] to [137], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[139] Use of an antibody-drug conjugate for production of a medicine for treating a disease through being administered in combination with an immune checkpoint inhibitor, wherein a drug-linker represented by the following formula:

[0029]

[Formula 10]



[0030]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond in the antibody-drug conjugate.

[140] The use according to [139], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[141] The use according to [140], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[142] The use according to [140] or [141], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[143] The use according to [140] or [141], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented

by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[144] The use according to any one of [139] to [143], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[145] The use according to any one of [139] to [143], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[146] The use according to any one of [139] to [143], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[147] The use according to any one of [139] to [146], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[148] The use according to [147], wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

[149] The use according to [147], wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[150] The use according to [147], wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[151] The use according to any one of [139] to [150], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active

components in different formulations, and are administered simultaneously or at different times.

[152] The use according to any one of [139] to [150], wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered.

[153] The use according to any one of [139] to [152], wherein the use is for treating cancer.

[154] The use according to [153], wherein the cancer is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[155] The use according to [154], wherein the cancer is colorectal cancer.

[156] The use according to [154], wherein the cancer is breast cancer.

[157] The use according to any one of [139] to [156], wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[158] The use according to any one of [139] to [157], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[159] The use according to any one of [139] to [158], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[160] The use according to [159], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[161] The use according to [159], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[162] The use according to any one of [139] to [161], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

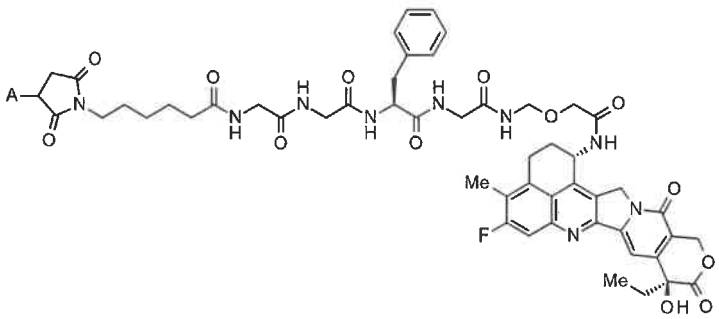
(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[163] The use according to any one of [139] to [162], wherein the immune checkpoint inhibitor deactivates an immunosuppression signal generated through elevation of the expression level of PD-L1 on cancer cells promoted by the antibody-drug conjugate, and thereby the antibody-drug conjugate exhibits a higher antitumor effect.

[164] Use of an antibody-drug conjugate for production of a medicine for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein a drug-linker represented by the following formula:

[0031]

[Formula 11]



[0032]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond in the antibody-drug conjugate.

[165] The use according to [164], wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[166] The use according to [164] or [165], wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[167] The use according to [166], wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[168] The use according to [166], wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[169] The use according to any one of [164] to [168], wherein the use is for production of a medicine for use in treatment of a disease that can be ameliorated through at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[170] The use according to any one of [164] to [169], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, or an anti-B7-H3 antibody.

[171] The use according to [170], wherein the antibody in the antibody-drug conjugate is an anti-HER2 antibody.

[172] The use according to [170] or [171], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[173] The use according to [170] or [171], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[174] The use according to any one of [164] to [173], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[175] The use according to any one of [164] to [173], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[176] The use according to any one of [164] to [173], wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[177] The use according to any one of [164] to [176], wherein the disease is at least one selected from the

group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

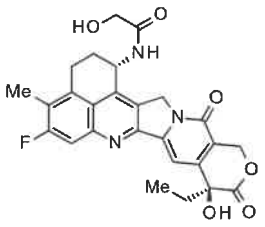
[178] The use according to [177], wherein the disease is colorectal cancer.

[179] The use according to [177], wherein the disease is breast cancer.

[180] Use of a compound for production of a medicine for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect, wherein the compound is represented by the following formula:

[0033]

[Formula 12]



[0034]

and released in a tumor.

[181] The use according to [180], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[182] The use according to [180] or [181], wherein the compound has a promoting effect on the formation of immune memory against tumor.

[183] The use according to any one of [180] to [182], wherein the compound has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[184] The use according to any one of [180] to [183], wherein the disease is at least one selected from the group consisting of lung cancer, urothelial cancer,

colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[0035]

Advantageous Effects of Invention

[0036]

The present invention can provide a pharmaceutical composition and a therapeutic method which exert a particularly superior antitumor effect and safety through administering a specific antibody-drug conjugate and an immune checkpoint inhibitor in combination. In addition, the present invention can provide a pharmaceutical composition and a therapeutic method for treating a disease that can be ameliorated through a promoting effect on the formation of immune memory against tumor wherein a specific antibody-drug conjugate is included.

Brief Description of Drawings

[0037]

[Figure 1] Figure 1 shows an amino acid sequence of a heavy chain of a humanized anti-HER2 antibody (SEQ ID NO: 1).

[Figure 2] Figure 2 shows an amino acid sequence of a light chain of a humanized anti-HER2 antibody (SEQ ID NO: 2).

[Figure 3] Figure 3 is a diagram showing life-prolonging effects of different agents on mice with subcutaneously transplanted CT26.WT-hHER2 cells. Comparison was made on life-prolonging effect between a single administration group with each of an antibody-drug conjugate (1) and an anti-PD-1 antibody (clone RMP1-14) and a combined administration group.

[Figure 4] Figure 4 is a diagram showing life-prolonging effects of different agents on mice with subcutaneously transplanted CT26.WT-hHER2 cells. Comparison was made on life-prolonging effect between a single administration group with each of an antibody-drug conjugate (1) and an anti-PD-1 antibody (clone RMP1-14) and a combined administration group.

[Figure 5] Figure 5 is a diagram showing transition of tumor volume in antibody-drug conjugate (1)-treated cured mice and control mice with subcutaneously transplanted

(retransplanted) CT26.WT-hHER2 cells or CT26.WT-mock cells.

[Figure 6] Figure 6 is a diagram showing immune response to an antigen derived from CT26.WT-hHER2 cells (the number of IFNγ-producing splenocytes) in antibody-drug conjugate (1)-treated cured mice and control mice with subcutaneously transplanted (retransplanted) CT26.WT-hHER2 cells.

[Figure 7] Figure 7 is a diagram showing immune response to an antigen derived from CT26.WT-mock cells (the number of IFNγ-producing splenocytes) in antibody-drug conjugate (1)-treated cured mice and control mice with subcutaneously transplanted (retransplanted) CT26.WT-hHER2 cells.

[Figure 8] Figure 8 is a diagram showing immune response to an antigen derived from CT26.WT-hHER2 cells (the number of IFNγ-producing splenocytes) in antibody-drug conjugate (1)-treated cured mice and control mice with subcutaneously transplanted (retransplanted) CT26.WT-mock cells.

[Figure 9] Figure 9 is a diagram showing immune response to an antigen derived from CT26.WT-mock cells (the number of IFNγ-producing splenocytes) in antibody-drug conjugate (1)-treated cured mice and control mice with subcutaneously transplanted (retransplanted) CT26.WT-mock cells.

[Figure 10] Figure 10 is a series of diagrams showing expression levels of CD86 in bone marrow-derived dendritic cells treated with a compound (A) and those treated with DMSO as determined with flow cytometry.

[Figure 11] Figure 11 is a series of diagrams showing expression levels of MHC class II in bone marrow-derived dendritic cells treated with a compound (A) and those treated with DMSO as determined with flow cytometry.

[Figure 12] Figure 12 is a diagram showing the number of dendritic cells among intratumor lymphocytes as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 13] Figure 13 is a diagram showing the number of CD86-positive cells among intratumor dendritic cells as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 14] Figure 14 is a diagram showing expression levels of CD86 on intratumor dendritic cells as determined with flow cytometry and expressed as MFI for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 15] Figure 15 is a diagram showing expression levels of MHC class I on cancer cells (human HER2-positive cells) as determined with flow cytometry and expressed as MFI for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 16] Figure 16 is a diagram showing expression levels of PD-L1 on cancer cells (human HER2-positive cells) as determined with flow cytometry and expressed as MFI for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 17] Figure 17 is a diagram showing expression levels of MHC class I as determined with flow cytometry for cancer cells treated with a compound (A) and those treated with DMSO.

[Figure 18] Figure 18 is a diagram showing transition of tumor volume for an antibody-drug conjugate (1)-administered group of mouse models of nude mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 19] Figure 19 is a diagram showing transition of tumor volume for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells, a control antibody-drug

conjugate-administered group thereof, and a control group thereof.

[Figure 20] Figure 20 is a diagram showing transition of tumor volume for single administration groups of mice with subcutaneously transplanted EMT6-hHER2 cells with each of an antibody-drug conjugate (1) and an anti-PD-1 antibody (clone RMP1-14), and a combined administration group thereof.

[Figure 21] Figure 21 is a diagram showing life-prolonging effects of different agents on mice with subcutaneously transplanted CT26.WT-hHER2 cells. Comparison was made on life-prolonging effect between a single administration group with each of an antibody-drug conjugate (1) and an anti-PD-L1 antibody (clone 10F.9G2) and a combined administration group.

[Figure 22] Figure 22 is a diagram showing life-prolonging effects of different agents on mice with subcutaneously transplanted EMT6-hHER2 cells. Comparison was made on life-prolonging effect between a single administration group with each of an antibody-drug conjugate (1) and an anti-PD-L1 antibody (clone 10F.9G2) and a combined administration group.

[Figure 23] Figure 23 is a diagram showing transition of tumor volume for single administration groups of mice with subcutaneously transplanted CT26.WT-hHER2 cells with each of an antibody-drug conjugate (1) and an anti-CD4 antibody, and a combined administration group thereof.

[Figure 24] Figure 24 is a diagram showing transition of tumor volume for single administration groups of mice with subcutaneously transplanted CT26.WT-hHER2 cells with each of an antibody-drug conjugate (1) and an anti-CD8 antibody, and a combined administration group thereof.

[Figure 25] Figure 25 is a diagram showing the fraction of CD8-positive T cells among intratumor living cells as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 26] Figure 26 is a diagram showing the fraction of Granzyme B-positive cells among intratumor CD8-positive T cells as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 27] Figure 27 is a diagram showing the fraction of CD8-positive T cells being Granzyme B-positive among intratumor living cells as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 28] Figure 28 is a diagram showing the fraction of CD4-positive T cells among intratumor living cells as determined with flow cytometry for an antibody-drug conjugate (1)-administered group of mice with

subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 29] Figure 29 is a series of images of excised tumors stained with an anti-CD8 antibody for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof.

[Figure 30] Figure 30 is a diagram showing the number of CD8-positive cells per unit area in tumors for an antibody-drug conjugate (1)-administered group of mice with subcutaneously transplanted CT26.WT-hHER2 cells and a control group thereof, as counted through analysis of images of excised tumors stained with an anti-CD8 antibody.

[Figure 31] Figure 31 is a diagram showing expression levels of MHC class I as determined with flow cytometry for cancer cells treated with a compound (A), those treated with DM1-SMe, those treated with DM4-SMe, those treated with MMAE, and those treated with DMSO.

[Figure 32] Figure 32 is a diagram showing transition of tumor volume for single administration groups of mice with subcutaneously transplanted EMT6-hHER2 cells with each of an antibody-drug conjugate (1) and an anti-CTLA-4 antibody (clone 9H10), and a combined administration group thereof.

Description of Embodiments

[0038]

Hereinafter, preferred modes for carrying out the present invention are described with reference to the drawings. The embodiments described below are given merely for illustrating one example of a typical embodiment of the present invention and are not intended to limit the scope of the present invention.

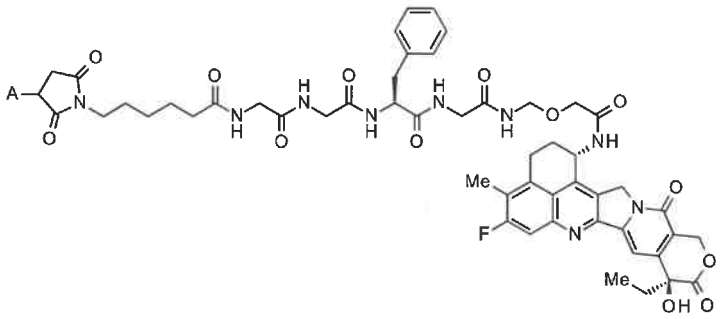
[0039]

[Antibody-drug conjugate]

The antibody-drug conjugate used in the present invention is an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0040]

[Formula 13]



[0041]

wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond.

[0042]

In the present invention, the partial structure consisting of a linker and a drug in the antibody-drug

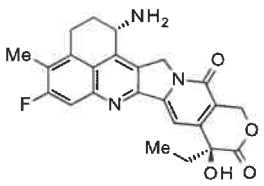
conjugate is referred to as a "drug-linker". The drug-linker is connected to a thiol group (in other words, the sulfur atom of a cysteine residue) formed at an interchain disulfide bond site (two sites between heavy chains, and two sites between a heavy chain and a light chain).

[0043]

The drug-linker of the present invention includes exatecan (IUPAC name: (1S,9S)-1-amino-9-ethyl-5-fluoro-1,2,3,9,12,15-hexahydro-9-hydroxy-4-methyl-10H,13H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10,13-dione, (also expressed as chemical name: (1S,9S)-1-amino-9-ethyl-5-fluoro-2,3-dihydro-9-hydroxy-4-methyl-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-10,13(9H,15H)-dione)), which is a topoisomerase I inhibitor, as a component. Exatecan is a camptothecin derivative having an antitumor effect, represented by the following formula:

[0044]

[Formula 14]



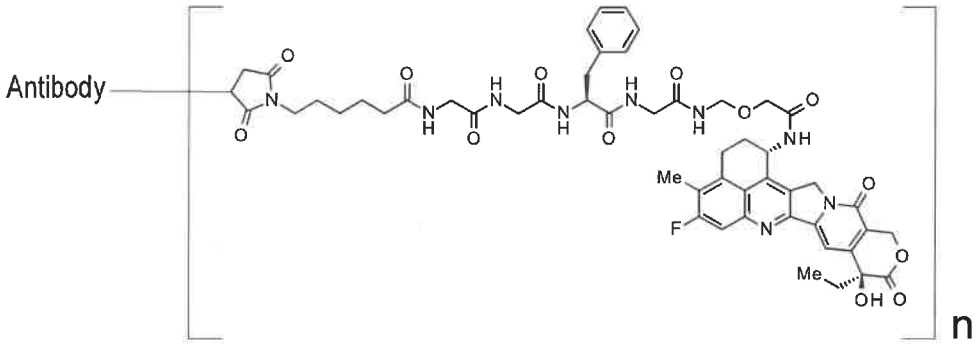
[0045]

[0046]

The antibody-drug conjugate used in the present invention can be also represented by the following formula.

[0047]

[Formula 15]



[0048]

Here, the drug-linker is conjugated to an antibody via a thioether bond. The meaning of n is the same as that of what is called the average number of conjugated drug molecules (DAR; Drug-to-Antibody Ratio), and indicates the average number of units of the drug-linker conjugated per antibody molecule.

[0049]

The antibody-drug conjugate used in the present invention has an antitumor immunity-activating effect.

[0050]

In the present invention, the term "antitumor immunity-activating" refers to a property of promoting exertion of an antitumor effect by activating at least one selected from the group consisting of T cells and B cells (Bracci L. et al., Cell Death Differ. (2014) 21,

15-25, Chen DS. Et al., Immunity (2013) 39, 1-10, Andersen MH. et al., Journal of Investigative Dermatology (2006) 126, 32-41).

[0051]

The situation that the antibody-drug conjugate used in the present invention is promoting exertion of an antitumor effect by activating at least one selected from the group consisting of T cells and B cells can be confirmed through comparison for the antibody-drug conjugate used in the present invention between an antitumor effect in mice with normal immune functions and that in mice with immune functions of T cells and B cells impaired (nude mice).

[0052]

The antibody-drug conjugate used in the present invention has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[0053]

The "promoting effect on growth of intratumor CD8-positive T cells" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the fraction of CD45-, CD3-, CD8-positive cells (CD8-positive T cells) among living

cells with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof. Alternatively, the effect can be confirmed by analyzing images of an excised tumor stained with an anti-CD8 antibody and counting the number of CD8-positive cells per unit area in the tumor to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof.

[0054]

The "activating effect on intratumor CD8-positive T cells" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the fraction of Granzyme B-positive cells among CD8-positive T cells with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof. Alternatively, the effect can be confirmed by determining the fraction of Granzyme B-positive cells among living cells with flow cytometry to examine the increase rate.

[0055]

The antibody-drug conjugate used in the present invention has a promoting effect on the formation of immune memory against tumor. This effect contributes to the above-described "antitumor immunity-activating effect".

[0056]

On being presented with a tumor-derived antigen from dendritic cells or cancer cells, T cells are activated to cause an immune response, exerting an antitumor effect.

[0057]

In the present invention, the phrase "formation of immune memory against tumor" refers to the phenomenon that T cells presented with a tumor-derived antigen generate memory T cells therefrom and thereby memory of an immune response against the antigen is formed. The phenomenon allows exertion of a sustained antitumor effect against tumor having the antigen, and further allows exertion of an antitumor effect again on the recurrence of tumor having the antigen.

[0058]

The "promoting effect on the formation of immune memory against tumor" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by administering the antibody-drug conjugate to cancer-bearing mice and retransplanting tumor to mice which have undergone complete tumor regression to determine the tumor proliferation (regression)-suppressing rate. Alternatively, the effect can be confirmed by excising the spleen from each of the mice and adding a tumor-derived antigen to the spleen to determine the increase rate of an immune response (e.g., the number of IFNγ-producing splenocytes).

[0059]

The antibody-drug conjugate used in the present invention has a promoting effect on the formation of immune memory not only against tumor expressing an antigen for the antibody in the antibody-drug conjugate, but also against tumor not expressing the antigen for the antibody in the antibody-drug conjugate in the same individual.

[0060]

In the case that the antibody in the antibody-drug conjugate is an anti-HER2 antibody, for example, the antibody-drug conjugate used in the present invention not only has a promoting effect on the formation of immune memory against tumor expressing HER2, but also has a promoting effect on the formation of immune memory against tumor not expressing HER2 in the same individual.

[0061]

In addition, the antibody-drug conjugate used in the present invention has at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

These effects contribute to the above-described "promoting effect on the formation of immune memory

against tumor", and eventually contribute to the above-described "antitumor immunity-activating effect".

[0062]

The "promoting effect on increase of the number of dendritic cells in a tumor" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the fraction of CD11c-, MHC class II-, CD45-positive cells (dendritic cells, DCs) among CD45-positive cells (lymphocytic cells) with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof.

[0063]

The "activating effect on dendritic cells" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the fraction of dendritic cells expressing CD86 (activation marker) with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof. Alternatively, the effect can be confirmed by determining the expression level (MFI (mean fluorescence intensity)) of CD86 on dendritic cells with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof.

[0064]

The "promoting effect on elevation of the expression level of MHC class I on cancer cells" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the expression level (MFI) of MHC class I on cancer cells with flow cytometry to examine the increase rate for an antibody-drug conjugate-administered group of cancer-bearing mice and a control group thereof.

[0065]

The antibody-drug conjugate used in the present invention occasionally has a promoting effect on elevation of the expression level of PD-L1 on cancer cells. Immune checkpoint inhibitors deactivate an immunosuppressive signal generated through the effect, and thereby the antibody-drug conjugate can exhibit a higher antitumor effect. Accordingly, the antibody-drug conjugate used in the present invention is expected to exhibit a higher antitumor effect if being used in combination with an immune checkpoint inhibitor.

[0066]

The "promoting effect on elevation of the expression level of PD-L1 on cancer cells" possessed by the antibody-drug conjugate used in the present invention can be confirmed, for example, by determining the expression level (MFI) of PD-L1 on cancer cells with flow cytometry to examine the increase rate for an antibody-drug

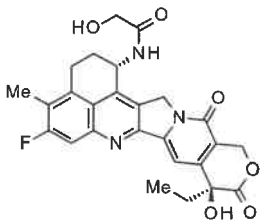
conjugate-administered group of cancer-bearing mice and a control group thereof.

[0067]

After migrating into cancer cells, the antibody-drug conjugate used in the present invention is cleaved at the linker portion to release a compound represented by the following formula:

[0068]

[Formula 16]



[0069]

(hereinafter, referred to as the "compound (A)").

[0070]

[0071]

The compound (A) is inferred to be the original source of the antitumor activity of the antibody-drug conjugate used in the present invention, and has been confirmed to have a topoisomerase I inhibitory effect (Ogitani Y. et al., Clinical Cancer Research, 2016, Oct 15;22(20):5097-5108, Epub 2016 Mar 29).

[0072]

The compound (A) has an activating effect on dendritic cells and a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[0073]

The "activating effect on dendritic cells" possessed by the compound (A) can be confirmed, for example, by determining the expression level of CD86 with flow cytometry to examine the increase rate for bone marrow-derived dendritic cells treated with the compound (A) and those treated with DMSO.

[0074]

The "promoting effect on elevation of the expression level of MHC class I on cancer cells" possessed by the compound (A) can be confirmed, for example, by determining the expression level of MHC class I with flow cytometry to examine the increase rate for cancer cells treated with the compound (A) and those treated with DMSO.

[0075]

The "activating effect on dendritic cells" and "promoting effect on elevation of the expression level of MHC class I on cancer cells" possessed by the compound (A) are effects associated with the "activating effect on dendritic cells" and "promoting effect on elevation of the expression level of MHC class I on cancer cells" possessed by the antibody-drug conjugate used in the present invention. As described above, the compound (A) is a compound which is released from the antibody-drug conjugate used in the present invention after the antibody-drug conjugate used in the present invention migrates into cancer cells.

[0076]

Accordingly, pharmaceutical compositions which release the compound (A) in a tumor are expected to have at least one effect selected from the group consisting of:

(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells,

as the antibody-drug conjugate used in the present invention.

[0077]

Further, pharmaceutical compositions which release the compound (A) in a tumor are expected to have a promoting effect on the formation of immune memory against tumor, as the antibody-drug conjugate used in the present invention.

[0078]

As described above, the compound (A) is a compound which is generated from the antibody-drug conjugate used in the present invention after the antibody-drug conjugate used in the present invention migrates into cancer cells.

[0079]

Accordingly, pharmaceutical compositions which release the compound (A) in a tumor are expected to have

at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells,

as the antibody-drug conjugate used in the present invention, and in addition are expected to have an "antitumor immunity-activating effect".

[0080]

The antibody-drug conjugate used in the present invention is known to have a bystander effect (Ogitani Y. et al., Cancer Science (2016) 107, 1039-1046).

The bystander effect is exerted through a process such that the antibody-drug conjugate used in the present invention is internalized in cancer cells expressing a target and the compound (A) released then exerts an antitumor effect also on cancer cells which are present therearound and not expressing the target.

The bystander effect possessed by the antibody-drug conjugate used in the present invention is exerted as an excellent antitumor effect even when using in combination with an immune checkpoint inhibitor.

[Antibody for use in production of antibody-drug conjugate]

The antibody for use in production of the antibody-drug conjugate according to the present invention may be

derived from any species, and is preferably an antibody derived from a human, a rat, a mouse, or a rabbit. In cases when the antibody is derived from species other than human species, it is preferably chimerized or humanized using a well known technique. The antibody of the present invention may be a polyclonal antibody or a monoclonal antibody and is preferably a monoclonal antibody.

[0081]

The antibody for use in production of the antibody-drug conjugate according to the present invention is an antibody preferably having a characteristic of being capable of targeting cancer cells, and is preferably an antibody possessing, for example, a property of recognizing a cancer cell, a property of binding to a cancer cell, a property of internalizing in a cancer cell, and/or cytocidal activity against cancer cells.

[0082]

The binding activity of the antibody against cancer cells can be confirmed using flow cytometry. The internalization of the antibody into tumor cells can be confirmed using (1) an assay of visualizing an antibody incorporated in cells under a fluorescence microscope using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Cell Death and Differentiation (2008) 15, 751-761), (2) an assay of measuring a fluorescence intensity incorporated in cells

using a secondary antibody (fluorescently labeled) binding to the therapeutic antibody (Molecular Biology of the Cell, Vol. 15, 5268-5282, December 2004), or (3) a Mab-ZAP assay using an immunotoxin binding to the therapeutic antibody wherein the toxin is released upon incorporation into cells to inhibit cell growth (Bio Techniques 28: 162-165, January 2000). As the immunotoxin, a recombinant complex protein of a diphtheria toxin catalytic domain and protein G may be used.

[0083]

The antitumor activity of the antibody can be confirmed in vitro by determining inhibitory activity against cell growth. For example, a cancer cell line overexpressing a target protein for the antibody is cultured, and the antibody is added at varying concentrations into the culture system to determine inhibitory activity against focus formation, colony formation, and spheroid growth. The antitumor activity can be confirmed in vivo, for example, by administering the antibody to a nude mouse with a transplanted cancer cell line highly expressing the target protein, and determining change in the cancer cell.

[0084]

Since the compound conjugated in the antibody-drug conjugate exerts an antitumor effect, it is preferred but not essential that the antibody itself should have an

antitumor effect. For the purpose of specifically and selectively exerting the cytotoxic activity of the antitumor compound against cancer cells, it is important and also preferred that the antibody should have the property of internalizing to migrate into cancer cells.
[0085]

The antibody for use in production of the antibody-drug conjugate according to the present invention can be obtained by a procedure known in the art. For example, the antibody of the present invention can be obtained using a method usually carried out in the art, which involves immunizing animals with an antigenic polypeptide and collecting and purifying antibodies produced *in vivo*. The origin of the antigen is not limited to humans, and the animals may be immunized with an antigen derived from a non-human animal such as a mouse, a rat and the like. In this case, the cross-reactivity of antibodies binding to the obtained heterologous antigen with human antigens can be tested to screen for an antibody applicable to a human disease.
[0086]

Alternatively, antibody-producing cells which produce antibodies against the antigen are fused with myeloma cells according to a method known in the art (e.g., Kohler and Milstein, Nature (1975) 256, p. 495-497; and Kennet, R. ed., Monoclonal Antibodies, p. 365-

367, Plenum Press, N.Y. (1980)) to establish hybridomas, from which monoclonal antibodies can in turn be obtained.
[0087]

The antigen can be obtained by genetically engineering host cells to produce a gene encoding the antigenic protein. Specifically, vectors that permit expression of the antigen gene are prepared and transferred to host cells so that the gene is expressed. The antigen thus expressed can be purified. The antibody can also be obtained by a method of immunizing animals with the above-described genetically engineered antigen-expressing cells or a cell line expressing the antigen.
[0088]

The antibody for use in production of the antibody-drug conjugate according to the present invention is preferably a recombinant antibody obtained by artificial modification for the purpose of decreasing heterologous antigenicity to humans such as a chimeric antibody or a humanized antibody, or is preferably an antibody having only the gene sequence of an antibody derived from a human, that is, a human antibody. These antibodies can be produced using a known method.
[0089]

As the chimeric antibody, an antibody in which antibody variable and constant regions are derived from different species, for example, a chimeric antibody in which a mouse- or rat-derived antibody variable region is

connected to a human-derived antibody constant region can be exemplified (Proc. Natl. Acad. Sci. USA, 81, 6851-6855, (1984)).

[0090]

As the humanized antibody, an antibody obtained by integrating only the complementarity determining region (CDR) of a heterologous antibody into a human-derived antibody (Nature (1986) 321, pp. 522-525), and an antibody obtained by grafting a part of the amino acid residues of the framework of a heterologous antibody as well as the CDR sequence of the heterologous antibody to a human antibody by a CDR-grafting method (WO 90/07861), and an antibody humanized using a gene conversion mutagenesis strategy (U.S. Patent No. 5821337) can be exemplified.

[0091]

As the human antibody, an antibody generated by using a human antibody-producing mouse having a human chromosome fragment including genes of a heavy chain and light chain of a human antibody (see Tomizuka, K. et al., Nature Genetics (1997) 16, p.133-143; Kuroiwa, Y. et. al., Nucl. Acids Res. (1998) 26, p.3447-3448; Yoshida, H. et. al., Animal Cell Technology:Basic and Applied Aspects vol.10, p.69-73 (Kitagawa, Y., Matsuda, T. and Iijima, S. eds.), Kluwer Academic Publishers, 1999; Tomizuka, K. et. al., Proc. Natl. Acad. Sci. USA (2000) 97, p.722-727, etc.) can be exemplified. As an alternative, an antibody

obtained by phage display, the antibody being selected from a human antibody library (see Wormstone, I. M. et. al, Investigative Ophthalmology & Visual Science. (2002)43 (7), p.2301-2308; Carmen, S. et. al., Briefings in Functional Genomics and Proteomics (2002), 1(2), p.189-203; Siriwardena, D. et. al., Ophthalmology (2002) 109(3), p.427-431, etc.) can be exemplified.

[0092]

In the present invention, modified variants of the antibody for use in production of the antibody-drug conjugate according to the present invention are also included. The modified variant refers to a variant obtained by subjecting the antibody according to the present invention to chemical or biological modification. Examples of the chemically modified variant include variants including a linkage of a chemical moiety to an amino acid skeleton, variants including a linkage of a chemical moiety to an N-linked or O-linked carbohydrate chain, etc. Examples of the biologically modified variant include variants obtained by post-translational modification (such as N-linked or O-linked glycosylation, N- or C-terminal processing, deamidation, isomerization of aspartic acid, or oxidation of methionine), and variants in which a methionine residue has been added to the N terminus by being expressed in a prokaryotic host cell. Further, an antibody labeled so as to enable the detection or isolation of the antibody or an antigen

according to the present invention, for example, an enzyme-labeled antibody, a fluorescence-labeled antibody, and an affinity-labeled antibody are also included in the meaning of the modified variant. Such a modified variant of the antibody according to the present invention is useful for improving the stability and blood retention of the antibody, reducing the antigenicity thereof, detecting or isolating an antibody or an antigen, and so on.

[0093]

Further, by regulating the modification of a glycan which is linked to the antibody according to the present invention (glycosylation, defucosylation, etc.), it is possible to enhance antibody-dependent cellular cytotoxic activity. As the technique for regulating the modification of a glycan of antibodies, WO 99/54342, WO 00/61739, WO 02/31140, etc. are known. However, the technique is not limited thereto. In the antibody according to the present invention, antibodies in which the modification of a glycan is regulated are also included.

[0094]

It is known that a lysine residue at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell is deleted (Journal of Chromatography A, 705: 129-134 (1995)), and it is also known that two amino acid residues (glycine and lysine)

at the carboxyl terminus of the heavy chain of an antibody produced in a cultured mammalian cell are deleted and a proline residue newly located at the carboxyl terminus is amidated (Analytical Biochemistry, 360: 75-83 (2007)). However, such deletion and modification of the heavy chain sequence do not affect the antigen-binding affinity and the effector function (the activation of complement, antibody-dependent cellular cytotoxicity, etc.) of the antibody. Therefore, in the antibody according to the present invention, antibodies subjected to such modification and functional fragments of the antibody are also included, and deletion variants in which one or two amino acids have been deleted at the carboxyl terminus of the heavy chain, variants obtained by amidation of deletion variants (for example, a heavy chain in which the carboxyl terminal proline residue has been amidated), and the like are also included. The type of deletion variant having a deletion at the carboxyl terminus of the heavy chain of the antibody according to the present invention is not limited to the above variants as long as the antigen-binding affinity and the effector function are conserved. The two heavy chains constituting the antibody according to the present invention may be of one type selected from the group consisting of a full-length heavy chain and the above-described deletion variant, or may be of two types in combination selected therefrom. The ratio of the

amount of each deletion variant can be affected by the type of cultured mammalian cells which produce the antibody according to the present invention and the culture conditions; however, an antibody in which one amino acid residue at the carboxyl terminus has been deleted in both of the two heavy chains in the antibody according to the present invention can be preferably exemplified.

[0095]

As isotypes of the antibody according to the present invention, for example, IgG (IgG1, IgG2, IgG3, IgG4) can be exemplified, and IgG1 or IgG2 can be exemplified preferably.

[0096]

Examples of antibodies applicable to production of the antibody-drug conjugate according to the present invention can include, but are not particularly limited to, an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, an anti-B7-H3 antibody, an anti-CD3 antibody, an anti-CD30 antibody, an anti-CD33 antibody, an anti-CD37 antibody, an anti-CD56 antibody, an anti-CD98 antibody, an anti-DR5 antibody, an anti-EGFR antibody, an anti-EPHA2 antibody, an anti-FGFR2 antibody, an anti-FGFR4 antibody, an anti-FOLR1 antibody, an anti-VEGF antibody, an anti-CD20 antibody, an anti-CD22 antibody, an anti-CD70 antibody, an anti-PSMA antibody, an anti-CEA antibody, and an anti-Mesothelin antibody,

and an anti-HER2 antibody, an anti-HER3 antibody, an anti-TROP2 antibody, and an anti-B7-H3 antibody can be preferably exemplified, and an anti-HER2 antibody can be more preferably exemplified.

[0097]

In the present invention, the term "anti-HER2 antibody" refers to an antibody which specifically binds to HER2 (Human Epidermal Growth Factor Receptor Type 2; ErbB-2), and preferably has an activity of internalizing in HER2-expressing cells by binding to HER2.

[0098]

Examples of the anti-HER2 antibody include trastuzumab (U.S. Patent No. 5821337) and pertuzumab (International Publication No. WO 01/00245), and trastuzumab can be preferably exemplified.

[0099]

In the present invention, the term "trastuzumab" is also called HERCEPTIN(registered trademark), huMAb4D5-8, or rhuMAb4D5-8 and is a humanized anti-HER2 antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 (Figure 1) and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2 (Figure 2).

[0100]

A preferred anti-HER2 antibody for use in production of the antibody-drug conjugate according to the present invention is:

(1) an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2; or

(2) an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[0101]

In the present invention, the term "anti-HER3 antibody" refers to an antibody which specifically binds to HER3 (Human Epidermal Growth Factor Receptor Type 3; ErbB-3), and preferably has an activity of internalizing in HER3-expressing cells by binding to HER3.

[0102]

Examples of the anti-HER3 antibody include patritumab (U3-1287), U1-59 (International Publication No. WO 2007/077028), MM-121 (seribantumab), an anti-ERBB3 antibody described in International Publication No. WO 2008/100624, RG-7116 (lumretuzumab), and LJM-716 (elgemtumab), and patritumab and U1-59 can be preferably exemplified.

[0103]

In the present invention, the term "anti-TROP2 antibody" refers to an antibody which specifically binds to TROP2 (TACSTD2: Tumor-associated calcium signal transducer 2; EGP-1), and preferably has an activity of internalizing in TROP2-expressing cells by binding to TROP2.

[0104]

Examples of the anti-TROP2 antibody include hTINA1-H1L1 (International Publication No. WO 2015/098099).

[0105]

In the present invention, the term "anti-B7-H3 antibody" refers to an antibody which specifically binds to B7-H3, and preferably has an activity of internalizing in B7-H3-expressing cells by binding to B7-H3.

[0106]

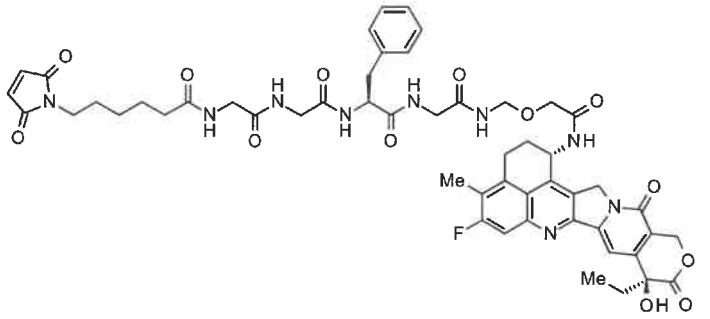
Examples of the anti-B7-H3 antibody include M30-H1-L4 (International Publication No. WO 2014/057687).

[Drug-linker intermediate for use in production of antibody-drug conjugate]

A drug-linker intermediate for use in production of the antibody-drug conjugate according to the present invention is represented by the following formula.

[0107]

[Formula 17]



[0108]

The drug-linker intermediate can be expressed as the chemical name N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]glycylglycyl-L-phenylalanyl-N-[(2-{[(1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-2,3,9,10,13,15-hexahydro-1H,12H-benzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl]amino}-2-oxoethoxy)methyl]glycinamide, and can be produced with reference to descriptions in International Publication No. WO 2014/057687, International Publication No. WO 2015/098099, International Publication No. WO 2015/115091, International Publication No. WO 2015/155998, and so on.

[0109]

[Conjugation between antibody and drug-linker intermediate]

The antibody-drug conjugate used in the present invention can be produced by reacting the above-described drug-linker intermediate and an antibody having a thiol group (or referred to as a sulfhydryl group).

[0110]

The antibody having a sulfhydryl group can be obtained by a method well known in the art (Hermanson, G. T, Bioconjugate Techniques, pp. 56-136, pp. 456-493, Academic Press (1996)). For example, by using 0.3 to 3 molar equivalents of a reducing agent such as tris(2-carboxyethyl)phosphine hydrochloride (TCEP) per interchain disulfide within the antibody and reacting with the antibody in a buffer solution containing a chelating agent such as ethylenediamine tetraacetic acid (EDTA), an antibody having a sulfhydryl group with partially or completely reduced interchain disulfides within the antibody can be obtained.

[0111]

Further, by using 2 to 20 molar equivalents of the drug-linker intermediate per the antibody having a sulfhydryl group, an antibody-drug conjugate in which 2 to 8 drug molecules are conjugated per antibody molecule can be produced.

[0112]

The average number of conjugated drug molecules per antibody molecule of the antibody-drug conjugate produced can be determined, for example, by a method of calculation based on measurement of UV absorbance for the antibody-drug conjugate and the conjugation precursor thereof at two wavelengths of 280 nm and 370 nm (UV method), or a method of calculation based on quantification through HPLC measurement for fragments

obtained by treating the antibody-drug conjugate with a reducing agent (HPLC method).

[0113]

Conjugation between the antibody and the drug-linker intermediate and calculation of the average number of conjugated drug molecules per antibody molecule of the antibody-drug conjugate can be performed with reference to descriptions in International Publication No. WO 2014/057687, International Publication No. WO 2015/098099, International Publication No. WO 2015/115091, International Publication No. WO 2015/155998, and so on.

[0114]

In the present invention, the term "anti-HER2 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in an antibody-drug conjugate is an anti-HER2 antibody.

[0115]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-HER2 antibody-drug conjugate used in the present invention is preferably 2 to 8, more preferably 3 to 8, even more preferably 7 to 8, even more preferably 7.5 to 8, and even more preferably about 8.

[0116]

The anti-HER2 antibody-drug conjugate used in the present invention can be produced with reference to

descriptions in International Publication No. WO 2015/115091 and so on.

[0117]

In the present invention, the term "anti-HER3 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in an antibody-drug conjugate is an anti-HER3 antibody.

[0118]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-HER3 antibody-drug conjugate used in the present invention is preferably 2 to 8, more preferably 3 to 8, even more preferably 7 to 8, even more preferably 7.5 to 8, and even more preferably about 8.

[0119]

The anti-HER3 antibody-drug conjugate used in the present invention can be produced with reference to descriptions in International Publication No. WO 2015/155998 and so on.

[0120]

In the present invention, the term "anti-TROP2 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in an antibody-drug conjugate is an anti-TROP2 antibody.

[0121]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-TROP2

antibody-drug conjugate used in the present invention is preferably 2 to 8, more preferably 3 to 5, even more preferably 3.5 to 4.5, and even more preferably about 4.

[0122]

The anti-TROP2 antibody-drug conjugate used in the present invention can be produced with reference to descriptions in International Publication No. WO 2015/098099 and so on.

[0123]

In the present invention, the term "anti-B7-H3 antibody-drug conjugate" refers to an antibody-drug conjugate such that the antibody in an antibody-drug conjugate is an anti-B7-H3 antibody.

[0124]

The average number of units of the drug-linker conjugated per antibody molecule in the anti-B7-H3 antibody-drug conjugate used in the present invention is preferably 2 to 8, more preferably 3 to 5, even more preferably 3.5 to 4.5, and even more preferably about 4.

[0125]

The anti-B7-H3 antibody-drug conjugate used in the present invention can be produced with reference to descriptions in International Publication No. WO 2014/057687 and so on.

[0126]

[Immune checkpoint inhibitor]

In the present invention, the term "immune checkpoint inhibitor" refers to an agent which inhibits the immune suppression system to activate tumor immunity.

[0127]

Preferred examples of the immune checkpoint inhibitor used in the present invention can include, but not particularly limited to, an anti-PD-1 antibody, an anti-PD-L1 antibody, and an anti-CTLA-4 antibody, and an anti-PD-1 antibody and an anti-PD-L1 antibody can be more preferably exemplified.

[0128]

In the present invention, the term "anti-PD-1 antibody" refers to an antibody which specifically binds to PD-1 (Programmed cell death-1; CD279; PDCD1), and has an activity of reducing, inhibiting, and/or interfering with signal transduction caused by interaction between PD-1 and PD-L1 or PD-L2 as a binding partner . The anti-PD-1 antibody used in the present invention is not particularly limited as long as the clinical efficacy and safety thereof have been confirmed, and nivolumab (International Publication No. WO 2006/121168, etc.) and pembrolizumab (International Publication No. WO 2008/156712, etc.) can be preferably exemplified. For the purpose of confirming the effect of use in combination with the antibody-drug conjugate used in the present invention in a preclinical study, a commercially

available anti-PD-1 antibody for research (e.g., clone RMP1-14) and so on can be used.

[0129]

In the present invention, the term "anti-PD-L1 antibody" refers to an antibody which specifically binds to PD-L1 (Programmed cell death ligand 1; CD274; B7-H1), and has an activity of reducing, inhibiting, and/or interfering with signal transduction caused by interaction between PD-L1 and PD-1 or B7.1 (CD80) as a binding partner. The anti-PD-L1 antibody used in the present invention is not particularly limited as long as the clinical efficacy and safety thereof have been confirmed, and atezolizumab (International Publication No. WO 2010/077634, etc.), durvalumab (International Publication No. WO 2011/066389, etc.), and avelumab (International Publication No. WO 2013/079174, etc.) can be preferably exemplified. For the purpose of confirming the effect of use in combination with the antibody-drug conjugate used in the present invention in a preclinical study, a commercially available anti-PD-L1 antibody for research (e.g., clone 10F.9G2) and so on can be used.

[0130]

In the present invention, the term "anti-CTLA-4 antibody" refers to an antibody which specifically binds to CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4; CD152), and has an activity of reducing, inhibiting, and/or interfering with signal transduction caused by

interaction between CTLA-4 and B7.1 (CD80) or B7.2 (CD86) as a binding partner. The anti-CTLA-4 antibody used in the present invention is not particularly limited as long as the clinical efficacy and safety thereof have been confirmed, and ipilimumab (International Publication No. WO 2001/014424, etc.) and tremelimumab (International Publication No. WO 2000/037504, etc.) can be preferably exemplified. For the purpose of confirming the effect of use in combination with the antibody-drug conjugate used in the present invention in a preclinical study, a commercially available anti-CTLA-4 antibody for research (e.g., clone 9H10) and so on can be used.

[0131]

[Medicines]

Described in the following are a pharmaceutical composition and a therapeutic method wherein the antibody-drug conjugate according to the present invention and an immune checkpoint inhibitor are administered in combination, and a pharmaceutical composition and a therapeutic method for use in treatment of a disease that can be ameliorated through an antitumor immunity-activating effect wherein the antibody-drug conjugate according to the present invention is included.

[0132]

The pharmaceutical composition and therapeutic method of the present invention may be characterized in that the antibody-drug conjugate and the immune

checkpoint inhibitor are separately contained as active components in different formulations, and are administered simultaneously or at different times, or characterized in that the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered. The pharmaceutical composition and therapeutic method according to the present invention may be such that the antibody-drug conjugate according to the present invention is contained as an active component in a single formulation and administered for treating a disease that can be ameliorated through an antitumor immunity-activating effect.

[0133]

The pharmaceutical composition and therapeutic method of the present invention can be used for treating cancer, and can be preferably used for treating at least one disease selected from the group consisting of lung cancer (including non-small cell lung cancer), urothelial cancer, colorectal cancer (also called colon and rectal cancer, and including colon cancer and rectal cancer), prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer (also called gastric adenocarcinoma), esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer,

endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[0134]

The pharmaceutical composition and therapeutic method of the present invention can be selectively used as an agent for drug therapy, which is a main method for treating cancer, and as a result, can delay development of cancer cells, inhibit growth thereof, and further kill cancer cells. These effects can allow cancer patients to be free from symptoms caused by cancer or achieve improvement in QOL of cancer patients and attain a therapeutic effect by sustaining the lives of the cancer patients. Even if the pharmaceutical composition and therapeutic method of the present invention do not accomplish killing cancer cells, they can achieve higher QOL of cancer patients while achieving longer-term survival, by inhibiting or controlling the growth of cancer cells.

[0135]

In such drug therapy, the pharmaceutical composition and therapeutic method of the present invention can be used as an agent alone and in addition, they can be used

as an agent in combination with an additional therapy in adjuvant therapy and can be combined with surgical operation, radiotherapy, hormone therapy, or the like. Furthermore, they can also be used as an agent for drug therapy in neoadjuvant therapy.

[0136]

In addition to the therapeutic use as described above, for example, a prophylactic effect such as suppressing the growth of small metastatic cancer cells and further killing them can also be expected for the pharmaceutical composition and therapeutic method according to the present invention. For example, an effect of inhibiting and killing cancer cells in a body fluid in the course of metastasis or an effect of, for example, inhibiting and killing small cancer cells immediately after implantation in any tissue can be expected. Accordingly, inhibition of cancer metastasis or a prophylactic effect can be expected, particularly, after surgical removal of cancer.

[0137]

The pharmaceutical composition and therapeutic method of the present invention can be expected to exert a therapeutic effect by application as systemic therapy to patients, and additionally, by local application to cancer tissues.

[0138]

The pharmaceutical composition and therapeutic method of the present invention can be preferably used for a mammal, but are more preferably used for a human. [0139]

The pharmaceutical composition of the present invention can be administered as a pharmaceutical composition containing at least one pharmaceutically suitable ingredient. Substances used in the pharmaceutical composition of the present invention can be suitably selected and applied from formulation additives or the like that are generally used in the art, in view of the dosage or administration concentration. For example, the pharmaceutical composition above typically contains at least one pharmaceutical carrier (for example, sterilized liquid). Herein, the liquid includes, for example, water and oil (petroleum oil and oil of animal origin, plant origin, or synthetic origin). The oil may be, for example, peanut oil, soybean oil, mineral oil, or sesame oil. Water is a more typical carrier when the pharmaceutical composition above is intravenously administered. Saline solution, an aqueous dextrose solution, and an aqueous glycerol solution can be also used as a liquid carrier, in particular, for an injection solution. A suitable pharmaceutical vehicle can be selected from ones known in the art. If desired, the composition above may also contain a trace amount of a moisturizing agent, an emulsifying agent, or a pH

buffering agent. Examples of suitable pharmaceutical carriers are disclosed in "Remington's Pharmaceutical Sciences" by E. W. Martin. The formulations correspond to the administration mode.

[0140]

Various delivery systems are known and they can be used for administering the pharmaceutical composition of the present invention. Examples of the administration route can include intradermal, intramuscular, intraperitoneal, intravenous, and subcutaneous routes, but are not limited thereto. The administration can be made by injection or bolus injection, for example. According to a specific preferred embodiment, the administration of the antibody-drug conjugate and immune checkpoint inhibitor used in the present invention is performed by injection. Parenteral administration is a preferred administration route.

[0141]

According to a representative embodiment, the pharmaceutical composition is prescribed, as a pharmaceutical composition suitable for intravenous administration to humans, according to conventional procedures. The composition for intravenous administration is typically a solution in a sterile and isotonic aqueous buffer solution. If necessary, the pharmaceutical composition may contain a solubilizing agent and local anesthetics to alleviate pain at an

injection site (for example, lignocaine). Generally, the ingredient above is provided individually as any one of a lyophilized powder or an anhydrous concentrate contained in a container which is obtained by sealing in an ampoule or a sachet having an amount of the active agent or as a mixture in a unit dosage form. When the pharmaceutical composition is to be administered by injection, it may be administered from an injection bottle containing water or saline of sterile pharmaceutical grade. When the pharmaceutical composition is administered by injection, an ampoule of sterile water or saline for injection may be provided such that the aforementioned ingredients are admixed with each other before administration.

[0142]

The pharmaceutical composition and therapeutic method of the present invention may include a cancer treating agent other than the antibody-drug conjugate and immune checkpoint inhibitor according to the present invention. The pharmaceutical composition and therapeutic method of the present invention can be administered in combination with other cancer treating agents. The anti-cancer effect may be enhanced accordingly. Other anti-cancer agents used for such purpose may be administered to an individual simultaneously with, separately from, or subsequently to the pharmaceutical composition of the present invention, and may be administered while varying the administration

interval for each. Examples of cancer treating agents include 5-fluorouracil (5-FU), pertuzumab, trastuzumab, paclitaxel, carboplatin, cisplatin, gemcitabine, capecitabine, irinotecan (CPT-11), docetaxel, pemetrexed, sorafenib, vinblastin, vinorelbine, everolims, tanespimycin, bevacizumab, oxaliplatin, lapatinib, trastuzumab emtansine (T-DM1) or agents described in International Publication No. WO 2003/038043, LH-RH alagogues (leuprorelin, goserelin, or the like), estramustine phosphate, estrogen antagonists (tamoxifen, raloxifene, or the like), and aromatase inhibitors (anastrozole, letrozole, exemestane, or the like), but are not limited as long as they are agents having an antitumor activity.

[0143]

The pharmaceutical composition can be formulated into a lyophilization formulation or a liquid formulation as a formulation having the desired composition and required purity. When formulated as a lyophilization formulation, it may be a formulation containing suitable formulation additives that are used in the art. Also for a liquid formulation, it can be formulated as a liquid formulation containing various formulation additives that are used in the art.

[0144]

The composition and concentration of the pharmaceutical composition may vary depending on the

administration method. However, the antibody-drug conjugate and immune checkpoint inhibitor contained in the pharmaceutical composition of the present invention can exhibit a pharmaceutical effect even at a small dosage when the antibody-drug conjugate has a higher affinity for an antigen, that is, a higher affinity (= lower Kd value) in terms of the dissociation constant (that is, Kd value) for the antigen. Thus, for determining the dosage of the antibody-drug conjugate and immune checkpoint inhibitor, the dosage can be determined in view of the situation relating to the affinity with the antigen. When the antibody-drug conjugate and immune checkpoint inhibitor according to the present invention are administered to a human, for example, about 0.001 to 100 mg/kg can be administered once or administered in several portions with intervals of 1 to 180 days.
[0145]

Examples of administration methods for the antibody-drug conjugate according to the present invention include a method of administering 0.8 mg/kg to 8 mg/kg once every three weeks. Examples of the dose include 0.8 mg/kg, 1.6 mg/kg, 3.2 mg/kg, 5.4 mg/kg, 6.4 mg/kg, 7.4 mg/kg, and 8 mg/kg. Although it is sufficient to administer once every three weeks (q3w), administration may be performed once a week (q1w), once every two weeks (q2w), or once every four weeks (q4w).

Examples

[0146]

The present invention is specifically described in view of the examples shown below. However, the present invention is not limited to these. Further, it is by no means to be interpreted in a limited way.

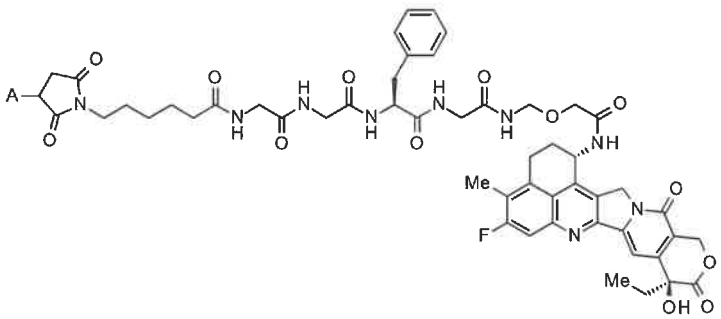
[0147]

[Production Example 1: Preparation of antibody-drug conjugate]

In accordance with a production method described in International Publication No. WO 2015/115091 with use of a humanized anti-HER2 antibody (trastuzumab), an antibody-drug conjugate in which a drug-linker represented by the following formula:

[0148]

[Formula 18]



[0149]

wherein A represents the connecting position to an antibody,

is conjugated to the anti-HER2 antibody via a thioether bond (hereinafter, referred to as "the antibody-drug conjugate (1)") was produced.

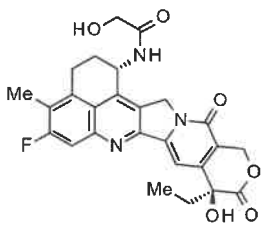
[0150]

[Production Example 2: Preparation of compound (A)]

In accordance with a production method described in International Publication No. WO 2014/057687, a compound represented by the following formula:

[0151]

[Formula 19]



[0152]

(compound (A)) was produced.

[0153]

[Evaluation Example 1: Life prolongation test]

Mouse: 6-week-old female BALB/c mice (BALB/c AnNCrlCrlj) (Charles River Laboratories Japan, Inc.) were subjected to experiment.

Assay and calculation expression: The major axis and minor axis of a tumor were measured twice a week by using an electronic digital caliper (CD15-CX, Mitutoyo Corp.), and the tumor volume (mm^3) was calculated. The calculation expression is as shown below.

Tumor volume (mm^3) = 0.5 × Major axis (mm) × [Minor axis (mm)]^2

From the viewpoint of animal testing ethics, individuals whose tumor volume exceeded 3000 mm^3 were euthanized.

[0154]

The antibody-drug conjugate (1) (Drug-to-Antibody Ratio: 7.6) was diluted with special solvent (10 mM Histidine, 10% Trehalose, 0.02% Polysorbate 20, pH 5.5) for use. An anti-PD-1 antibody (clone RMP1-14) was purchased from Bio X Cell, and diluted with DPBS (Sigma-Aldrich Co. LLC) for use. In administration, a dose of 10 mL/kg was intravenously administered to the tail vein of each mouse.

[0155]

A human HER2 gene was transfected into the mouse colorectal cancer cell line CT26.WT (CRL2638) purchased from American Type Culture Collection with a retrovirus vector to prepare CT26.WT-hHER2 cells for use. These cells were expressing human HER2 protein on their cell membranes. The CT26.WT-hHER2 cells were suspended in physiological saline, and 5.0×10^6 cells were subcutaneously transplanted to the right axilla of each BALB/c mouse, and the mice were randomly grouped 6 days thereafter (Day 0). The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg on Days 0 and 7, twice in total.

The anti-PD-1 antibody was intravenously administered to the tail vein of each mouse at a dose of 2.5 mg/kg on Days 0, 3, 7, 10, and 14, five times in total. A combined administration group with the antibody-drug conjugate (1) and the anti-PD-1 antibody was established, and a group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was six, and tumor volumes were measured until Day 43.
[0156]

The results are shown in Figure 3. Kaplan-Meier curves are shown therein, where the timing when tumor volume exceeded 3000 mm^3 was regarded as the end point. The ordinate depicts survival rates (%) and the abscissa depicts days from the day of initial administration. For the control group, drop-out was found from Day 17, and all of the mice were determined as subjects of euthanasia by Day 24. For the antibody-drug conjugate (1) group, in contrast, drop-out was found from Day 28, and three mice survived until Day 43. For the anti-PD-1 antibody group, drop-out was found from Day 21, and two mice survived until Day 43. Moreover, for the combined administration group with these two agents, all the mice survived until Day 43. Weight loss was observed for none of the mice in all of the groups in this test. From the results, the antitumor effect of single administration of each agent was confirmed, and it was further confirmed that such

effect is dramatically enhanced through use of the two agents in combination.

[0157]

[Evaluation Example 2: Life prolongation test]

A test was conducted in the same manner as in Evaluation Example 1. The anti-PD-1 antibody was intravenously administered to the tail vein of each mouse at a dose of 5 mg/kg on Days 0, 3, 7, and 10, four times in total, where the number of mice in each group was 20, and tumor volumes were measured until Day 38. Comparison on pharmaceutical effect between the control group and each of the antibody-drug conjugate (1) group and the anti-PD-1 antibody group, and comparison on pharmaceutical effect between each of the antibody-drug conjugate (1) group and the anti-PD-1 antibody group and the combined administration group with both agents were performed by using the Kaplan-Meier method/logrank test (comparison among multiple groups). The day when estimated tumor volume exceeded 3000 mm^3 (day of euthanasia) was defined as the day of event occurrence (day of death). P values adjusted for multiplicity were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

The results are shown in Figure 4. The antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control group (P =

0.0001). The anti-PD-1 antibody group exhibited a significantly superior antitumor effect to the control group ($P = 0.0010$). Further, the combined administration group exhibited a significantly superior antitumor effect to the antibody-drug conjugate (1) group ($P = 0.0006$). The combined administration group exhibited a significantly superior antitumor effect to the anti-PD-1 antibody group ($P < 0.0001$).

[0158]

[Evaluation Example 3: Retransplantation test]

The antibody-drug conjugate (1) was administered to mice with subcutaneously transplanted CT26.WT-hHER2 cells in the same manner as in Evaluation Example 1. The mice were randomly grouped 5 days after the transplantation. From these mice, mice whose tumor completely disappeared were selected (hereinafter, referred to as "antibody-drug conjugate (1)-treated cured mice"). Untreated mice were used for a control (hereinafter, referred to as "control mice").

Subsequently, 5.0×10^6 cells of CT26.WT-hHER2 cells or CT26.WT-mock cells were subcutaneously transplanted to the left axilla of each of the antibody-drug conjugate (1)-treated cured mice and the control mice (retransplantation, Day 0), and tumor volumes were measured until Day 17. The number of mice in each group was nine.

The results are shown in Figure 5. The ordinate depicts tumor volumes (mm^3) and the abscissa depicts days from the day of retransplantation. Tumor growth was found for the control mice with retransplanted CT26.WT-hHER2 cells and those with retransplanted CT26.WT-mock cells. In contrast, almost no tumor growth, thus, tumor rejection was found for the antibody-drug conjugate (1)-treated cured mice with retransplanted CT26.WT-hHER2 cells and those with retransplanted CT26.WT-mock cells. From these results, administration of the antibody-drug conjugate (1) was confirmed to cause the formation of immune memory against the tumor.

[0159]

[Evaluation Example 4: ELISPOT analysis]

This analysis was performed by using Murine IFNγ Single-Color Enzymatic ELISPOT Assay. The spleen was excised from each of the mice used in Evaluation Example 3, and 1.0×10^6 cells/mL of splenocytes were prepared therefrom with CTL test medium. CT26.WT-hHER2 cells and CT26.WT-mock cells were each treated with 10 μg/mL of mitomycin C for 2 hours and washed, and the cells were then collected, and 1.0×10^6 cells/mL of cells were prepared with CTL test medium, which was used as an antigen. The splenocytes and the antigen were added to an anti-IFNγ antibody-coated PVDF-membrane plate each at 100 μL/well, and co-cultured at 37°C for 24 hours, and then the number of IFNγ-producing splenocytes was counted.

Comparison between the control group and the antibody-drug conjugate (1) group was performed by using the Wilcoxon rank sum test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

The results are shown in Figures 6 to 9. The CT26.WT-hHER2 cell-derived antigen was found to give a significantly larger number of IFNγ-producing splenocytes for the splenocytes of the antibody-drug conjugate (1)-treated cured mice with retransplanted CT26.WT-hHER2 cells than for the splenocytes of the control mice ($P = 0.0012$, Figure 6). The CT26.WT-mock cell-derived antigen was also found to give a significantly larger number of IFNγ-producing splenocytes ($P = 0.0008$, Figure 7).

Further, even in the cases involving retransplantation of CT26.WT-mock cells, the CT26.WT-hHER2 cell-derived antigen was found to give a significantly larger number of IFNγ-producing splenocytes for the splenocytes of the antibody-drug conjugate (1)-treated cured mice than for the splenocytes of the control mice ($P = 0.0116$, Figure 8). The CT26.WT-mock cell-derived antigen was also found to give a significantly larger number of IFNγ-producing splenocytes ($P = 0.0052$, Figure 9).

These results suggested that T cells which recognize a CT26.WT cell-derived antigen other than human HER2 had

been induced in the antibody-drug conjugate (1)-treated cured mice.

[0160]

The results of Evaluation Examples 3 and 4 demonstrated that the antibody-drug conjugate (1) had a promoting effect on the formation of immune memory against tumor. The effect was found not only for tumor expressing HER2 but also for tumor derived from the same origin and not expressing HER2.

[0161]

Thus, it was revealed that the antibody-drug conjugate used in the present invention has a promoting effect on the formation of immune memory, not only against tumor expressing the antigen for the antibody in the antibody-drug conjugate, but also against tumor not expressing the antigen for the antibody in the antibody-drug conjugate in the same individual.

[0162]

[Evaluation Example 5: Evaluation of effect on in vitro dendritic cells]

BALB/c mice were euthanized, and bone marrow cells were then separated from each femur, and cultured with an RPMI 1640 medium containing 10% FBS, 55 μM 2-mercaptoethanol, 100 U/mL penicillin, 100 U/mL streptomycin, 1 mM sodium pyruvate, 1 × non-essential amino acid, 2 mM L-glutamine, and 10 ng/mL mouse GM-CSF for 11 days to induce bone marrow-derived dendritic cells.

To the culture solution for the induced dendritic cells, the compound (A) was added to a concentration of 0.0625 μM, 0.125 μM, 0.25 μM, 0.5 μM, or 1 μM. For a control, DMSO in a quantity equal to that of the compound (A) was added. After 24 hours, staining was performed by using a Pacific Blue labeled anti-mouse CD45 Antibody (103126, BioLegend), PE labeled anti-mouse CD86 (B7-2) (553692, Becton Dickinson), APC labeled anti-mouse CD11c (550261, Becton Dickinson), and FITC labeled anti-mouse MHC Class II (I-A/I-E) (11-5321-85, Thermo Fisher Scientific), and analysis was performed by using an FACS Canto II. Dead cells had been stained with a LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit purchased from Thermo Fisher Scientific, and excluded from the analysis.

[0163]

Figures 10 and 11 show measurement results of flow cytometry for CD11c-positive cells in terms of expression levels of CD86 and MHC class II, respectively. It was found that treatment with the compound (A) elevated expression levels of both CD86 and MHC class II, which are mature/activation markers for dendritic cells, as compared with the case with DMSO as the control.

[0164]

The results of Evaluation Example 5 demonstrated that the compound (A) has an activating effect on dendritic cells.

[0165]

[Evaluation Example 6: Analysis of intratumor dendritic cells]

CT26.WT-hHER2 cells were transplanted to mice in the same manner as in Evaluation Example 1, and the mice were randomly grouped 8 days thereafter (Day 0). The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg on Day 0. A group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was seven. The mice were euthanized on Day 8, and tumors were excised. Single cell suspensions were prepared from the tumors by using a Tumor Dissociation Kit, mouse, purchased from Miltenyi Biotec, and stained and analyzed in the same manner as in Evaluation Example 5. Comparison between the control group and the antibody-drug conjugate (1) group was performed by using Student's t-test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0166]

The results are shown in Figures 12 to 14.

It was found that the fraction of CD11c-, MHC class II-, CD45-positive cells (dendritic cells, DC) among CD45-positive cells (lymphocytic cells) in tumors

significantly increased by administration of the antibody-drug conjugate (1) (Figure 12).

Further, it was found that the number of dendritic cells expressing CD86 (activation marker) significantly increased by administration of the antibody-drug conjugate (1) (Figure 13).

Furthermore, it was found that the expression level of CD86 on dendritic cells determined in terms of MFI (mean fluorescence intensity) was significantly elevated by administration of the antibody-drug conjugate (1) (Figure 14).

These results confirmed that administration of the antibody-drug conjugate (1) to cancer-bearing mice results in increase of the number of dendritic cells among intratumor lymphocytes, increase of the number of CD86-positive cells among intratumor dendritic cells, and elevation of the expression level of CD86 on dendritic cells.

[0167]

It has been demonstrated from the results of Evaluation Example 5 that the compound (A), which is a drug released from the antibody-drug conjugate (1), itself has an activating effect on dendritic cells. The "activating effect on dendritic cells" possessed by the compound (A) is an effect associated with the "activating effect on dendritic cells" possessed by the antibody-drug conjugate used in the present invention. The compound

(A) is a compound which is generated from the antibody-drug conjugate used in the present invention after the antibody-drug conjugate used in the present invention migrates into cancer cells. Accordingly, the compound (A) is expected to have the same effect even in an antibody-drug conjugate in which the antibody portion is not an anti-HER2 antibody.

[0168]

[Evaluation Example 7: Analysis of intratumor cancer cells]

Cell suspensions were prepared in the same manner as in Evaluation Example 6, and then staining was performed with PE labeled anti-human Her2/neu (340552, Becton Dickinson), APC labeled anti-mouse CD274 (B7-H1, PD-L1) (124312, BioLegend), and FITC labeled anti-mouse H-2Dd (110606, BioLegend), and expression levels of MHC class I and expression levels of PD-L1 on cancer cells were determined with flow cytometry. Dead cells had been stained with a LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit purchased from Thermo Fisher Scientific, and excluded from the analysis. Comparison between the control group and the antibody-drug conjugate (1) group was performed by using Student's t-test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0169]

The results are shown in Figures 15 and 16.

It was found that the expression level of MHC class I on cancer cells (human HER2-positive cells) was significantly elevated by administration of the antibody-drug conjugate (1) (Figure 15). MHC class I is a molecule necessary when T cells recognize cancer cells. Hence, it was suggested that the antibody-drug conjugate (1) activates antitumor immunity through promoting elevation of the expression level of MHC class I on cancer cells.

It was further found that the expression level of PD-L1 on cancer cells was significantly elevated by the antibody-drug conjugate (1) (Figure 16). PD-L1 is known to act on PD-1 on T cells to elicit an immunosuppressive signal. Hence, it was suggested that the antibody-drug conjugate (1) activates antitumor immunity through promoting elevation of the expression level of PD-L1 on cancer cells, and combined use with a PD-1 antibody is expected to deactivate the suppressive signal, resulting in a higher antitumor effect.

[0170]

[Evaluation Example 8: Analysis of in vitro cancer cells]

To culture solution for CT26.WT-hHER2 cells, the compound (A) was added to a concentration of 0.0625 μM, 0.125 μM, 0.25 μM, 0.5 μM, or 1 μM. For a control, DMSO in a quantity equal to that of the compound (A) was added. After 24 hours, staining was performed by using PE

labeled anti-human Her2/neu (340552, Becton Dickinson) and FITC labeled anti-mouse H-2Dd (110606, BioLegend), and expression levels of MHC class I on cancer cells were determined with flow cytometry. Dead cells had been stained with a LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit purchased from Thermo Fisher Scientific, and excluded from the analysis. The mean fluorescence intensity (MFI) of MHC class I was calculated, and MFI for cells treated with an Isotype control was subtracted from the MFI for stained cells, and the resulting value was used as adjusted MFI. Comparison between the control group and the compound (A) group was performed by using Dunnett's test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

The results are shown in Figure 17.

It was found that the expression level of MHC class I on CT26.WT-hHER2 cells was significantly elevated by the compound (A) (Figure 17). Hence, it was suggested that the compound (A) activates antitumor immunity through promoting elevation of the expression level of MHC class I on cancer cells.

[0171]

[Evaluation Example 9: Antitumor test using nude mice]

Mouse: 6-week-old female BALB/c-nu mice (CAnN.Cg-Foxn1[nu]/CrlCrlj [Foxn1nu/Foxn1nu]) (Charles River Laboratories Japan, Inc.) were subjected to experiment.

Assay and calculation expression: The major axis and minor axis of a tumor were measured twice a week by using an electronic digital caliper (CD15-CX, Mitutoyo Corp.), and the tumor volume (mm^3) was calculated. The calculation expression is as shown below.

Tumor volume (mm^3) = 0.5 × Major axis (mm) × [Minor axis (mm)]^2

From the viewpoint of animal testing ethics, individuals whose tumor volume exceeded 3000 mm^3 were euthanized.

[0172]

The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg. CT26.WT-hHER2 cells were suspended in physiological saline, and 5.0×10^6 cells were subcutaneously transplanted to the right axilla of each BALB/c-nu mouse, and the mice were randomly grouped 3 days thereafter (Day 0). The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg on Days 0 and 7, twice in total. A group with administration of the solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was 12, and tumor volumes were measured until Day 13.

[0173]

The results are shown in Figure 18. The ordinate depicts tumor volumes (mm^3) and the abscissa depicts days

from the day of initial administration. The antitumor effect by administration of the antibody-drug conjugate (1), which had been found for BALB/c mice, was not found for the BALB/c-nu mice. From the finding that the number of T cells and that of B cells were reduced and the functions were impaired in the BALB/c-nu mice, it was inferred that these cells play an important role for the antitumor effect of the antibody-drug conjugate (1).

[0174]

[Evaluation Example 10: Antitumor test]

In the same manner as in Evaluation Example 1, transition of tumor volume in mice with subcutaneously transplanted CT26.WT-hHER2 cells was determined for an antibody-drug conjugate (1)-administered group, a control antibody-drug conjugate-administered group, and a control group.

The control antibody-drug conjugate (Drug-to-Antibody Ratio: 7.8), using a human IgG1 antibody which binds to molecules other than those derived from mice and humans, was diluted with special solvent for use. Grouping was performed 5 days after the transplantation (Day 0). The control antibody-drug conjugate or antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg on Days 0 and 7, twice in total. The number of mice in each group was 10, and tumor volumes were measured until Day 10. Comparison on pharmaceutical

effect between the control antibody-drug conjugate group and the antibody-drug conjugate (1) group was performed by using the Wilcoxon rank sum test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0175]

The results are shown in Figure 19. The ordinate depicts tumor volumes (mm^3) and the abscissa depicts days from the day of initial administration. On Day 10, the antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control antibody-drug conjugate group ($P = 0.0003$). From the result, the antitumor effect of the antibody-drug conjugate (1) was found to be target-dependent.

[0176]

[Evaluation Example 11: Antitumor test]

In the same manner as in Evaluation Example 1, transition of tumor volume in mice with subcutaneously transplanted EMT6-hHER2 cells was determined for single administration groups with each of the antibody-drug conjugate (1) and an anti-PD-1 antibody (clone RMP1-14), and a combined administration group. The EMT6-hHER2 cells were prepared through transfection of a human HER2 gene into the mouse breast cancer cell line EMT6 (CRL-2755) purchased from American Type Culture Collection by using a lentivirus vector. These cells were expressing

human HER2 protein on their cell membranes. The EMT6-hHER2 cells were suspended in physiological saline, and 1.0×10^6 cells were subcutaneously transplanted to the right axilla of each 5-week-old BALB/c mouse, and the mice were randomly grouped 4 days after the transplantation (Day 0). The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg once on Day 0. An anti-PD-1 antibody (clone RMP1-14) was prepared with D-PBS(-) (WAKO), and intravenously administered to the tail vein of each mouse at a dose of 5.0 mg/kg on Days 0, 3, 7, and 10, four times in total. A combined administration group with the antibody-drug conjugate (1) and the anti-PD-1 antibody was established, and a group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was 11, and tumor volumes were measured until Day 17. Comparison on pharmaceutical effect between the control group and each of the antibody-drug conjugate (1) group and the anti-PD-1 antibody group, and comparison on pharmaceutical effect between each of the antibody-drug conjugate (1) group and the anti-PD-1 antibody group and the combined administration group with both agents were performed by using Dunnett's test (comparison among multiple groups). P values adjusted for multiplicity were expressed as numerical values to the fourth decimal place, and values

of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0177]

The results are shown in Figure 20. The ordinate depicts tumor volumes (mm^3) and the abscissa depicts days from the day of initial administration. On Day 17, the antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control group ($P < 0.0001$). The anti-PD-1 antibody group exhibited a significantly superior antitumor effect to the control group ($P < 0.0001$). Further, the combined administration group exhibited a significantly superior antitumor effect to the antibody-drug conjugate (1) group ($P = 0.0136$). The combined administration group exhibited a significantly superior antitumor effect to the anti-PD-1 antibody group ($P = 0.0372$). Weight loss was observed for none of the mice in all of the groups in this test. From the results, the antitumor effect of single administration of each agent was confirmed, and it was further confirmed that such effect is dramatically enhanced through use of the two agents in combination.

[0178]

[Evaluation Example 12: Life prolongation test]

In the same manner as in Evaluation Example 1, life-prolonging effects on mice with subcutaneously transplanted CT26.WT-hHER2 cells were determined for single administration groups with each of the antibody-

drug conjugate (1) and an anti-PD-L1 antibody, and a combined administration group. An anti-PD-L1 antibody (clone 10F.9G2) was purchased from Bio X Cell, and diluted with InVivoPure pH 6.5 Dilution Buffer (Bio X Cell) for use. The mice were randomly grouped 6 days after the transplantation (Day 0), and the antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg on Days 0 and 7, twice in total. The anti-PD-L1 antibody was intravenously administered to the tail vein of each mouse at a dose of 5 mg/kg on Days 0 and 3, twice in total. A combined administration group with the antibody-drug conjugate (1) and the anti-PD-L1 antibody was established, and a group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was 15, and tumor volumes were measured until Day 38. The day when estimated tumor volume exceeded 3000 mm^3 (day of euthanasia) was defined as the day of event occurrence (day of death), and comparison on survival time between the control group and each of the antibody-drug conjugate (1) group and the anti-PD-L1 antibody group, and comparison on survival time between each of the antibody-drug conjugate (1) group and the anti-PD-L1 antibody group and the combined administration group with both agents were performed by using the Kaplan-Meier method/logrank test (comparison among multiple groups).

P values adjusted for multiplicity were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0179]

The results are shown in Figure 21. The antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control group ($P = 0.0069$). The anti-PD-L1 antibody group exhibited a significantly superior antitumor effect to the control group ($P = 0.0037$). Further, the combined administration group exhibited a significantly superior antitumor effect to the antibody-drug conjugate (1) group ($P = 0.0059$). The combined administration group exhibited a significantly superior antitumor effect to the anti-PD-L1 antibody group ($P = 0.0091$). Weight loss was observed for none of the mice in all of the groups in this test. From the results, the antitumor effect of single administration of each agent was confirmed, and it was further confirmed that such effect is dramatically enhanced through use of the two agents in combination.

[0180]

[Evaluation Example 13: Life prolongation test]

EMT6-hHER2 cells were subcutaneously transplanted to mice in the same manner as in Evaluation Example 11, and life-prolonging effects were determined for single administration groups with each of the antibody-drug

conjugate (1) and an anti-PD-L1 antibody, and a combined administration group in the same manner as in Evaluation Example 12. The mice were randomly grouped 5 days after the transplantation (Day 0), and the antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg once on Day 0. The anti-PD-L1 antibody was intravenously administered to the tail vein of each mouse at a dose of 5 mg/kg on Days 0 and 3, twice in total. A combined administration group with the antibody-drug conjugate (1) and the anti-PD-L1 antibody was established, and a group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was six, and tumor volumes were measured until Day 60. Comparison on survival time between the control group and each of the antibody-drug conjugate (1) group and the anti-PD-L1 antibody group, and comparison on survival time between each of the antibody-drug conjugate (1) group and the anti-PD-L1 antibody group and the combined administration group with both agents were performed by using the Kaplan-Meier method/logrank test (comparison among multiple groups). P values adjusted for multiplicity were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0181]

The results are shown in Figure 22. The antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control group (P = 0.0006). The anti-PD-L1 antibody group exhibited a significantly superior antitumor effect to the control group (P = 0.0227). Further, the combined administration group exhibited a significantly superior antitumor effect to the antibody-drug conjugate (1) group (P = 0.0039). Weight loss was observed for none of the mice in all of the groups in this test. From the results, the antitumor effect of single administration of each agent was confirmed, and it was further confirmed that such effect is dramatically enhanced through use of the two agents in combination.

[0182]

[Evaluation Example 14: In vivo CD4/8 depletion test]

In the same manner as in Evaluation Example 1, transition of tumor volume in mice with subcutaneously transplanted CT26.WT-hHER2 cells was determined for single administration groups with each of the antibody-drug conjugate (1) and an anti-CD4 antibody, and a combined administration group, and for single administration groups with each of the antibody-drug conjugate (1) and an anti-CD8 antibody, and a combined administration group. The antibody-drug conjugate (1) was prepared to reach 10 mg/kg, and the anti-CD4 antibody (Bio X Cell, clone GK1.5) and anti-CD8 antibody (Bio X

Cell, clone 53.6.7), each of which is a depletion antibody, were each prepared to reach 1 mg/mL with D-PBS(-) immediately before administration, and each of them was intravenously administered to the tail vein of each mouse at a dose of 200 μg/head on Days 0 and 7. A group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. Grouping was performed 5 days after the transplantation (Day 0), tumor volumes were measured until Day 11.

[0183]

The results are shown in Figures 23 and 24. The tumor volume on Day 11 was 651 mm^3 for the antibody-drug conjugate (1) group, and 561 mm^3 for the combined administration group with the antibody-drug conjugate (1) and the anti-CD4 antibody, suggesting that CD4-positive cells do not contribute to the antitumor effect of the antibody-drug conjugate (1) (Figure 23). The tumor volume on Day 11 was 651 mm^3 for the antibody-drug conjugate (1) group, and 2247 mm^3 for the combined administration group with the antibody-drug conjugate (1) and the anti-CD8 antibody, suggesting that CD8-positive cells contribute to the antitumor effect of the antibody-drug conjugate (1) (Figure 24). Tumor volume had exceeded 3000 mm^3 in some individuals in the anti-CD4 antibody group and the anti-CD8 antibody group, and hence the individuals were euthanized during the test. Thus,

each of the tumor growth curves ends at the timing of euthanasia. In combination with the finding from the tumor model using immunodeficient nude mice in Evaluation Example 9 that T cells or B cells are partly involved in the pharmaceutical effect of the antibody-drug conjugate (1), the present results suggested that CD8-positive T cells contribute to the antitumor effect of the antibody-drug conjugate (1).

[0184]

[Evaluation Example 15: Analysis of intratumor T cells]

The fraction of CD8-positive T cells among intratumor living cells, the fraction of Granzyme B-positive cells among intratumor CD8-positive T cells, the fraction of CD8-positive T cells being Granzyme B-positive among intratumor living cells, and the fraction of CD4-positive T cells among intratumor living cells were determined with flow cytometry for the case that the antibody-drug conjugate (1) was administered to mice with subcutaneously transplanted CT26.WT-hHER2 cells. Cell suspensions were prepared in the same manner as in Evaluation Example 6, and then staining was performed with a Pacific Blue labeled anti-mouse CD45 antibody (103126, BioLegend), PE labeled anti-mouse CD3e antibody (553064, Becton Dickinson), PerCP/Cy5.5 labeled anti-mouse CD4 antibody (100434, BioLegend), PE-Cy 7 labeled anti-mouse CD8a antibody (552877, Becton Dickinson), and Alexa FluorR 647 labeled anti-human/mouse Granzyme B

antibody (515405, BioLegend), and assayed with flow cytometry. Dead cells had been stained with a LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit purchased from Thermo Fisher Scientific, and excluded from the analysis. Comparison between the control group and the antibody-drug conjugate (1) group was performed by using Student's t-test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences. [0185]

The results are shown in Figures 25 to 28. It was found that the fraction of CD45-, CD3-, CD8-positive cells (CD8-positive T cells) among living cells significantly increased by administration of the antibody-drug conjugate (1) (Figure 25). It was found that the fraction of Granzyme B-positive cells among CD8-positive T cells significantly increased by administration of the antibody-drug conjugate (1) (Figure 26). It was found that the fraction of CD8-positive T cells being Granzyme B-positive among living cells significantly increased by administration of the antibody-drug conjugate (1) (Figure 27). Although the fraction of CD45-, CD3-, CD4-positive cells (CD4-positive T cells) among living cells had an increasing tendency by administration of the antibody-drug conjugate (1), the difference was not significant (Figure 28).

Thus, these results suggested that the antibody-drug conjugate (1) activates antitumor immunity by increasing the number of intratumor CD8-positive T cells and promoting activation thereof.

[0186]

[Evaluation Example 16: CD8 IHC analysis]

The number of CD8-positive cells per unit area in tumors was counted by using IHC for the case that the antibody-drug conjugate (1) was administered to mice with subcutaneously transplanted CT26.WT-hHER2 cells. In the same manner as in Evaluation Example 6, the control and the antibody-drug conjugate (1) were administered, and the mice were euthanized 8 days after the administration. The number of mice in each group was five, and tumors were excised from the intermediate number of mice, namely, three mice, and each soaked in paraformaldehyde/phosphate buffer to produce a paraffin block. Each paraffin block was stained with an anti-CD8 antibody (clone: 4SM16), and the sample image was taken by using a NanoZoomer 2.0-HT (Hamamatsu Photonics K.K.), and the whole region of tissue was analyzed by using the image analysis software Tissue Studio3.0 (Definiens).

[0187]

The results are shown in Figures 29 and 30. It was found that the number of CD8-positive cells per unit area in tumors tended to increase by the action of the antibody-drug conjugate (1).

[Evaluation Example 17: Analysis of in vitro cancer cells]

Expression levels of MHC class I were determined for cancer cells treated with different compounds. In the same manner as in Evaluation Example 8, each of the compound (A), DM1-SMe, DM4-SMe (J. Med. Chem. (2014), 57, 16, 6949-6964), and MMAE (Molecular Cancer Therapeutics (2011), 10, 9, 1728-1739) was added to a concentration of 20 nM, 100 nM, or 500 nM, and the expression level of MHC class I on cancer cells was determined with flow cytometry. The experiment was performed in triplicate. Comparison between the control group and each of the agent groups at each concentration was performed by using Dunnett's test, P values were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences (***: $P < 0.001$, **: $P < 0.01$).

[0188]

The results are shown in Figure 31. At the concentrations examined (20 nM, 100 nM, 500 nM), all of the agents evaluated were found to significantly elevate the expression level of MHC class I on CT26.WT-hHER2 cells as compared with the control group. Among them, the compound (A) was found to exhibit the maximum elevation effect on expression of MHC class I on CT26.WT-hHER2 cells.

[0189]

[Evaluation Example 18: Antitumor test]

In the same manner as in Evaluation Example 11, transition of tumor volume in mice with subcutaneously transplanted EMT6-hHER2 cells was determined for single administration groups with each of the antibody-drug conjugate (1) and an anti-CTLA-4 antibody, and a combined administration group. An anti-CTLA-4 antibody (clone 9H10) was purchased from Bio X Cell, and diluted with D-PBS(-) for use. Grouping was performed 5 days after the transplantation (Day 0). The antibody-drug conjugate (1) was intravenously administered to the tail vein of each mouse at a dose of 10 mg/kg once on Day 0. The anti-CTLA-4 antibody was intravenously administered to the tail vein of each mouse at a dose of 5.0 mg/kg on Days 0, 3, and 7, three times in total. A combined administration group with the antibody-drug conjugate (1) and the anti-CTLA-4 antibody was established, and a group with administration of the special solvent for the antibody-drug conjugate (1) was established as a control group. The number of mice in each group was 10, and tumor volumes were measured until Day 14. Comparison on pharmaceutical effect between the control group and each of the antibody-drug conjugate (1) group and the anti-CTLA-4 antibody group, and comparison on pharmaceutical effect between each of the antibody-drug conjugate (1) and the anti-CTLA-4 antibody group and the combined administration group with both agents were performed by

using Dunnett's test (comparison among multiple groups). P values adjusted for multiplicity were expressed as numerical values to the fourth decimal place, and values of $P < 0.05$ (two-tailed test) were regarded as significant differences.

[0190]

The results are shown in Figure 32. The ordinate depicts tumor volumes (mm^3) and the abscissa depicts days from the day of initial administration. On Day 14, the antibody-drug conjugate (1) group exhibited a significantly superior antitumor effect to the control group ($P = 0.0011$). The anti-CTLA-4 antibody group exhibited a significantly superior antitumor effect to the control group ($P = 0.0006$). Further, the combined administration group exhibited a significantly superior antitumor effect to the antibody-drug conjugate (1) group ($P = 0.0115$). The combined administration group exhibited a significantly superior antitumor effect to the anti-CTLA-4 antibody group ($P = 0.0309$). From the results, the antitumor effect of single administration of each agent was confirmed, and it was further confirmed that such effect is dramatically enhanced through use of the two agents in combination.

[0191]

From the above experimental results, the antibody-drug conjugate according to the present invention was revealed to exhibit a dramatically excellent antitumor

effect through being administered in combination with an immune checkpoint inhibitor. In addition, the antibody-drug conjugate according to the present invention was demonstrated to have an antitumor immunity-activating effect. Accordingly, the antibody-drug conjugate can provide a pharmaceutical composition and therapeutic method superior in antitumor effect and safety.

Free Text of Sequence Listing

[0192]

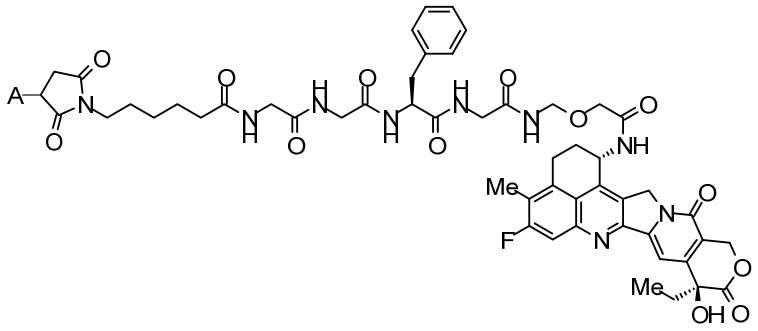
SEQ ID NO: 1 - Amino acid sequence of a heavy chain of the humanized anti-HER2 antibody

SEQ ID NO: 2 - Amino acid sequence of a light chain of the humanized anti-HER2 antibody

CLAIMS

[Claim 1]

Use of an antibody-drug conjugate and an immune checkpoint inhibitor for the manufacture of a medicament for a therapeutic method for treating cancer wherein the antibody-drug conjugate and the immune checkpoint inhibitor are to be administered in combination, and the antibody-drug conjugate is an antibody-drug conjugate in which a drug-linker represented by the following formula:



wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond,

the antibody in the antibody-drug conjugate is an anti-HER2 antibody and the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[Claim 2]

The use according to claim 1, wherein the anti-HER2 antibody is an antibody comprising a heavy chain

consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[Claim 3]

The use according to claim 1, wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[Claim 4]

The use according to any one of claims 1 to 3, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[Claim 5]

The use according to any one of claims 1 to 3, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[Claim 6]

The use according to any one of claims 1 to 3, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.

[Claim 7]

The use according to any one of claim 1 to 6, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

[Claim 8]

The use according to claim 7, wherein the anti-PD-1 antibody is nivolumab.

[Claim 9]

The use according to claim 7, wherein the anti-PD-1 antibody is pembrolizumab.

[Claim 10]

The use according to any one of claim 1 to 6, wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[Claim 11]

The use according to claim 10, wherein the anti-PD-L1 antibody is atezolizumab.

[Claim 12]

The use according to claim 10, wherein the anti-PD-L1 antibody is durvalumab.

[Claim 13]

The use according to claim 10, wherein the anti-PD-L1 antibody is avelumab.

[Claim 14]

The use according to any one of claim 1 to 6, wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[Claim 15]

The use according to claim 14, wherein the anti-CTLA-4 antibody is ipilimumab.

[Claim 16]

The use according to claim 14, wherein the anti-CTLA-4 antibody is tremelimumab.

[Claim 17]

The use according to any one of claims 1 to 16, wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active components in different formulations, and are to be administered simultaneously or at different times.

[Claim 18]

The use according to any one of claims 1 to 16, wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and to be administered.

[Claim 19]

The use according to any one of claims 1 to 18, wherein the cancer is at least one selected from the group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial

cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[Claim 20]

The use according to claim 19, wherein the cancer is colorectal cancer.

[Claim 21]

The use according to claim 19, wherein the cancer is breast cancer.

[Claim 22]

The use according to claim 19, wherein the cancer is lung cancer.

[Claim 23]

The use according to claim 19, wherein the cancer is urothelial cancer.

[Claim 24]

The use according to claim 19, wherein the cancer is bladder cancer.

[Claim 25]

The use according to claim 19, wherein the cancer is gastric cancer.

[Claim 26]

The use according to claim 19, wherein the cancer is esophagogastric junction adenocarcinoma.

[Claim 27]

The use according to any one of claims 1 to 26, wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[Claim 28]

The use according to any one of claims 1 to 27, wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

(1) a promoting effect on growth of intratumor CD8-positive T cells; and

(2) an activating effect on intratumor CD8-positive T cells.

[Claim 29]

The use according to any one of claims 1 to 28, wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.

[Claim 30]

The use according to claim 29, wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.

[Claim 31]

The use according to claim 29, wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.

[Claim 32]

The use according to any one of claims 1 to 31, wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:

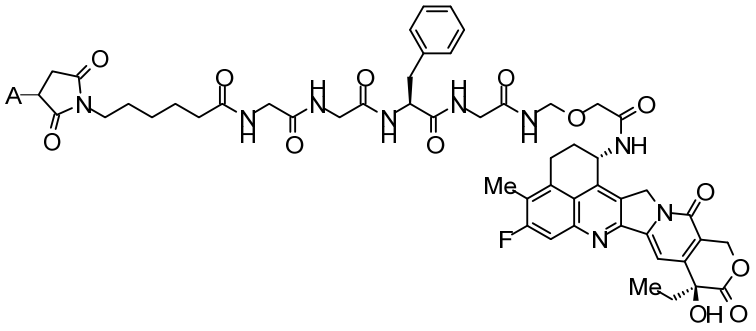
(1) a promoting effect on increase of the number of dendritic cells in a tumor;

(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.

[Claim 33]

Use of an antibody-drug conjugate for the manufacture of a medicament for a therapeutic method for treating cancer, wherein the antibody-drug conjugate is to be administered in combination with an immune checkpoint inhibitor, wherein a drug-linker represented by the following formula:



wherein A represents the connecting position to an antibody,

is conjugated to the antibody via a thioether bond in the antibody-drug conjugate,

the antibody in the antibody-drug conjugate is an anti-HER2 antibody and
the immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[Claim 34]
The use according to claim 33, wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence consisting of amino acid residues 1 to 449 of SEQ ID NO: 1 and a light chain consisting of an amino acid sequence consisting of amino acid residues 1 to 214 of SEQ ID NO: 2.

[Claim 35]
The use according to claim 33, wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of the amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of the amino acid sequence represented by SEQ ID NO: 2.

[Claim 36]
The use according to any one of claim 33 to claim 35, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 2 to 8.

[Claim 37]
The use according to any one of claim 33 to claim 35, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7 to 8.

[Claim 38]
The use according to any one of claim 33 to claim 35, wherein the average number of units of the drug-linker conjugated per antibody molecule in the antibody-drug conjugate is in the range of from 7.5 to 8.
[Claim 39]
The use according to any one of claim 33 to claim 38, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.
[Claim 40]
The use according to claim 39, wherein the anti-PD-1 antibody is nivolumab.
[Claim 41]
The use according to claim 39, wherein the anti-PD-1 antibody is pembrolizumab.
[Claim 42]
The use according to any one of claim 33 to claim 38, wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.
[Claim 43]
The use according to claim 42, wherein the anti-PD-L1 antibody is atezolizumab.
[Claim 44]
The use according to claim 42, wherein the anti-PD-L1 antibody is durvalumab.

[Claim 45]

The use according to claim 42, wherein the anti-PD-L1 antibody is avelumab.

[Claim 46]

The use according to any one of claim 33 to claim 38, wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

[Claim 47]

The use according to claim 46, wherein the anti-CTLA-4 antibody is ipilimumab.

[Claim 48]

The use according to claim 46, wherein the anti-CTLA-4 antibody is tremelimumab.

[Claim 49]

The use according to any one of claim 33 to claim 48, wherein the antibody-drug conjugate and the immune checkpoint inhibitor are separately contained as active components in different formulations, and are administered simultaneously or at different times.

[Claim 50]

The use according to any one of claim 33 to claim 49, wherein the antibody-drug conjugate and the immune checkpoint inhibitor are contained as active components in a single formulation and administered.

[Claim 51]

The use according to any one of claim 33 to claim 50, wherein the cancer is at least one selected from the

group consisting of lung cancer, urothelial cancer, colorectal cancer, prostate cancer, ovarian cancer, pancreatic cancer, breast cancer, bladder cancer, gastric cancer, esophagogastric junction adenocarcinoma, gastrointestinal stromal tumor, uterine cervix cancer, esophageal cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, endometrial cancer, uterine cancer, salivary gland cancer, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, neuroepithelial tissue tumor, nerve sheath tumor, head-and-neck cancer, skin cancer, pharyngeal cancer, gallbladder cancer, bile duct cancer, mesothelioma, Paget's disease, and sarcoma.

[Claim 52]

The use according to claim 51, wherein the cancer is colorectal cancer.

[Claim 53]

The use according to claim 51, wherein the cancer is breast cancer.

[Claim 54]

The use according to any one of claim 33 to claim 53, wherein the antibody-drug conjugate has an antitumor immunity-activating effect.

[Claim 55]
The use according to any one of claim 33 to claim 53, wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:
(1) a promoting effect on growth of intratumor CD8-positive T cells; and
(2) an activating effect on intratumor CD8-positive T cells.
[Claim 56]
The use according to any one of claim 33 to claim 53, wherein the antibody-drug conjugate has a promoting effect on the formation of immune memory against tumor.
[Claim 57]
The use according to claim 56, wherein the tumor is expressing an antigen for the antibody in the antibody-drug conjugate.
[Claim 58]
The use according to claim 56, wherein a part of the cells of the tumor are not expressing an antigen for the antibody in the antibody-drug conjugate.
[Claim 59]
The use according to any one of claim 33 to claim 58, wherein the antibody-drug conjugate has at least one effect selected from the group consisting of:
(1) a promoting effect on increase of the number of dendritic cells in a tumor;
(2) an activating effect on dendritic cells; and

(3) a promoting effect on elevation of the expression level of MHC class I on cancer cells.