COMBINATION OF ANTIBODY-DRUG CONJUGATE AND ATR INHIBITOR

[Technical Field]

The present disclosure relates to a pharmaceutical product for administration of a specific antibody-drug conjugate, having an antitumor drug conjugated to an anti-HER2 antibody via a linker structure, in combination with an ATR inhibitor, and to a therapeutic use and method wherein the specific antibody-drug conjugate and the ATR inhibitor are administered in combination to a subject.

[Background]

ATR (ataxia telangiectasia and rad3-related kinase) is a serine/threonine protein kinase and member of the phosphatidylinositol 3-kinase related kinase (PIKK) family. During normal DNA replication, ATR is recruited at stalled replication forks, which can progress to double strand breaks if left unrepaired. ATR is recruited to single strand DNA coated with Replication Protein A (RPA) following single strand DNA damage or the resection of double strand breaks during DNA replication. Recruitment and activation of ATR leads to cell cycle arrest in S-phase while the DNA is repaired and the stalled replication fork resolved, or nuclear fragmentation and entry into programmed cell death (apoptosis).

As a result, ATR inhibitors are expected to cause growth inhibition in tumor cells dependent upon ATR for DNA repair e.g. ATM-deficient tumors. In addition to such monotherapy activity, ATR inhibitors are also predicted to potentiate the activity of DNA damage-inducing therapies (through inhibition of ATR-dependent DNA repair processes) when used in combination. Examples of ATR inhibitors are disclosed, for example, in WO2011/154737.

Inactivation of Schlafen 11 (SLFN11) in cancer cells has also been shown to result in resistance to anticancer agents that cause DNA damage and replication stress. Thus, SLFN11 may serve as a determinant of sensitivity to different classes of DNA-damaging agents including but not restricted to topoisomerase I inhibitors. *See* Zoppoli et al., PNAS 2012; 109: 15030-35; Murai et al., Oncotarget 2016; 7: 76534-50; Murai et al., Mol. Cell 2018; 69: 371-84.

Antibody-drug conjugates (ADCs), which are composed of a cytotoxic drug conjugated to an antibody, can deliver the drug selectively to cancer cells, and are therefore expected to cause accumulation of the drug within cancer cells and to kill the cancer cells (Ducry, L., et al., Bioconjugate Chem. (2010) 21, 5-13; Alley, S. C., et al., Current Opinion in Chemical Biology (2010) 14, 529-537; Damle N. K. Expert Opin. Biol. Ther. (2004) 4, 1445-1452; Senter P. D., et al., Nature Biotechnology (2012) 30, 631-637; Burris HA., et al., J. Clin. Oncol. (2011) 29(4): 398-405).

One such antibody-drug conjugate is trastuzumab deruxtecan, which is composed of a HER2-targeting antibody and a derivative of exatecan (Ogitani Y. et al., Clinical Cancer Research (2016) 22(20), 5097-5108; Ogitani Y. et al., Cancer Science (2016) 107, 1039-1046).

Despite the therapeutic potential of antibody-drug conjugates and ATR inhibitors, no literature is published that describes a test result demonstrating an excellent effect of combined use of the antibody-drug conjugate and an ATR inhibitor or any scientific basis suggesting such a test result. Moreover, in the absence of test results, a possibility exists that combined administration of the antibody-drug conjugate together with another cancer treating agent such as an ATR inhibitor could lead to negative interactions and/or sub-additive therapeutic outcomes, and thus an excellent or superior effect obtained by such combination treatment could not be expected.

Accordingly, a need remains for improved therapeutic compositions and methods, that can enhance efficacy of existing cancer treating agents, increase durability of therapeutic response and/or reduce dose-dependent toxicity.

[Summary of Disclosure]

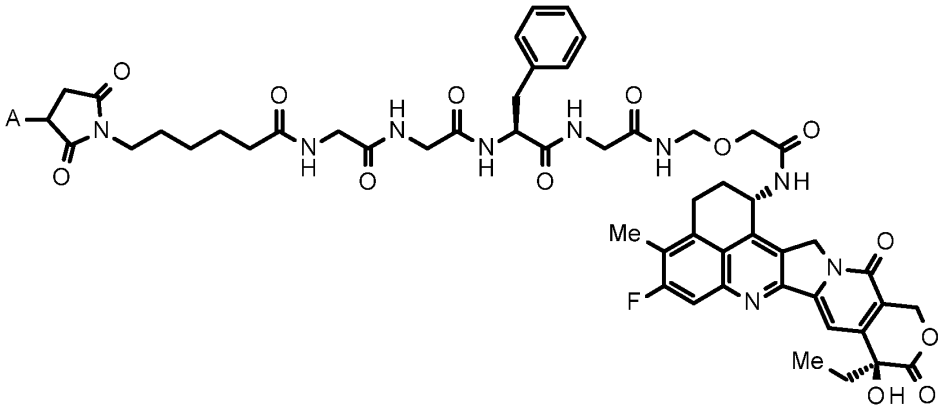
The antibody-drug conjugate used in the present disclosure (an anti-HER2 antibody-drug conjugate that includes a derivative of the topoisomerase I inhibitor

exatecan) has been confirmed to exhibit an excellent antitumor effect in the treatment of certain cancers such as breast cancer and gastric cancer, when administered singly. However, it is desired to provide a medicine and treatment which can obtain a superior antitumor effect in the treatment of cancers, such as enhanced efficacy, increased durability of therapeutic response and/or reduced dose-dependent toxicity. By inhibiting the DNA damage response to replication stress and double strand breaks introduced by the antibody-drug conjugate of the present disclosure, an ATR inhibitor may further enhance antitumor efficacy when administered in combination with the antibody-drug conjugate.

The present disclosure provides a pharmaceutical product which can exhibit an excellent antitumor effect in the treatment of cancers, through administration of an anti-HER2 antibody-drug conjugate in combination with an ATR inhibitor. The present disclosure also provides a therapeutic use and method wherein the anti-HER2 antibody-drug conjugate and ATR inhibitor are administered in combination to a subject.

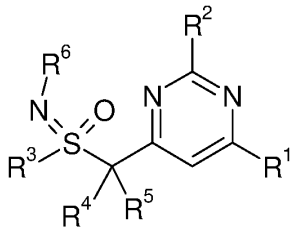
Specifically, the present disclosure relates to the following [1] to [54]:

[1] a pharmaceutical product comprising an anti-HER2 antibody-drug conjugate and an ATR inhibitor for administration in combination, wherein the anti-HER2 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 an anti-HER2 antibody via a thioether bond;

[2] the pharmaceutical product according to [1], wherein the ATR inhibitor is a compound represented by the following formula (I):

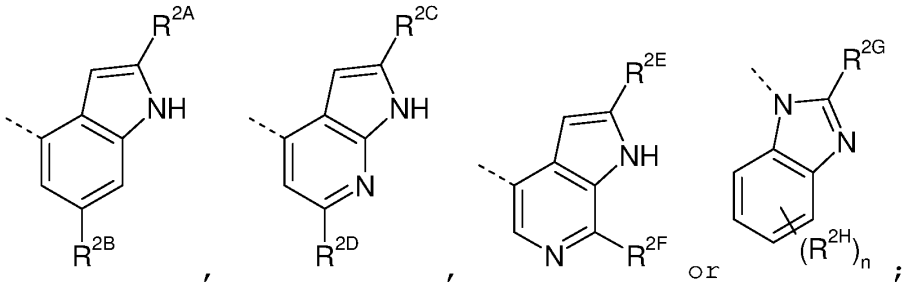


(I)

wherein:

R^{1} is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

R^{2} is



n is 0 or 1;

R^{2A}, **R^{2C}**, **R^{2E}** and **R^{2F}** each independently are hydrogen or methyl;

R^{2B} and **R^{2D}** each independently are hydrogen or methyl;

R^{2G} is selected from $-NHR^7$ and $-NHCOR^8$;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and **R^5** are each independently hydrogen or methyl, or **R^4** and **R^5** together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 membered heterocyclic ring containing one heteroatom selected from O and N;

R^6 is hydrogen;

R^7 is hydrogen or methyl;

R^8 is methyl,

or a pharmaceutically acceptable salt thereof;

[3] the pharmaceutical product according to [2] wherein, in formula (I), **R^4** and **R^5** together with the atom to which they are attached form Ring A, and Ring A is a C_{3-}

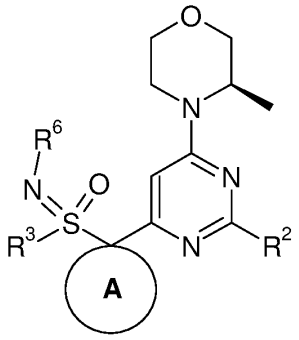
$_{6}$cycloalkyl or a saturated 4-6 heterocyclic ring containing one heteroatom selected from O and N;

[4] the pharmaceutical product according to [2] or [3] wherein, in formula (I), Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring;

[5] the pharmaceutical product according to any one of [2] to [4] wherein, in formula (I), **R^{2A}** is hydrogen; **R^{2B}** is hydrogen; **R^{2C}** is hydrogen; **R^{2D}** is hydrogen; **R^{2E}** is hydrogen; and **R^{2F}** is hydrogen;

[6] the pharmaceutical product according to any one of [2] to [5] wherein, in formula (I), R^1 is 3-methylmorpholin-4-yl;

[7] the pharmaceutical product according to any one of [2] to [6] wherein the compound of formula (I) is a compound of formula (Ia):



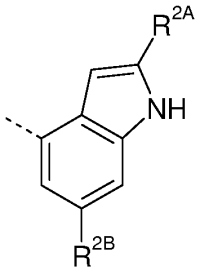
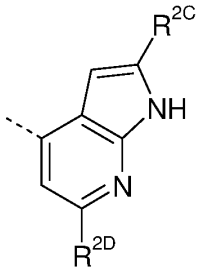
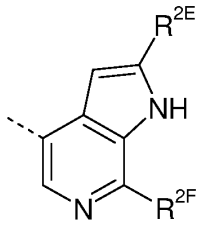
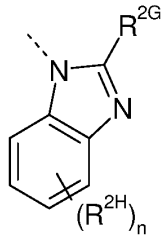
(Ia)

or a pharmaceutically acceptable salt thereof;

[8] the pharmaceutical product according to [7] wherein, in formula (Ia):

Ring A is cyclopropyl ring;

R^2 is

;

n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is $-NHR^7$;

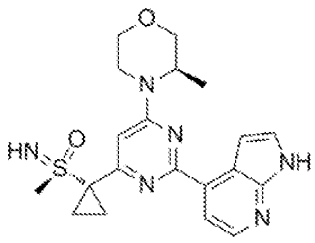
R^{2H} is fluoro;

R^3 is a methyl group;

R^6 is hydrogen; and

R^7 is hydrogen or methyl;

[9] the pharmaceutical product according to [2], wherein the ATR inhibitor is AZD6738, also known as ceralasertib or AZ13386215, represented by the following formula:



or a pharmaceutically acceptable salt thereof;

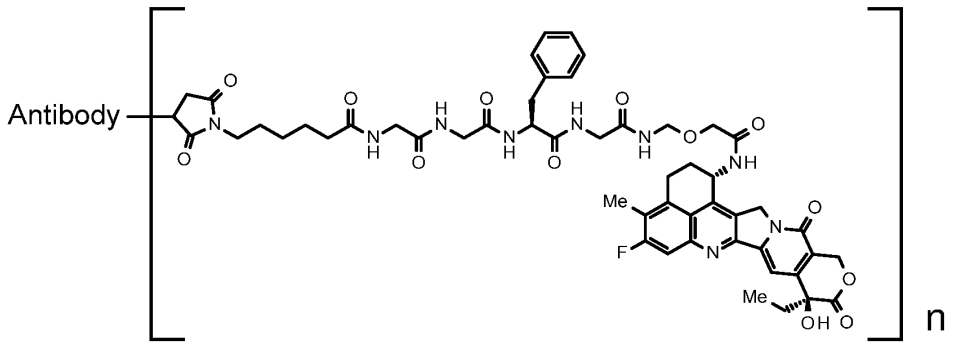
[10] the pharmaceutical product according to any one of [1] to [9], wherein the anti-HER2 antibody is an antibody comprising a heavy chain comprising CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 3 [= amino acid residues 26 to 33 of SEQ ID NO: 1], CDRH2 consisting of an amino acid sequence represented by SEQ ID NO: 4 [= amino acid residues 51 to 58 of SEQ ID NO: 1] and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 5 [= amino acid residues 97 to 109 of SEQ ID NO: 1], and a light chain comprising CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 6 [= amino acid residues 27 to 32 of SEQ ID NO: 2], CDRL2 consisting of an amino acid sequence consisting of amino acid residues 1 to 3 of SEQ ID NO: 7 [= amino acid residues 50 to 52 of SEQ ID NO: 2] and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 8 [=amino acid residues 89 to 97 of SEQ ID NO: 2];

[11] the pharmaceutical product according to any one of [1] to [9], wherein the anti-HER2 antibody is an antibody comprising a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 9 [= amino acid residues 1 to 120 of SEQ ID NO: 1] and a light chain comprising a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10 [= amino acid residues 1 to 107 of SEQ ID NO: 2];

[12] the pharmaceutical product according to any one of [1] to [9], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of an amino acid sequence represented by SEQ ID NO: 2;

[13] the pharmaceutical product according to any one of [1] to [9], wherein the anti-HER2 antibody is an antibody comprising a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 11 [= amino acid residues 1 to 449 of SEQ ID NO: 1] and a light chain consisting of an amino acid sequence represented by SEQ ID NO: 2;

[14] the pharmaceutical product according to any one of [1] to [13], wherein the anti-HER2 antibody-drug conjugate is represented by the following formula:



wherein 'Antibody' indicates the anti-HER2 antibody conjugated to the drug-linker via a thioether bond, and n indicates an average number of units of the drug-linker

conjugated per antibody molecule in the antibody-drug conjugate, wherein n is in the range of from 7 to 8;

[15] the pharmaceutical product according to any one of [1] to [14], wherein the anti-HER2 antibody-drug conjugate is trastuzumab deruxtecan (DS-8201);

[16] the pharmaceutical product according to any one of [1] to [15] wherein the product is a composition comprising the anti-HER2 antibody-drug conjugate and the ATR inhibitor, for simultaneous administration;

[17] the pharmaceutical product according to any one of [1] to [15] wherein the product is a combined preparation comprising the anti-HER2 antibody-drug conjugate and the ATR inhibitor, for sequential or simultaneous administration;

[18] the pharmaceutical product according to any one of [1] to [17], wherein the product is for treating cancer;

[19] the pharmaceutical product according to [18], wherein the cancer is at least one selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, lung cancer, esophageal cancer, head-and-neck cancer, esophagogastric junction adenocarcinoma, biliary tract cancer, Paget's disease, pancreatic cancer, ovarian cancer, uterine carcinosarcoma, urothelial cancer, prostate cancer, bladder cancer, gastrointestinal stromal tumor, digestive tract stromal tumor, uterine cervix cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, corpus uteri carcinoma, kidney cancer, vulval cancer, thyroid cancer,

penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, glioma, glioblastoma multiforme, osteosarcoma, sarcoma, and melanoma;

[20] the pharmaceutical product according to [19], wherein the cancer is breast cancer;

[21] the pharmaceutical product according to [20], wherein the breast cancer has a HER2 status score of IHC 3+;

[22] the pharmaceutical product according to [20], wherein the breast cancer is HER2 low-expressing breast cancer;

[23] the pharmaceutical product according to [20], wherein the breast cancer has a HER2 status score of IHC 2+;

[24] the pharmaceutical product according to [20], wherein the breast cancer has a HER2 status score of IHC 1+;

[25] the pharmaceutical product according to [20], wherein the breast cancer has a HER2 status score of IHC >0 and <1+;

[26] the pharmaceutical product according to [20], wherein the breast cancer is triple-negative breast cancer;

[27] the pharmaceutical product according to [18], wherein the cancer is gastric cancer;

[28] the pharmaceutical product according to [18], wherein the cancer is colorectal cancer;

[29] the pharmaceutical product according to [18], wherein the cancer is lung cancer;

[30] the pharmaceutical product according to [29], wherein the lung cancer is non-small cell lung cancer;

[31] the pharmaceutical product according to [18], wherein the cancer is pancreatic cancer;

[32] the pharmaceutical product according to [18], wherein the cancer is ovarian cancer;

[33] the pharmaceutical product according to [18], wherein the cancer is prostate cancer;

[34] the pharmaceutical product according to [18], wherein the cancer is kidney cancer;

[35] the pharmaceutical product according to [18], wherein cancer cells of the cancer are SLFN11-deficient;

[36] the pharmaceutical product according to [18], wherein SLFN11 expression is lower in the cancer cells of a patient relative to the patient's SLFN11-expressing non-cancer cells;

[37] a pharmaceutical product as defined in any one of [1] to [17], for use in treating cancer;

[38] the pharmaceutical product for the use according to [37], wherein the cancer is as defined in any one of [19] to [36];

[39] use of an anti-HER2 antibody-drug conjugate or an ATR inhibitor in the manufacture of a medicament for administration of the anti-HER2 antibody-drug conjugate and the ATR inhibitor in combination, wherein the anti-

HER2 antibody-drug conjugate and the ATR inhibitor are as defined in any one of [1] to [15], for treating cancer;

[40] the use according to [39], wherein the cancer is as defined in any one of [19] to [36];

[41] the use according to [39] or [40] wherein the medicament is a composition comprising the anti-HER2 antibody-drug conjugate and the ATR inhibitor, for simultaneous administration;

[42] the use according to [39] or [40] wherein the medicament is a combined preparation comprising the anti-HER2 antibody-drug conjugate and the ATR inhibitor, for sequential or simultaneous administration;

[43] an anti-HER2 antibody-drug conjugate for use, in combination with an ATR inhibitor, in the treatment of cancer, wherein the anti-HER2 antibody-drug conjugate and the ATR inhibitor are as defined in any one of [1] to [15];

[44] the anti-HER2 antibody-drug conjugate for the use according to [43], wherein the cancer is as defined in any one of [19] to [36];

[45] the anti-HER2 antibody-drug conjugate for the use according to [43] or [44], wherein the use comprises administration of the anti-HER2 antibody-drug conjugate and the ATR inhibitor sequentially;

[46] the anti-HER2 antibody-drug conjugate for the use according to [43] or [44], wherein the use comprises administration of the anti-HER2 antibody-drug conjugate and the ATR inhibitor simultaneously;

[47] an ATR inhibitor for use, in combination with an anti-HER2 antibody-drug conjugate, in the treatment of cancer, wherein the anti-HER2 antibody-drug conjugate and the ATR inhibitor are as defined in any one of [1] to [15];

[48] the ATR inhibitor for the use according to [47], wherein the cancer is as defined in any one of [19] to [36];

[49] the ATR inhibitor for the use according to [47] or [48], wherein the use comprises administration of the anti-HER2 antibody-drug conjugate and the ATR inhibitor sequentially;

[50] the ATR inhibitor for the use according to [47] or [48], wherein the use comprises administration of the anti-HER2 antibody-drug conjugate and the ATR inhibitor simultaneously;

[51] a method of treating cancer comprising administering an anti-HER2 antibody-drug conjugate and an ATR inhibitor as defined in any one of [1] to [15] in combination to a subject in need thereof;

[52] the method according to [51], wherein the cancer is as defined in any one of [19] to [36];

[53] the method according to [51] or [52], wherein the method comprises administering the anti-HER2 antibody-drug conjugate and the ATR inhibitor sequentially; and

[54] the method according to [51] or [52], wherein the method comprises administering the anti-HER2 antibody-drug conjugate and the ATR inhibitor simultaneously.

[Advantageous Effects of Disclosure]

The present disclosure provides a pharmaceutical product wherein an anti-HER2 antibody-drug conjugate, having an antitumor drug conjugated to an anti-HER2 antibody via a linker structure, and an ATR inhibitor are administered in combination, and a therapeutic use and method wherein the specific antibody-drug conjugate and the ATR inhibitor are administered in combination to a subject. Thus, the present disclosure can provide a medicine and treatment which can obtain a superior antitumor effect in the treatment of cancers.

[Brief Description of Drawings]

[Figure 1] Figure 1 is a diagram showing the amino acid sequence of a heavy chain of an anti-HER2 antibody (SEQ ID NO: 1).

[Figure 2] Figure 2 is a diagram showing the amino acid sequence of a light chain of an anti-HER2 antibody (SEQ ID NO: 2).

[Figure 3] Figure 3 is a diagram showing the amino acid sequence of a heavy chain CDRH1 (SEQ ID NO: 3 [= amino acid residues 26 to 33 of SEQ ID NO: 1]).

[Figure 4] Figure 4 is a diagram showing the amino acid sequence of a heavy chain CDRH2 (SEQ ID NO: 4 [= amino acid residues 51 to 58 of SEQ ID NO: 1]).

[Figure 5] Figure 5 is a diagram showing the amino acid sequence of a heavy chain CDRH3 (SEQ ID NO: 5 [= amino acid residues 97 to 109 of SEQ ID NO: 1]).

[Figure 6] Figure 6 is a diagram showing the amino acid sequence of a light chain CDRL1 (SEQ ID NO: 6 [= amino acid residues 27 to 32 of SEQ ID NO: 2]).

[Figure 7] Figure 7 is a diagram showing an amino acid sequence comprising the amino acid sequence of a light chain CDRL2 (SAS) (SEQ ID NO: 7 [= amino acid residues 50 to 56 of SEQ ID NO: 2]).

[Figure 8] Figure 8 is a diagram showing the amino acid sequence of a light chain CDRL3 (SEQ ID NO: 8 [= amino acid residues 89 to 97 of SEQ ID NO: 2]).

[Figure 9] Figure 9 is a diagram showing the amino acid sequence of a heavy chain variable region (SEQ ID NO: 9 [= amino acid residues 1 to 120 of SEQ ID NO: 1]).

[Figure 10] Figure 10 is a diagram showing the amino acid sequence of a light chain variable region (SEQ ID NO: 10 [= amino acid residues 1 to 107 of SEQ ID NO: 2]).

[Figure 11] Figure 11 is a diagram showing the amino acid sequence of a heavy chain (SEQ ID NO: 11 [= amino acid residues 1 to 449 of SEQ ID NO: 1]).

[Figures 12A to 12D] Figures 12A to D are diagrams showing combination matrices obtained with high-throughput screens combining DS-8201 with AZD6738 (AZ13386215; ATR inhibitor) in breast cancer cell lines with diverse HER2 expression and one gastric cell line with high HER2 expression.

[Figure 13] Figure 13 is a diagram showing synergy matrices for combinations with DS-8201 and AZD6738 in HER2-high KPL4 cell line, in terms of (A) relative total cell counts as percentage of control, and (B) Loewe, Bliss and HSA scores.

[Figure 14] Figure 14 is a diagram showing change in total cells remaining after treatment compared to time zero for combinations of DS-8201 with AZD6738 in (A) HER2-high KPL4 cell line and (B) HER2-negative MDA-MB-468 cell line.

[Figure 15] Figure 15 is a diagram showing induction of ATM-dependent KAP1 pSer824 signalling, DNA double strand break damage (γH2AX) biomarkers or percentage of cell number (vs solvent control) for combinations of DS-8201 with AZD6738 in (A) HER2-high KPL4 cell line or (B) HER2-low MDA-MB-468 cell line.

[Figure 16] Figure 16 is a diagram showing change in tumor volume over time for treatment groups of female nude mice having NCI-N87 tumors implanted subcutaneously, treated with DS-8201 at 1 mg/kg or 3 mg/kg alone and in combination with AZD6738 at 25 mg/kg BID.

[Figure 17] Figure 17 is a diagram showing antibody blot images combining DS-8201 or exatecan mesylate, with AZD6738 in (A) NCI-N87 (gastric cancer) and (B) KPL4 (breast carcinoma) cell lines.

[Figures 18A and 18B] Figures 18A and 18B are diagrams showing combination matrices obtained with screens combining DS-8201 with AZD6738 (ceralasertib) in primary

$CD34^+$ bone marrow-derived hematopoietic stem and progenitor cells induced to differentiate into erythroid, myeloid, or megakaryocytic lineages.

[Figure 19] In Figure 19, (A) and (B) are diagrams showing combination matrices obtained with high-throughput screens combining DS-8201 with AZD6738 in HER2-low NCI-H522 (lung cancer) cell line.

In order that the present disclosure can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

Before describing the present disclosure in detail, it is to be understood that this disclosure is not limited to specific compositions or method steps, as such can vary. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein.

Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as

used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range.

It is understood that wherever aspects are described herein with the language "comprising", otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided. The terms "inhibit", "block", and "suppress" are used interchangeably herein and refer to any statistically significant decrease in biological activity, including full blocking of the activity. For example, "inhibition"

can refer to a decrease of about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% in biological activity. Cellular proliferation can be assayed using art recognized techniques which measure rate of cell division, and/or the fraction of cells within a cell population undergoing cell division, and/or rate of cell loss from a cell population due to terminal differentiation or cell death (*e.g.*, thymidine incorporation).

The term "subject" refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

The term "pharmaceutical product" refers to a preparation which is in such form as to permit the biological activity of the active ingredients, either as a composition containing all the active ingredients (for simultaneous administration), or as a combination of separate compositions (a combined preparation) each containing at least one but not all of the active ingredients (for administration sequentially or simultaneously), and which contains no additional components which are unacceptably toxic to a subject to which the product would be administered. Such product can be sterile. By "simultaneous administration" is meant that the active ingredients are administered at the same

time. By "sequential administration" is meant that the active ingredients are administered one after the other, in either order, at a time interval between the individual administrations. The time interval can be, for example, less than 24 hours, preferably less than 6 hours, more preferably less than 2 hours.

Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and (2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In certain aspects, a subject is successfully "treated" for cancer according to the methods of the present disclosure if the patient shows, *e.g.*, total, partial, or transient remission of a certain type of cancer.

The terms "cancer", "tumor", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancers include but are not limited to, breast cancer, gastric cancer, colorectal cancer, lung cancer, esophageal cancer, head-and-neck cancer, esophagogastric junction adenocarcinoma,

biliary tract cancer, Paget's disease, pancreatic cancer, ovarian cancer, uterine carcinosarcoma, urothelial cancer, prostate cancer, bladder cancer, gastrointestinal stromal tumor, digestive tract stromal tumor, uterine cervix cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, corpus uteri carcinoma, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, glioma, glioblastoma multiforme, osteosarcoma, sarcoma, and melanoma. Cancers include hematological malignancies such as acute myeloid leukemia, multiple myeloma, chronic lymphocytic leukemia, diffuse large B cell lymphoma, Burkitt's lymphoma, follicular lymphoma and solid tumors such as breast cancer, lung cancer, neuroblastoma and colon cancer.

The term "cytotoxic agent" as used herein is defined broadly and refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells (cell death), and/or exerts anti-neoplastic/anti-proliferative effects. For example, a cytotoxic agent prevents directly or indirectly the development, maturation, or spread of neoplastic tumor cells. The term includes also such agents that cause a cytostatic effect only and not a mere cytotoxic effect. The term includes chemotherapeutic agents as specified below, as well as other HER2 antagonists, anti-angiogenic agents, tyrosine kinase inhibitors, protein kinase A inhibitors, members of the cytokine family, radioactive

isotopes, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin.
The term "chemotherapeutic agent" is a subset of the term "cytotoxic agent" comprising natural or synthetic chemical compounds.

In accordance with the methods or uses of the present disclosure, compounds of the present disclosure may be administered to a patient to promote a positive therapeutic response with respect to cancer. The term "positive therapeutic response" with respect to cancer treatment refers to an improvement in the symptoms associated with the disease. For example, an improvement in the disease can be characterized as a complete response. The term "complete response" refers to an absence of clinically detectable disease with normalization of any previous test results.
Alternatively, an improvement in the disease can be categorized as being a partial response. A "positive therapeutic response" encompasses a reduction or inhibition of the progression and/or duration of cancer, the reduction or amelioration of the severity of cancer, and/or the amelioration of one or more symptoms thereof resulting from the administration of compounds of the present disclosure. In specific aspects, such terms refer to one, two or three or more results following the administration of compounds of the instant disclosure:
(1) a stabilization, reduction or elimination of the cancer cell population;

(2) a stabilization or reduction in cancer growth;

(3) an impairment in the formation of cancer;

(4) eradication, removal, or control of primary, regional and/or metastatic cancer;

(5) a reduction in mortality;

(6) an increase in disease-free, relapse-free, progression-free, and/or overall survival, duration, or rate;

(7) an increase in the response rate, the durability of response, or number of patients who respond or are in remission;

(8) a decrease in hospitalization rate,

(9) a decrease in hospitalization lengths,

(10) the size of the cancer is maintained and does not increase or increases by less than 10%, preferably less than 5%, preferably less than 4%, preferably less than 2%, and

(11) an increase in the number of patients in remission.

(12) a decrease in the number of adjuvant therapies (*e.g.*, chemotherapy or hormonal therapy) that would otherwise be required to treat the cancer.

Clinical response can be assessed using screening techniques such as PET, magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, flow cytometry or fluorescence-activated cell sorter (FACS) analysis, histology, gross pathology, and blood chemistry, including but not limited to changes detectable by ELISA, RIA, chromatography, and the like.

In addition to these positive therapeutic responses, the subject undergoing therapy can experience the beneficial effect of an improvement in the symptoms associated with the disease.

As used herein, the term "the expression level of SLFN11 is" some amount, e.g. 0%, means that the stated amount of cancer cells in the patient's cancer tissue express SLFN11. Similarly, as used herein, the term "the expression level of SLFN11 is <" some amount, e.g. 10%, means that less than the stated amount of cancer cells in the patient's cancer tissue express SLFN11. The expression level of SLFN11 may be, for example, <25%, <20%, <15%, <10%, <9%, <8%, <7%, <6%, <5%, <4%, <3%, <2%, <1% or 0%.

As used herein, the term "SLFN11-deficient" refers to an expression level of SLFN11 in the relevant patient, animal, tissue, cell, etc. that is inadequate to exhibit the normal phenotype associated with the gene, or for the protein to exhibit its physiological function. In the context of preclinical models, cells or animals in which the SLFN11 gene is knocked out (KO) are examples of "SLFN11-deficient".

In this specification the generic term "C_{p-q}alkyl" includes both straight-chain and branched-chain alkyl groups. However references to individual alkyl groups such as "propyl" are specific for the straight chain version only (i.e. *n*-propyl and isopropyl) and references

to individual branched-chain alkyl groups such as "*tert*-butyl" are specific for the branched chain version only.

The prefix C_{p-q} in C_{p-q}alkyl and other terms (where p and q are integers) indicates the range of carbon atoms that are present in the group, for example C_{1-4}alkyl includes C_1alkyl (methyl), C_2alkyl (ethyl), C_3alkyl (propyl as *n*-propyl and isopropyl) and C_4alkyl (n-butyl, sec-butyl, isobutyl and *tert*-butyl).

The term C_{p-q}alkoxy comprises -O-C_{p-q}alkyl groups.

The term C_{p-q}alkanoyl comprises -C(O)alkyl groups.

The term halo includes fluoro, chloro, bromo and iodo.

"Carbocyclyl" is a saturated, unsaturated or partially saturated monocyclic ring system containing from 3 to 6 ring atoms, wherein a ring CH_2 group may be replaced with a C=O group. "Carbocyclyl" includes "aryl", "C_{p-q}cycloalkyl" and "C_{p-q}cycloalkenyl".

"Aryl" is an aromatic monocyclic carbocyclyl ring system.

"C_{p-q}cycloalkenyl" is an unsaturated or partially saturated monocyclic carbocyclyl ring system containing at least 1 C=C bond and wherein a ring CH_2 group may be replaced with a C=O group.

"C_{p-q}cycloalkyl" is a saturated monocyclic carbocyclyl ring system and wherein a ring CH_2 group may be replaced with a C=O group.

"Heterocyclyl" is a saturated, unsaturated or partially saturated monocyclic ring system containing

from 3 to 6 ring atoms of which 1, 2 or 3 ring atoms are chosen from nitrogen, sulfur or oxygen, which ring may be carbon or nitrogen linked and wherein a ring nitrogen or sulfur atom may be oxidised and wherein a ring CH_2 group may be replaced with a C=O group. "Heterocyclyl" includes "heteroaryl", "cycloheteroalkyl" and "cycloheteroalkenyl".

"Heteroaryl" is an aromatic monocyclic heterocyclyl, particularly having 5 or 6 ring atoms, of which 1, 2 or 3 ring atoms are chosen from nitrogen, sulfur or oxygen where a ring nitrogen or sulfur may be oxidised.

"Cycloheteroalkenyl" is an unsaturated or partially saturated monocyclic heterocyclyl ring system, particularly having 5 or 6 ring atoms, of which 1, 2 or 3 ring atoms are chosen from nitrogen, sulfur or oxygen, which ring may be carbon or nitrogen linked and wherein a ring nitrogen or sulfur atom may be oxidised and wherein a ring CH_2 group may be replaced with a C=O group.

"Cycloheteroalkyl" is a saturated monocyclic heterocyclic ring system, particularly having 5 or 6 ring atoms, of which 1, 2 or 3 ring atoms are chosen from nitrogen, sulfur or oxygen, which ring may be carbon or nitrogen linked and wherein a ring nitrogen or sulfur atom may be oxidised and wherein a ring CH_2 group may be replaced with a C=O group.

This specification may make use of composite terms to describe groups comprising more than one functionality. Unless otherwise described herein, such

terms are to be interpreted as is understood in the art. For example carbocyclylC_{p-q}alkyl comprises C_{p-q}alkyl substituted by carbocyclyl, heterocyclylC_{p-q}alkyl comprises C_{p-q}alkyl substituted by heterocyclyl, and bis(C_{p-q}alkyl)amino comprises amino substituted by 2 C_{p-q}alkyl groups which may be the same or different. HaloC_{p-q}alkyl is a C_{p-q}alkyl group that is substituted by 1 or more halo substituents and particularly 1, 2 or 3 halo substituents. Similarly, other generic terms containing halo such as haloC_{p-q}alkoxy may contain 1 or more halo substituents and particularly 1, 2 or 3 halo substituents.

HydroxyC_{p-q}alkyl is a C_{p-q}alkyl group that is substituted by 1 or more hydroxyl substituents and particularly by 1, 2 or 3 hydroxy substituents. Similarly other generic terms containing hydroxy such as hydroxyC_{p-q}alkoxy may contain 1 or more and particularly 1, 2 or 3 hydroxy substituents.

C_{p-q}alkoxyC_{p-q}alkyl is a C_{p-q}alkyl group that is substituted by 1 or more C_{p-q}alkoxy substituents and particularly 1, 2 or 3 C_{p-q}alkoxy substituents. Similarly other generic terms containing C_{p-q}alkoxy such as C_{p-q}alkoxyC_{p-q}alkoxy may contain 1 or more C_{p-q}alkoxy substituents and particularly 1, 2 or 3 C_{p-q}alkoxy substituents.

Where optional substituents are chosen from "1 or 2", from "1, 2, or 3" or from "1, 2, 3 or 4" groups or substituents it is to be understood that this definition

includes all substituents being chosen from one of the specified groups i.e. all substitutents being the same or the substituents being chosen from two or more of the specified groups i.e. the substitutents not being the same.

Compounds of the present disclosure have been named with the aid of computer software (ACD/Name version 10.06).

Suitable values for any R group or any part or substituent for such groups include:

for C_{1-3}alkyl: methyl, ethyl, propyl and *iso*-propyl;

for C_{1-6}alkyl: C_{1-3}alkyl, butyl, 2-methylpropyl, *tert*-butyl, pentyl, 2,2-dimethylpropyl, 3-methylbutyl and hexyl;

for C_{3-6}cycloalkyl: cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl;

for C_{3-6}cycloalkylC_{1-3}alkyl: cyclopropylmethyl, cyclopropylethyl, cyclobutylmethyl, cyclopentylmethyl and cyclohexylmethyl;

for aryl: phenyl;

for arylC_{1-3}alkyl: benzyl and phenethyl;

for carbocylyl: aryl, cyclohexenyl and C_{3-6}cycloalkyl;

for halo: fluoro, chloro, bromo and iodo;

for C_{1-3}alkoxy: methoxy, ethoxy, propoxy and isopropoxy;

for C_{1-6}alkoxy: C_{1-3}alkoxy, butoxy, *tert*–butoxy, pentyloxy, 1–ethylpropoxy and hexyloxy;

for C_{1-3}alkanoyl: acetyl and propanoyl;

for C_{1-6}alkanoyl: acetyl, propanoyl and 2–methylpropanoyl;

for heteroaryl:pyridinyl, imidazolyl, pyrimidinyl, thienyl, pyrrolyl, pyrazolyl, thiazolyl, thiazolyl, triazolyl, oxazolyl, isoxazolyl, furanyl, pyridazinyl and pyrazinyl;

for heteroarylC_{1-3}alkyl: pyrrolylmethyl, pyrrolylethyl, imidazolylmethyl, imidazolylethyl, pyrazolylmethyl, pyrazolylethyl, furanylmethyl, furanylethyl, thienylmethyl, theinylethyl, pyridinylmethyl, pyridinylethyl, pyrazinylmethyl, pyrazinylethyl, pyrimidinylmethyl, pyrimidinylethyl, pyrimidinylpropyl, pyrimidinylbutyl, imidazolylpropyl, imidazolylbutyl, 1,3,4–triazolylpropyl and oxazolylmethyl;

for heterocyclyl: heteroaryl, pyrrolidinyl, piperidinyl, piperazinyl, azetidinyl, morpholinyl, dihydro–*2H*–pyranyl, tetrahydropyridine and tetrahydrofuranyl;

for saturated heterocyclyl: oxetanyl, pyrrolidinyl, piperidinyl, piperazinyl, azetidinyl, morpholinyl, tetrahydropyranyl and tetrahydrofuranyl.

It should be noted that examples given for terms used in the description are not limiting.

As used herein, the phrase "effective amount" means an amount of a compound or composition which is sufficient enough to significantly and positively modify the symptoms and/or conditions to be treated (e.g., provide a positive clinical response). The effective amount of an active ingredient for use in a pharmaceutical product will vary with the particular condition being treated, the severity of the condition, the duration of the treatment, the nature of concurrent therapy, the particular active ingredient(s) being employed, the particular pharmaceutically-acceptable excipient(s)/carrier(s) utilized, and like factors within the knowledge and expertise of the attending physician. In particular, an effective amount of a compound of formula (I) for use in the treatment of cancer in combination with the antibody-drug conjugate is an amount such that the combination is sufficient to symptomatically relieve in a warm-blooded animal such as man, the symptoms of cancer, to slow the progression of cancer, or to reduce in patients with symptoms of cancer the risk of getting worse.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic

response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

Certain compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the disclosure encompasses all geometric and optical isomers of the compounds of formula (I) and mixtures thereof including racemates. Tautomers and mixtures thereof also form an aspect of the present disclosure.

Solvates and mixtures thereof also form an aspect of the present disclosure. For example, a suitable solvate of a compound of formula (I) is, for example, a hydrate such as a hemi-hydrate, a mono-hydrate, a di-hydrate or a tri-hydrate or an alternative quantity thereof.

It is to be understood that, insofar as certain of the compounds of formula (I) defined above may exist in optically active or racemic forms by virtue of one or more asymmetric carbon atoms or sulphur atoms, the disclosure includes in its definition any such optically active or racemic form which possesses the above-mentioned activity. The present disclosure encompasses all such stereoisomers having activity as herein defined. It is further to be understood that in the names of chiral compounds (R,S) denotes any scalemic or racemic mixture while (R) and (S) denote the enantiomers. In the absence of (R,S), (R) or (S) in the name it is to be understood that the name refers to any scalemic or racemic mixture, wherein a scalemic mixture contains R and S enantiomers in any relative proportions

and a racemic mixture contains R and S enantiomers in the ratio 50:50. The synthesis of optically active forms may be carried out by standard techniques of organic chemistry well known in the art, for example by synthesis from optically active starting materials or by resolution of a racemic form. Racemates may be separated into individual enantiomers using known procedures (see, for example, Advanced Organic Chemistry: 3rd Edition: author J March, p104–107). A suitable procedure involves formation of diastereomeric derivatives by reaction of the racemic material with a chiral auxiliary, followed by separation, for example by chromatography, of the diastereomers and then cleavage of the auxiliary species. Similarly, the above-mentioned activity may be evaluated using standard laboratory techniques.

It will be understood that compounds of formula (I) may encompass compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like. disclosure The present disclosure may use compounds of formula (I) as herein defined as well as to salts thereof. Salts for use in pharmaceutical products will be pharmaceutically acceptable salts, but other salts may be useful in the production of the compounds of formula (I) and their pharmaceutically acceptable salts.

Pharmaceutically acceptable salts of the disclosure may, for example, include acid addition salts of compounds of formula (I) as herein defined which are sufficiently basic to form such salts. Such acid addition salts include but are not limited to fumarate, methanesulfonate, hydrochloride, hydrobromide, citrate and maleate salts and salts formed with phosphoric and sulfuric acid. In addition where compounds of formula (I) are sufficiently acidic, salts are base salts and examples include but are not limited to, an alkali metal salt for example sodium or potassium, an alkaline earth metal salt for example calcium or magnesium, or organic amine salt for example triethylamine, ethanolamine, diethanolamine, triethanolamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, dibenzylamine or amino acids such as lysine.

The compounds of formula (I) may also be provided as *in vivo* hydrolysable esters. An *in vivo* hydrolysable ester of a compound of formula (I) containing carboxy or hydroxy group is, for example a pharmaceutically acceptable ester which is cleaved in the human or animal body to produce the parent acid or alcohol. Such esters can be identified by administering, for example, intravenously to a test animal, the compound under test and subsequently examining the test animal's body fluid.

Suitable pharmaceutically acceptable esters for carboxy include C_{1-6}alkoxymethyl esters for example methoxymethyl, C_{1-6}alkanoyloxymethyl esters for example

pivaloyloxymethyl, phthalidyl esters, C_{3-8}cycloalkcarbonyloxyC_{1-6}alkyl esters for example 1-cyclohexylcarbonyloxyethyl, (1,3-dioxolen-2-one)ylmethyl esters for example (5-methyl-1,3-dioxolen-2-one)ylmethyl, and C_{1-6}alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl; and may be formed at any carboxy group in the compounds of this disclosure.

Suitable pharmaceutically acceptable esters for hydroxy include inorganic esters such as phosphate esters (including phosphoramidic cyclic esters) and α-acyloxyalkyl ethers and related compounds which as a result of the *in vivo* hydrolysis of the ester breakdown to give the parent hydroxy groups. Examples of α-acyloxyalkyl ethers include acetoxymethoxy and 2,2-dimethylpropionyloxymethoxy. A selection of *in vivo* hydrolysable ester forming groups for hydroxy include C_{1-10}alkanoyl, for example acetyl, benzoyl, phenylacetyl, substituted benzoyl and phenylacetyl; C_{1-10}alkoxycarbonyl (to give alkyl carbonate esters), for example ethoxycarbonyl; di-C_{1-4}alkylcarbamoyl and *N*-(di-C_{1-4}alkylaminoethyl)-*N*-C_{1-4}alkylcarbamoyl (to give carbamates); di-C_{1-4}alkylaminoacetyl and carboxyacetyl. Examples of ring substituents on phenylacetyl and benzoyl include aminomethyl, C_{1-4}alkylaminomethyl and di-(C_{1-4}alkyl)aminomethyl, and morpholino or piperazino linked from a ring nitrogen atom via a methylene linking group to the 3- or 4- position of the benzoyl ring.

Other interesting *in vivo* hydrolysable esters include, for example, $R^AC(O)OC_{1-6}$alkyl–CO–, wherein R^A is for example, benzyloxy–C_{1-4}alkyl, or phenyl. Suitable substituents on a phenyl group in such esters include, for example, 4–C_{1-4}alkylpiperazino–C_{1-4}alkyl, piperazino–C_{1-4}alkyl and morpholino–C_{1-4}alkyl.

The compounds of the formula (I) may be also be administered in the form of a prodrug which is broken down in the human or animal body to give a compound of the formula (I). Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309–396, edited by K. Widder, et al. (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard–Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113–191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1–38 (1992);

d) H. Bundgaard, et al., Journal of Pharmaceutical Sciences, 77, 285 (1988); and

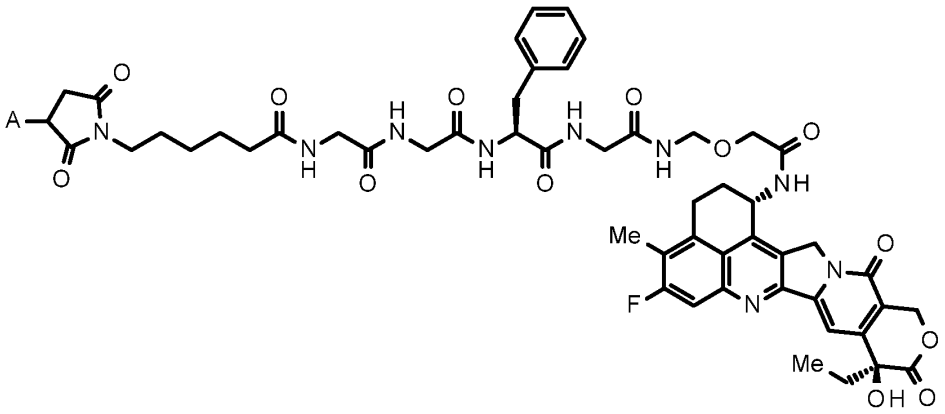
e) N. Kakeya, et al., Chem Pharm Bull, 32, 692 (1984).

[Description of Embodiments]

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

1. Antibody-drug conjugate

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

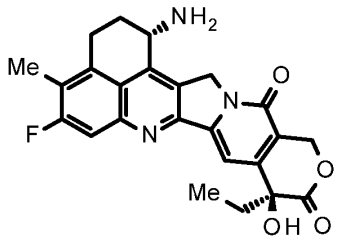


wherein A represents the connecting position to an antibody,
is conjugated to an anti-HER2 antibody via a thioether bond.

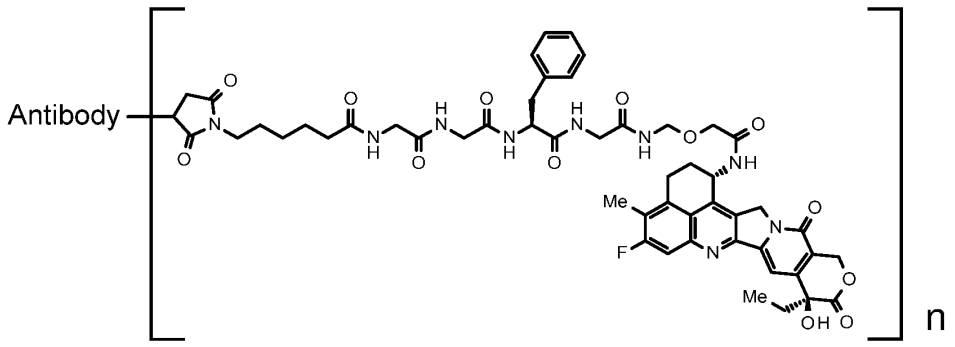
In the present disclosure, the partial structure consisting of a linker and a drug in the antibody-drug conjugate is referred to as a "drug-linker". The drug-linker is connected to a thiol group (in other words, the

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) in the antibody.

The drug-linker of the present disclosure 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:

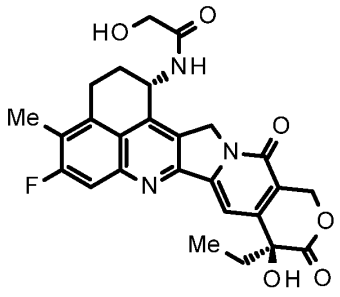


The anti-HER2 antibody-drug conjugate used in the present disclosure can be also represented by the following formula:



Here, the drug-linker is conjugated to an anti-HER2 antibody ('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.

After migrating into cancer cells, the anti-HER2 antibody-drug conjugate used in the present disclosure is cleaved at the linker portion to release a compound represented by the following formula:



This compound is inferred to be the original source of the antitumor activity of the antibody-drug conjugate used in the present disclosure, 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).

The anti-HER2 antibody-drug conjugate used in the present disclosure 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 whereby the antibody-drug conjugate used in the present disclosure is internalized in cancer cells expressing the target and the compound released then exerts an antitumor effect also on cancer cells which are present therearound and not expressing the target. This bystander effect is exerted as an excellent antitumor effect even when the anti-HER2 antibody-drug conjugate is used in combination with an ATR inhibitor according to the present disclosure.

2. Antibody in antibody-drug conjugate

The anti-HER2 antibody in the antibody-drug conjugate used in the present disclosure may be derived from any species, and is preferably an anti-HER2 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 anti-HER2 antibody may be a polyclonal antibody or a monoclonal antibody and is preferably a monoclonal antibody.

The antibody in the antibody-drug conjugate used in the present disclosure is an anti-HER2 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.

The binding activity of the anti-HER2 antibody against cancer cells can be confirmed using flow cytometry. The internalization of the antibody into cancer 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.

The antitumor activity of the anti-HER2 antibody can be confirmed in vitro by determining inhibitory activity against cell growth. For example, a cancer cell line overexpressing HER2 as 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.

Since the compound conjugated in the anti-HER2 antibody-drug conjugate exerts an antitumor effect, it is preferred but not essential that the anti-HER2 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 anti-HER2 antibody should have the property of internalizing to migrate into cancer cells.

The anti-HER2 antibody in the antibody-drug conjugate used in the present disclosure can be obtained by a procedure known in the art. For example, the antibody of the present disclosure 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.

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.

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.

The anti-HER2 antibody in the antibody-drug conjugate used the present disclosure 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.

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)).

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.

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.

In the present disclosure, modified variants of the anti-HER2 antibody in the antibody-drug conjugate used in the present disclosure are also included. The modified variant refers to a variant obtained by subjecting the antibody according to the present disclosure 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 disclosure, 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 disclosure 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.

Further, by regulating the modification of a glycan which is linked to the antibody according to the present disclosure (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, those disclosed in WO99/54342, WO00/61739, WO02/31140, WO2007/133855, WO2013/120066, etc. are known. However, the technique is not limited thereto. In the anti-HER2 antibody according to the present disclosure, antibodies in which the modification of a glycan is regulated are also included.

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 anti-HER2 antibody according to the present disclosure, 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 anti-HER2 antibody according to the present disclosure 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 disclosure 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 anti-HER2 antibody according to the present disclosure 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 disclosure can be exemplified as preferred.

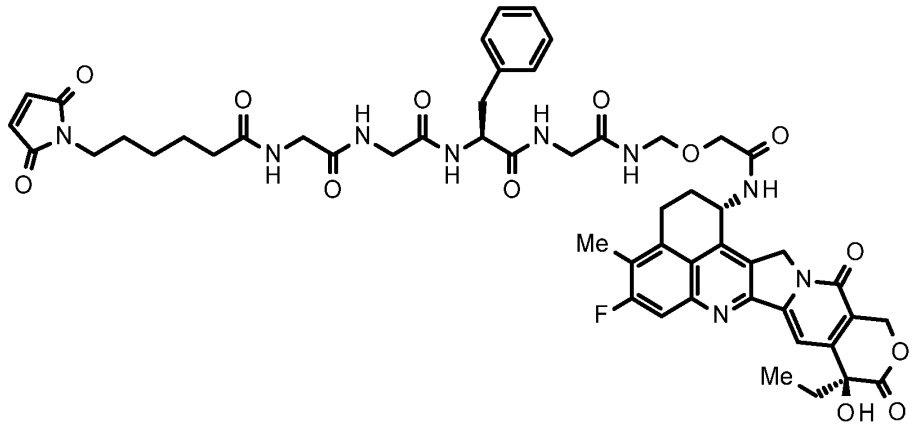
As isotypes of the anti-HER2 antibody according to the present disclosure, for example, IgG (IgG1, IgG2, IgG3, IgG4) can be exemplified, and IgG1 or IgG2 can be exemplified as preferred.

In the present disclosure, 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.

Examples of the anti-HER2 antibody include trastuzumab (U.S. Patent No. 5821337) and pertuzumab (WO01/00245), and trastuzumab can be exemplified as preferred.

3. Production of antibody-drug conjugate

A drug-linker intermediate for use in production of the anti-HER2 antibody-drug conjugate according to the present disclosure is represented by the following formula:



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 WO2014/057687, WO2015/098099, WO2015/115091, WO2015/155998, WO2019/044947 and so on.

The anti-HER2 antibody-drug conjugate used in the present disclosure can be produced by reacting the above-described drug-linker intermediate and an anti-HER2 antibody having a thiol group (also referred to as a sulfhydryl group).

The anti-HER2 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 anti-HER2 antibody having a sulfhydryl group with partially or completely reduced interchain disulfides within the antibody can be obtained.

Further, by using 2 to 20 molar equivalents of the drug-linker intermediate per anti-HER2 antibody having a sulfhydryl group, an anti-HER2 antibody-drug conjugate in which 2 to 8 drug molecules are conjugated per antibody molecule can be produced.

The average number of conjugated drug molecules per anti-HER2 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).

Conjugation between the anti-HER2 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 WO2014/057687,

WO2015/098099, WO2015/115091, WO2015/155998, WO2017/002776, WO2018/212136, and so on.

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

The anti-HER2 antibody is preferably an antibody comprising a heavy chain comprising CDRH1 consisting of an amino acid sequence consisting of amino acid residues 26 to 33 of SEQ ID NO: 1, CDRH2 consisting of an amino acid sequence consisting of amino acid residues 51 to 58 of SEQ ID NO: 1 and CDRH3 consisting of an amino acid sequence consisting of amino acid residues 97 to 109 of SEQ ID NO: 1, and a light chain comprising CDRL1 consisting of an amino acid sequence consisting of amino acid residues 27 to 32 of SEQ ID NO: 2, CDRL2 consisting of an amino acid sequence consisting of amino acid residues 50 to 52 of SEQ ID NO: 2 and CDRL3 consisting of an amino acid sequence consisting of amino acid residues 89 to 97 of SEQ ID NO: 2, and more preferably an antibody comprising a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence consisting of amino acid residues 1 to 120 of SEQ ID NO: 1 and a light chain comprising a light chain variable region consisting of an amino acid sequence consisting of amino acid residues 1 to 107 of SEQ ID NO: 2, and even more preferably an antibody comprising a heavy chain

consisting of an 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, or an antibody comprising a heavy chain 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 all amino acid residues 1 to 214 of SEQ ID NO: 2.

The average number of units of the drug-linker conjugated per antibody molecule in the anti-HER2 antibody-drug conjugate 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.

The anti-HER2 antibody-drug conjugate used in the present disclosure can be produced with reference to descriptions in WO2015/115091 and so on.

In preferred embodiments, the anti-HER2 antibody-drug conjugate is trastuzumab deruxtecan (DS-8201).

4. ATR inhibitor

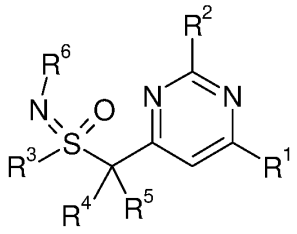
In the present disclosure, the term "ATR inhibitor" refers to an agent that inhibits ATR (ataxia telangiectasia and rad3-related kinase). The ATR inhibitor in the present disclosure may selectively inhibit the kinase ATR, or may non-selectively inhibit ATR and inhibit also kinase(s) other than ATR. The ATR inhibitor in the present disclosure is not particularly limited as long as it is an agent that has the described

characteristics, and preferred examples thereof can include those disclosed in WO2011/154737.

Other examples of ATR inhibitors which may be used according to the present disclosure are BAY-1895344, ETP-46464, and VE-821.

Preferably, the ATR inhibitor in the present disclosure inhibits ATR selectively.

According to preferred embodiments of the ATR inhibitor used in the present disclosure, the ATR inhibitor is a compound represented by the following formula (I):

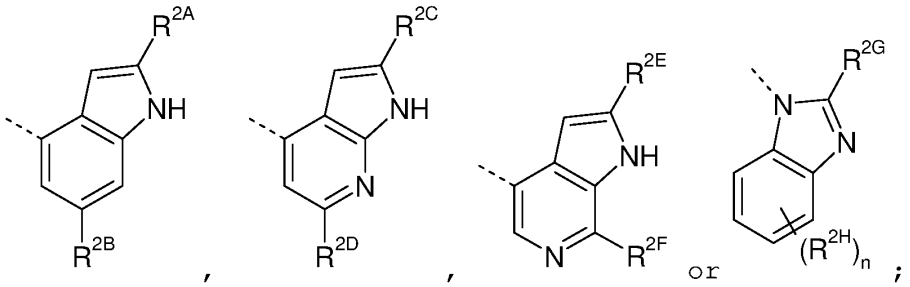


(I)

wherein:

R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

R^2 is



n is 0 or 1;

R^{2A}, R^{2C}, R^{2E} and R^{2F} each independently are hydrogen or methyl;

R^{2B} and R^{2D} each independently are hydrogen or methyl;

R^{2G} is selected from $-NHR^7$ and $-NHCOR^8$;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and R^5 are each independently hydrogen or methyl, or R^4 and R^5 together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 membered heterocyclic ring containing one heteroatom selected from O and N;

R^6 is hydrogen;

R^7 is hydrogen or methyl; and

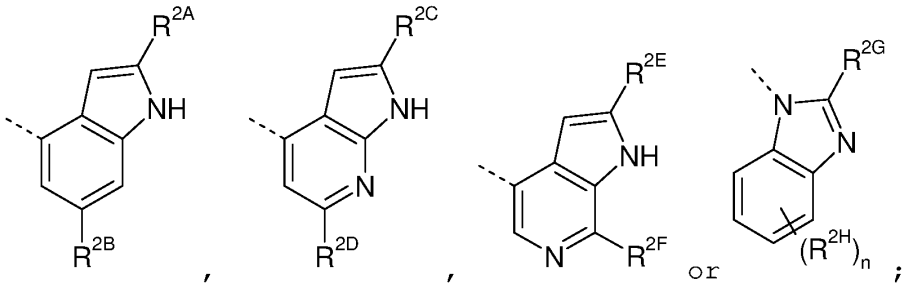
R^8 is methyl,

or a pharmaceutically acceptable salt thereof.

In preferred embodiments, the ATR inhibitor is a compound represented by formula (I) wherein:

R^1 is 3-methylmorpholin-4-yl;

R^2 is



n is 0 or 1;

R^{2A}, R^{2C}, R^{2E} and R^{2F} each independently are hydrogen or methyl;

R^{2B} and R^{2D} each independently are hydrogen or methyl;

R^{2G} is selected from $-NH_2$, $-NHMe$ and $-NHCOMe$;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and R^5 are each independently hydrogen or methyl, or R^4 and R^5 together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 membered heterocyclic ring containing one heteroatom selected from O and N; and

R^6 is hydrogen,

or a pharmaceutically acceptable salt thereof.

Additional embodiments of the ATR inhibitor are compounds of formula (I), and pharmaceutically acceptable salts thereof, in which Ring A, n, R^1, R^2, R^4, R^5, R^6, R^7 and R^8 are defined as follows. Such specific substituents may be used, where appropriate, with any of the definitions, claims or embodiments defined herein.

n

In one embodiment n is 0.

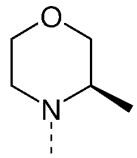
In another embodiment n is 1.

R^1

In one embodiment, R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl.

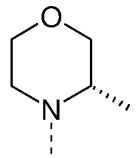
In a further embodiment, R^1 is 3-methylmorpholin-4-yl.

In a further embodiment, R^1 is



.

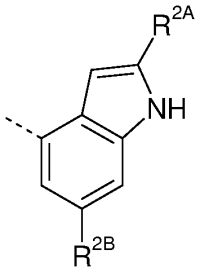
In a further embodiment, R^1 is



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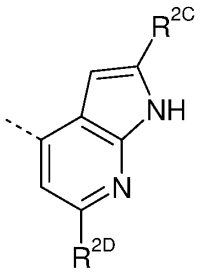
R^2

In one embodiment **R^2** is



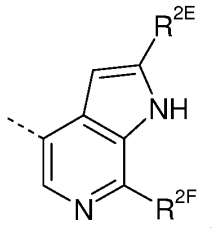
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In another embodiment **R^2** is



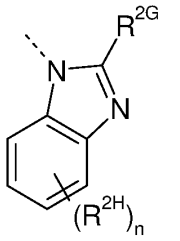
.

In another embodiment **R^2** is



.

In another embodiment **R²** is



.

R^{2A}

In one embodiment R^{2A} is hydrogen.

R^{2B}

In one embodiment R^{2B} is hydrogen.

R^{2C}

In one embodiment R^{2C} is hydrogen.

R^{2D}

In one embodiment R^{2D} is hydrogen.

R^{2E}

In one embodiment R^{2E} is hydrogen.

R^{2F}

In one embodiment R^{2F} is hydrogen.

R^{2G}

In one embodiment **R^{2G}** is selected from $-NHR^7$ and $-NHCOR^8$.

In another embodiment **R^{2G}** is $-NHR^7$.

In another embodiment **R^{2G}** is $-NHCOR^8$.

In another embodiment $\mathbf{R^{2G}}$ is selected from $-NH_2$, $-NHMe$ and $-NHCOMe$.

In another embodiment of the disclosure $\mathbf{R^{2G}}$ is $-NH_2$.

In another embodiment $\mathbf{R^{2G}}$ is $-NHMe$.

In another embodiment $\mathbf{R^{2G}}$ is $-NHCOMe$.

R^4 and R^5

In one embodiment R^4 and R^5 are hydrogen.

In another embodiment R^4 and R^5 are methyl.

In another embodiment R^4 and R^5 together with the atom to which they are attached form Ring A.

Ring A

In one embodiment Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 heterocyclic ring containing one heteroatom selected from O and N.

In another embodiment Ring A is a cyclopropyl, cyclobutyl, cyclopentyl, oxetanyl, tetrahydrofuryl, tetrahydropyranyl, azetidinyl, pyrrolidinyl or piperidinyl ring.

In another embodiment Ring A is a cyclopropyl, cyclobutyl, cylopentyl, tetrahydropyranyl or piperidinyl ring.

In another embodiment Ring A is a cyclopropyl, cylopentyl, tetrahydropyranyl or piperidinyl ring.

In another embodiment Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring.

In another embodiment Ring A is a cyclopropyl or tetrahydropyranyl ring.

In another embodiment Ring A is a piperidinyl ring.

In another embodiment Ring A is a tetrahydropyranyl ring.

In another embodiment Ring A is a cyclopropyl ring.

R^6

In one embodiment **R^6** is hydrogen.

R^7

In one embodiment **R^7** is hydrogen or methyl.

In another embodiment **R^7** is methyl.

In another embodiment **R^7** is hydrogen.

R^8

In one embodiment **R^{12}** is methyl.

In one embodiment of compounds of formula (I), or a pharmaceutically acceptable salt thereof:

R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl,;

n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is selected from $-NHR^7$ and $-NHCOR^8$;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and **R^5** together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 heterocyclic ring containing one heteroatom selected from O and N;

R^6 is hydrogen;

R^7 is hydrogen or methyl; and

R^8 is methyl.

In another embodiment:

R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is selected from $-NH_2$, –NHMe and –NHCOMe;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and R^5 together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 heterocyclic ring containing one heteroatom selected from O and N; and

R^6 is hydrogen.

In another embodiment:

R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

n is 0 or 1;

$\mathbf{R^{2A}}$ is hydrogen;

$\mathbf{R^{2B}}$ is hydrogen;

$\mathbf{R^{2C}}$ is hydrogen;

$\mathbf{R^{2D}}$ is hydrogen;

$\mathbf{R^{2E}}$ is hydrogen;

$\mathbf{R^{2F}}$ is hydrogen;

$\mathbf{R^{2G}}$ is selected from $-NHR^7$ and $-NHCOR^8$;

$\mathbf{R^{2H}}$ is fluoro;

$\mathbf{R^3}$ is methyl;

$\mathbf{R^4}$ and $\mathbf{R^5}$ together with the atom to which they are attached form Ring A;

Ring A is a cyclopropyl, cyclobutyl, cyclopentyl, oxetanyl, tetrahydrofuryl, tetrahydropyranyl, azetidinyl, pyrrolidinyl or piperidinyl ring;

$\mathbf{R^6}$ is hydrogen;

$\mathbf{R^7}$ is hydrogen or methyl; and

$\mathbf{R^8}$ is methyl.

In another embodiment:

$\mathbf{R^1}$ is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

n is 0 or 1;

$\mathbf{R^{2A}}$ is hydrogen;

$\mathbf{R^{2B}}$ is hydrogen;

$\mathbf{R^{2C}}$ is hydrogen;

$\mathbf{R^{2D}}$ is hydrogen;

$\mathbf{R^{2E}}$ is hydrogen;

R^{2F} is hydrogen;

R^{2G} is selected from $-NH_2$, $-NHMe$ and $-NHCOMe$;

R^{2H} is fluoro;

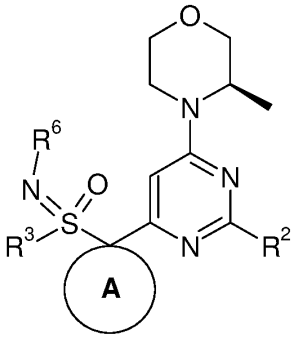
R^3 is methyl;

R^4 and R^5 together with the atom to which they are attached form Ring A;

Ring A is a cyclopropyl, cyclobutyl, cyclopentyl, oxetanyl, tetrahydrofuryl, tetrahydropyranyl, azetidinyl, pyrrolidinyl or piperidinyl ring; and

R^6 is hydrogen.

In another embodiment, compounds of formula (I) are compounds of formula (Ia):

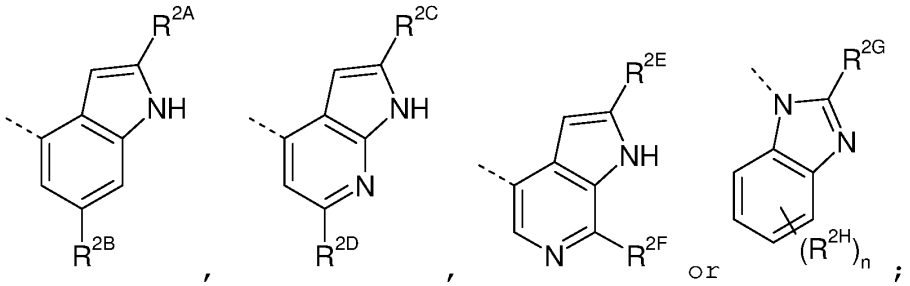


(Ia)

or a pharmaceutically acceptable salt thereof, in which:

Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring;

R^2 is



n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is selected from $-NHR^7$ and $-NHCOR^8$;

R^{2H} is fluoro;

R^3 is a methyl group;

R^6 is hydrogen;

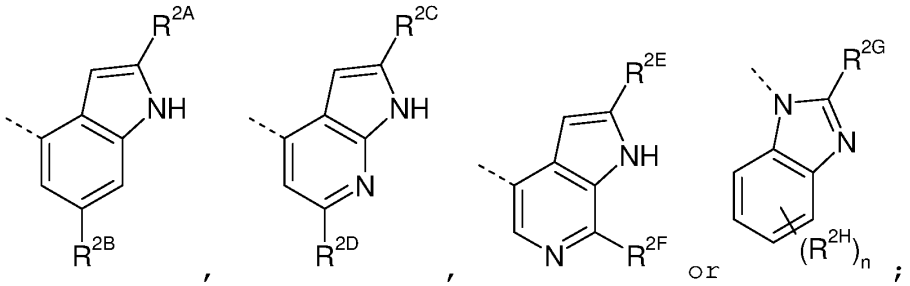
R^7 is hydrogen or methyl; and

R^8 is methyl.

In another embodiment, compounds of formula (I) are compounds of formula (Ia) or a pharmaceutically acceptable salt thereof, in which:

Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring;

R^2 is



n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is selected from $-NH_2$, –NHMe and –NHCOMe;

R^{2H} is fluoro;

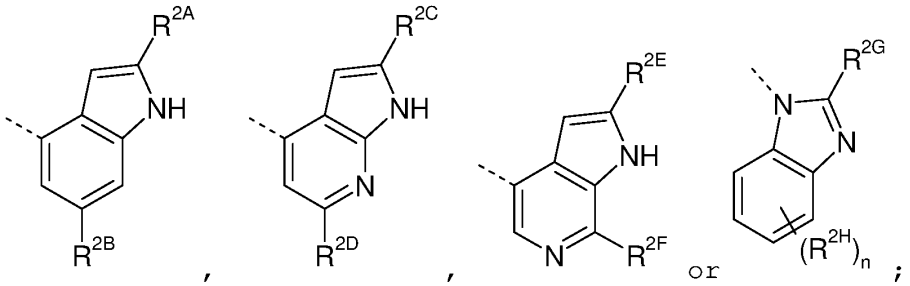
R^{3} is a methyl group; and

R^{6} is hydrogen.

In another embodiment, compounds of formula (I) are compounds of formula (Ia) or a pharmaceutically acceptable salt thereof, in which:

Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring;

R^{2} is



, , , or ;

n is 0 or 1;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is $-NHR^7$;

R^{2H} is fluoro;

R^3 is a methyl group;

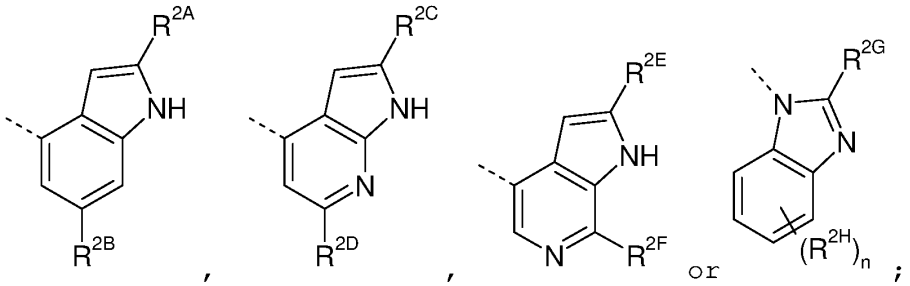
R^6 is hydrogen; and

R^7 is hydrogen.

In another embodiment, compounds of formula (I) are compounds of formula (Ia) or a pharmaceutically acceptable salt thereof, in which:

Ring A is a cyclopropyl ring;

R^2 is



n is 0;

R^{2A} is hydrogen;

R^{2B} is hydrogen;

R^{2C} is hydrogen;

R^{2D} is hydrogen;

R^{2E} is hydrogen;

R^{2F} is hydrogen;

R^{2G} is $-NHR^7$;

R^{2H} is fluoro;

R^3 is a methyl group;

R^6 is hydrogen; and

R^7 is methyl.

In other embodiments, the ATR inhibitor used in the disclosure is a compound selected from:

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[((R)-S-methylsulfonimidoyl)methyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;

N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-indole;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-indole;

1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-(S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-c]pyridine;

N-methyl-1-{4-[1-methyl-1-((S)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

N-methyl-1-{4-[1-methyl-1-((R)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[4-((S)-S-methylsulfonimidoyl)tetrahydro-2H-pyran-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[4-((R)-S-methylsulfonimidoyl)tetrahydro-2H-pyran-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[4-((S)-S-methylsulfonimidoyl)tetrahydro-2H-pyran-4-yl]pyrimidin-2-yl}-1H-indole;

4-fluoro-N-methyl-1-{4-[1-methyl-1-((S)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

4-fluoro-N-methyl-1-{4-[1-methyl-1-((R)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

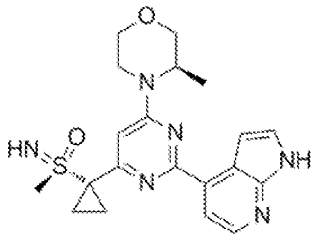
6-fluoro-N-methyl-1-{4-[1-methyl-1-((R)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;

5-fluoro-N-methyl-1-{4-[1-methyl-1-((R)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
5-fluoro-N-methyl-1-{4-[1-methyl-1-((S)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
6-fluoro-N-methyl-1-{4-[1-methyl-1-((S)-S-methylsulfonimidoyl)ethyl]-6-[(3R)-3-methylmorpholin-4-yl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
6-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
5-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
5-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
6-fluoro-N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine,
and pharmaceutically acceptable salts thereof.

In other embodiments, the ATR inhibitor used in the disclosure is a compound selected from:
4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[(R)-(S-methylsulfonimidoyl)methyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;

4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((S)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;
4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine;
N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-(R)-(S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine;
N-methyl-1-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-(S)-(S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-benzimidazol-2-amine,
and pharmaceutically acceptable salts thereof.

In a preferred embodiment the ATR inhibitor used in the disclosure is the compound AZD6738, 4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine, represented by the following formula:

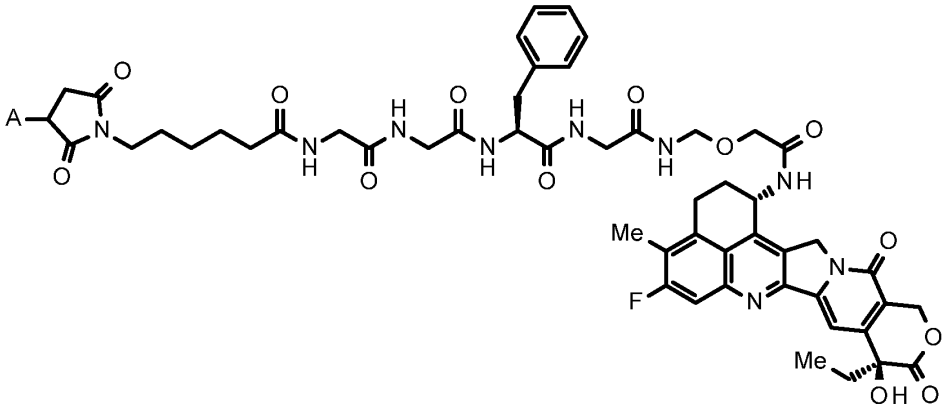


or a pharmaceutically acceptable salt thereof.

ATR inhibitors such as compounds of formula (I), including AZD6738, may be prepared by methods known in the art such as disclosed in WO2011/154737.

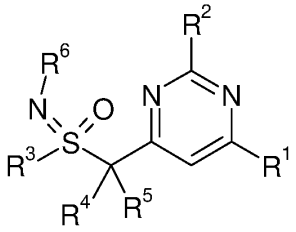
5. Combination of antibody-drug conjugate and ATR inhibitor

In a first combination embodiment of the disclosure, the anti-HER2 antibody-drug conjugate which is combined with the ATR inhibitor 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 an anti-HER2 antibody via a thioether bond.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above for the first combination embodiment is combined with an ATR inhibitor which is a compound represented by the following formula (I):

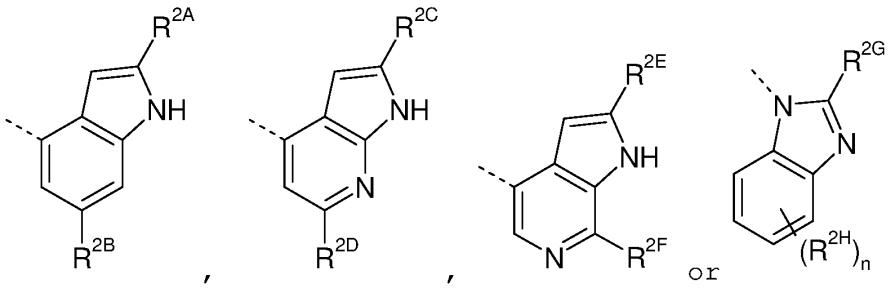


(I)

wherein:

R^1 is selected from morpholin-4-yl and 3-methylmorpholin-4-yl;

R^2 is



;

n is 0 or 1;

R^{2A}, **R^{2C}**, **R^{2E}** and **R^{2F}** each independently are hydrogen or methyl;

R^{2B} and **R^{2D}** each independently are hydrogen or methyl;

R^{2G} is selected from $-NHR^7$ and $-NHCOR^8$;

R^{2H} is fluoro;

R^3 is methyl;

R^4 and **R^5** are each independently hydrogen or methyl, or **R^4** and **R^5** together with the atom to which they are attached form Ring A;

Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 membered heterocyclic ring containing one heteroatom selected from O and N;

$\mathbf{R^6}$ is hydrogen;

$\mathbf{R^7}$ is hydrogen or methyl;

$\mathbf{R^8}$ is methyl,

or a pharmaceutically acceptable salt thereof.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor which is a compound represented by formula (I) as defined above wherein, in formula (I), $\mathbf{R^4}$ and $\mathbf{R^5}$ together with the atom to which they are attached form Ring A, and Ring A is a C_{3-6}cycloalkyl or a saturated 4–6 heterocyclic ring containing one heteroatom selected from O and N.

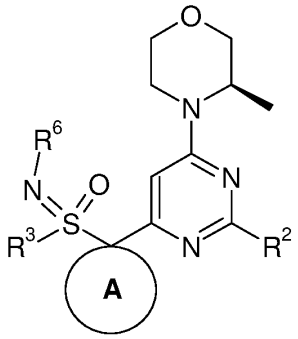
In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor as defined above wherein, in formula (I), $\mathbf{R^4}$ and $\mathbf{R^5}$ together with the atom to which they are attached form Ring A, and Ring A is a cyclopropyl, tetrahydropyranyl or piperidinyl ring.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor as defined above wherein, in formula (I), $\mathbf{R^{2A}}$ is hydrogen; $\mathbf{R^{2B}}$ is hydrogen; $\mathbf{R^{2C}}$ is hydrogen; $\mathbf{R^{2D}}$ is hydrogen; $\mathbf{R^{2E}}$ is hydrogen; and $\mathbf{R^{2F}}$ is hydrogen.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with

an ATR inhibitor as defined above wherein, in formula (I), R^1 is 3-methylmorpholin-4-yl.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor as defined above, wherein the compound of formula (I) is a compound of formula (Ia):



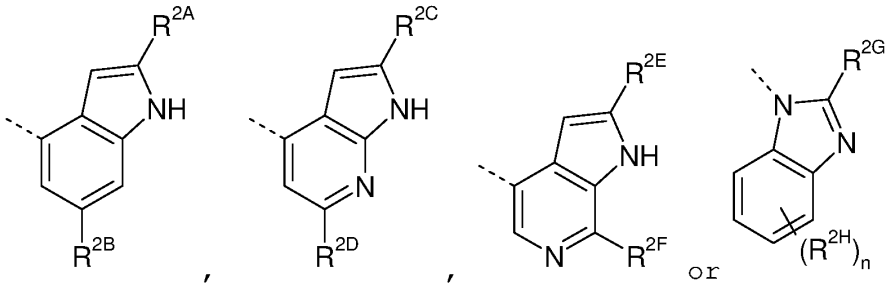
(Ia)

or a pharmaceutically acceptable salt thereof.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor as defined above wherein the compound of formula (I) is a compound of formula (Ia) wherein, in formula (Ia):

Ring A is cyclopropyl ring;

R^2 is



n is 0 or 1;

$\mathbf{R^{2A}}$ is hydrogen;

$\mathbf{R^{2B}}$ is hydrogen;

$\mathbf{R^{2C}}$ is hydrogen;

$\mathbf{R^{2D}}$ is hydrogen;

$\mathbf{R^{2E}}$ is hydrogen;

$\mathbf{R^{2F}}$ is hydrogen;

$\mathbf{R^{2G}}$ is $-NHR^7$;

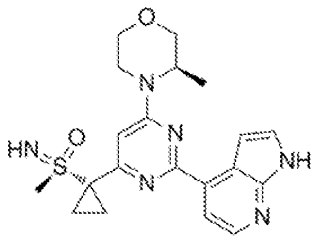
$\mathbf{R^{2H}}$ is fluoro;

$\mathbf{R^3}$ is a methyl group;

$\mathbf{R^6}$ is hydrogen; and

$\mathbf{R^7}$ is hydrogen or methyl.

In another combination embodiment, the anti-HER2 antibody-drug conjugate as defined above is combined with an ATR inhibitor as defined above, wherein the ATR inhibitor is AZD6738 represented by the following formula:

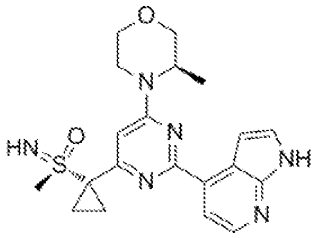


or a pharmaceutically acceptable salt thereof.

In an embodiment of each of the combination embodiments described above, the anti-HER2 antibody comprises a heavy chain comprising CDRH1 consisting of an amino acid sequence represented by SEQ ID NO: 3, CDRH2

consisting of an amino acid sequence represented by SEQ ID NO: 4 and CDRH3 consisting of an amino acid sequence represented by SEQ ID NO: 5, and a light chain comprising CDRL1 consisting of an amino acid sequence represented by SEQ ID NO: 6, CDRL2 consisting of an amino acid sequence consisting of amino acid residues 1 to 3 of SEQ ID NO: 7 and CDRL3 consisting of an amino acid sequence represented by SEQ ID NO: 8. In another embodiment of each of the combination embodiments described above, the anti-HER2 antibody comprises a heavy chain comprising a heavy chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 9 and a light chain comprising a light chain variable region consisting of an amino acid sequence represented by SEQ ID NO: 10. In another embodiment of each of the combination embodiments described above, the anti-HER2 antibody comprises a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 1 and a light chain consisting of an amino acid sequence represented by SEQ ID NO: 2. In another embodiment of each of the combination embodiments described above, the anti-HER2 antibody comprises a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 11 and a light chain consisting of an amino acid sequence represented by SEQ ID NO: 2.

In a particularly preferred combination embodiment of the disclosure, the anti-HER2 antibody-drug conjugate is trastuzumab deruxtecan (DS-8201) and the ATR inhibitor is the compound represented by the following formula:



also identified as AZD6738.

6. Therapeutic combined use and method

Described in the following are a pharmaceutical product and a therapeutic use and method wherein the anti-HER2 antibody-drug conjugate according to the present disclosure and an ATR inhibitor are administered in combination.

The pharmaceutical product and therapeutic use and method of the present disclosure may be characterized in that the anti-HER2 antibody-drug conjugate and the ATR 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 ATR inhibitor are contained as active components in a single formulation and administered.

In the pharmaceutical product and therapeutic method of the present disclosure, a single ATR inhibitor used in the present disclosure can be administered in combination with the anti-HER2 antibody-drug conjugate, or two or more different ATR inhibitors can be administered in combination with the antibody-drug conjugate.

The pharmaceutical product and therapeutic method of the present disclosure can be used for treating cancer, and can be preferably used for treating at least one cancer selected from the group consisting of breast cancer (including triple negative breast cancer and luminal breast cancer), gastric cancer (also called gastric adenocarcinoma), colorectal cancer (also called colon and rectal cancer, and including colon cancer and rectal cancer), lung cancer (including small cell lung cancer and non-small cell lung cancer), esophageal cancer, head-and-neck cancer (including salivary gland cancer and pharyngeal cancer), esophagogastric junction adenocarcinoma, biliary tract cancer (including bile duct cancer), Paget's disease, pancreatic cancer, ovarian cancer, uterine carcinosarcoma, urothelial cancer, prostate cancer, bladder cancer, gastrointestinal stromal tumor, uterine cervix cancer, squamous cell carcinoma, peritoneal cancer, liver cancer, hepatocellular cancer, corpus uteri carcinoma, kidney cancer, vulval cancer, thyroid cancer, penis cancer, leukemia, malignant lymphoma, plasmacytoma, myeloma, glioma, glioblastoma multiforme, osteosarcoma, sarcoma, and melanoma, and can be more preferably used for treating at least one cancer selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, lung cancer (preferably non-small cell lung cancer), pancreatic cancer, ovarian cancer, prostate cancer, and kidney cancer.

The presence or absence of HER2 tumor markers can be determined, for example, by collecting tumor tissue from a cancer patient to prepare a formalin-fixed, paraffin-embedded (FFPE) specimen and subjecting the specimen to a test for gene products (proteins), for example, with an immunohistochemical (IHC) method, a flow cytometer, or Western blotting, or to a test for gene transcription, for example, with an in situ hybridization (ISH) method, a quantitative PCR method (q-PCR), or microarray analysis, or by collecting cell-free circulating tumor DNA (ctDNA) from a cancer patient and subjecting the ctDNA to a test with a method such as next-generation sequencing (NGS).

The pharmaceutical product and therapeutic method of the present disclosure can be used for HER2-expressing cancer, which may be HER2-overexpressing cancer (high or moderate) or may be HER2 low-expressing cancer.

In the present disclosure, the term "HER2-overexpressing cancer" is not particularly limited as long as it is recognized as HER2-overexpressing cancer by those skilled in the art. Preferred examples of the HER2-overexpressing cancer can include cancer given a score of 3+ for the expression of HER2 in an IHC method, and cancer given a score of 2+ for the expression of HER2 in an IHC method and determined as positive for the expression of HER2 in an in situ hybridization method (ISH). The in situ hybridization method of the present disclosure includes a fluorescence in situ hybridization

method (FISH) and a dual color in situ hybridization method (DISH).

In the present disclosure, the term "HER2 low-expressing cancer" is not particularly limited as long as it is recognized as HER2 low-expressing cancer by those skilled in the art. Preferred examples of the HER2 low-expressing cancer can include cancer given a score of 2+ for the expression of HER2 in an IHC method and determined as negative for the expression of HER2 in an in situ hybridization method, and cancer given a score of 1+ for the expression of HER2 in an IHC method.

The method for scoring the degree of HER2 expression by the IHC method, or the method for determining positivity or negativity to HER2 expression by the in situ hybridization method is not particularly limited as long as it is recognized by those skilled in the art. Examples of the method can include a method described in the 4th edition of the guidelines for HER2 testing, breast cancer (developed by the Japanese Pathology Board for Optimal Use of HER2 for Breast Cancer).

The cancer, particularly in regard to the treatment of breast cancer, may be HER2-overexpressing (high or moderate) or low-expressing breast cancer, or triple-negative breast cancer, and/or may have a HER2 status score of IHC 3+, IHC 2+, IHC 1+ or IHC >0 and <1+.

The pharmaceutical product and therapeutic method of the present disclosure can be preferably used for a mammal, but are more preferably used for a human.

The antitumor effect of the pharmaceutical product and therapeutic method of the present disclosure can be confirmed by transplanting cancer cells to a test subject animal to prepare a model and measuring reduction in tumor volume or life-prolonging effect by application of the pharmaceutical product and therapeutic method of the present disclosure. And then, the effect of combined use of the antibody-drug conjugate used in the present disclosure and an ATR inhibitor can be confirmed by comparing antitumor effect with single administration of the antibody-drug conjugate used in the present disclosure and that of the ATR inhibitor.

The antitumor effect of the pharmaceutical product and therapeutic method of the present disclosure can be confirmed in a clinical trial using any of an evaluation method with Response Evaluation Criteria in Solid Tumors (RECIST), a WHO evaluation method, a Macdonald evaluation method, body weight measurement, and other approaches, and can be determined on the basis of indexes of complete response (CR), partial response (PR); progressive disease (PD), objective response rate (ORR), duration of response (DoR), progression-free survival (PFS), overall survival (OS), and so on.

By using the above methods, the superiority in antitumor effect of the pharmaceutical product and therapeutic method of the present disclosure to existing pharmaceutical products and therapeutic methods for cancer treatment can be confirmed.

The pharmaceutical product and therapeutic method of the present disclosure 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 quality of life (QOL) of cancer patients and attain a therapeutic effect by sustaining the lives of the cancer patients. Even if the pharmaceutical product and therapeutic method of the present disclosure 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.

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

The pharmaceutical product of the present disclosure can be administered containing at least one pharmaceutically suitable ingredient. Pharmaceutically suitable ingredients can be suitably selected and applied from formulation additives or the like that are generally used in the art, in accordance with the dosage, administration concentration, or the like of the antibody-drug conjugate used in the present disclosure and an ATR inhibitor. The anti-HER2 antibody-drug conjugate used in the present disclosure can be administered, for example, as a pharmaceutical product

containing a buffer such as histidine buffer, a vehicle such as sucrose and trehalose, and a surfactant such as Polysorbates 80 and 20. The pharmaceutical product containing the antibody-drug conjugate used in the present disclosure can be preferably used as an injection, can be more preferably used as an aqueous injection or a lyophilized injection, and can be even more preferably used as a lyophilized injection.

In the case that the pharmaceutical product containing the anti-HER2 antibody-drug conjugate used in the present disclosure is an aqueous injection, the aqueous injection can be preferably diluted with a suitable diluent and then given as an intravenous infusion. Examples of the diluent can include dextrose solution and physiological saline, dextrose solution can be preferably exemplified, and 5% dextrose solution can be more preferably exemplified.

In the case that the pharmaceutical product of the present disclosure is a lyophilized injection, a required amount of the lyophilized injection dissolved in advance in water for injection can be preferably diluted with a suitable diluent and then given as an intravenous infusion. Examples of the diluent can include dextrose solution and physiological saline, dextrose solution can be preferably exemplified, and 5% dextrose solution can be more preferably exemplified.

Examples of the administration route applicable to administration of the pharmaceutical product of the

present disclosure can include intravenous, intradermal, subcutaneous, intramuscular, and intraperitoneal routes, and intravenous routes are preferred.

The anti-HER2 antibody-drug conjugate used in the present disclosure can be administered to a human with intervals of 1 to 180 days, can be preferably administered with intervals of a week, two weeks, three weeks, or four weeks, and can be more preferably administered with intervals of three weeks. The anti-HER2 antibody-drug conjugate used in the present disclosure can be administered in a dose of about 0.001 to 100 mg/kg per administration, and can be preferably administered in a dose of 0.8 to 12.4 mg/kg per administration. For example, the anti-HER2 antibody-drug conjugate can be administered once every three weeks at a dose of 0.8 mg/kg, 1.6 mg/kg, 3.2 mg/kg, 5.4 mg/kg, 6.4 mg/kg, 7.4 mg/kg, or 8 mg/kg, and can be preferably administered once every three weeks at a dose of 5.4 mg/kg or 6.4 mg/kg.

For example, a formulation of an ATR inhibitor compound of formula (I) intended for oral administration to humans will generally contain, for example, from 1 mg to 1000 mg of the active ingredient, compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. For further information on Routes of Administration and Dosage Regimes, reference may be made to Chapter 25.3 in Volume 5 of Comprehensive Medicinal

Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose required for the therapeutic treatment of a particular disease state will necessarily be varied depending on the subject treated, the route of administration and the severity of the illness being treated. A daily dose of the ATR inhibitor in the range of 0.1–50 mg/kg may be employed. For example, in the case that the ATR inhibitor used in the present disclosure is the compound AZD6738 or a pharmaceutically acceptable salt thereof, the ATR inhibitor can be preferably orally administered twice per day in a dose of 20 mg, 40 mg, 60 mg, 80 mg, 120 mg, 160 mg, 200 mg or 240 mg per administration.

The pharmaceutical product and therapeutic method of the present disclosure can be used as adjuvant chemotherapy combined with surgery operation. The pharmaceutical product of the present disclosure may be administered for the purpose of reducing tumor size before surgical operation (referred to as preoperative adjuvant chemotherapy or neoadjuvant therapy), or may be administered for the purpose of preventing recurrence of tumor after surgical operation (referred to as postoperative adjuvant chemotherapy or adjuvant therapy).

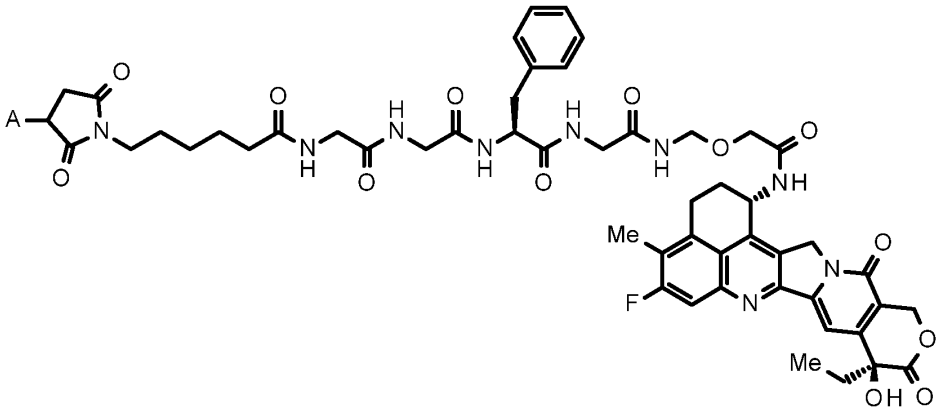
[Examples]

The present disclosure is specifically described in view of the examples shown below. However, the present

disclosure is not limited to these. Further, it is by no means to be interpreted in a limited way.

Example 1: Production of antibody-drug conjugate

In accordance with a production method described in WO2015/115091 and using an anti-HER2 antibody (an antibody comprising a heavy chain consisting of an amino acid sequence represented by SEQ ID NO: 11 (amino acid residues 1 to 449 of SEQ ID NO: 1) and a light chain consisting of an amino acid sequence consisting of all amino acid residues 1 to 214 of SEQ ID NO: 2), an anti-HER2 antibody-drug conjugate in which a drug-linker represented by the following formula:

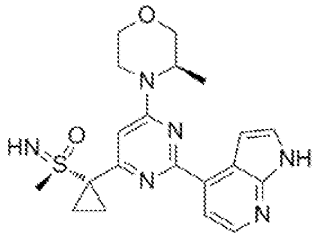


wherein A represents the connecting position to an antibody,

is conjugated to the anti-HER2 antibody via a thioether bond was produced (DS-8201: trastuzumab deruxtecan). The DAR of the antibody-drug conjugate is 7.7 or 7.8.

Example 2: Production of ATR inhibitor

In accordance with a production method described in WO2011/154737), an ATR inhibitor of formula (I) is prepared. Specifically, 4-{4-[(3R)-3-methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine:



(AZD6738)

can be prepared according to Example 2.02 of WO2011/154737.

Example 3: Antitumor test (1)

Combination of antibody-drug conjugate DS-8201 (trastuzumab deruxtecan) with ATR inhibitor AZD6738 (4-{4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine)

Method:

A high-throughput combination screen was run, in which breast cancer cell lines with diverse HER2 expression and one gastric cell line with high HER2 expression (Table 1)

were treated with combinations of DS-8201 and AZD6738 (ATR inhibitor).

Table 1

Cell line	HER2 expression	Cancer type
KPL4	High	Breast (HER2 +)
NCI-N87	High	Gastric
MDA-MB-468	Low	Breast (TNB)
HCC1937	Low	Breast (TNB)
HCC1954	High	Breast
HCC38	Amp/Low	Breast
T47D	Low	Breast (ER+)

The readout of the screen was a 7-day cell titer-glo cell viability assay, conducted as a 6 x 6 dose response matrix for each combination (5-point log serial dilution for DS-8201, and half log serial dilution for partners). In addition, trastuzumab and exatecan (DNA topoisomerase I inhibitor) were also screened in parallel with AZD6738. Combination activity was assessed based on a combination of the ΔEmax and HSA synergy scores.

Results:

Results are shown in Figures 12A to 12D and Table 2.

Figures 12A and 12B show matrices of measured cell viability signals. X axes represent drug A (DS-8201), and

Y axes represent drug B (AZD6738). Values in the box represent the ratio of cells treated with drug A + B compared to DMSO control at day 7. All values are normalised to cell viability values at day 0. Values between 0 and 100 represent % growth inhibition and values above 100 represent cell death.

Figures 12C and 12D show HSA excess matrices. Values in the box represent excess values calculated by the HSA (Highest Single Agent) model.

Table 2 below shows HSA synergy and Loewe additivity scores:

Table 2

Cell line	KPL4	NCI-N87	MDA-MB-468	HCC1937	HCC1954	HCC38	T47D
HSA synergy score	42.0	45.1	7.2	6.5	30.4	9.3	2.7
Loewe synergy score	41.0	44.0	7.2	6.5	28.7	7.5	2.7

Loewe Dose Additivity predicts the expected response if the two compounds act on the same molecular target by means of the same mechanism. It calculates additivity based on the assumption of zero interaction between the compounds and it is independent from the nature of the dose-response relationship.

HSA (Highest Single Agent) [Berenbaum 1989] quantifies the higher of the two single compound effects at their corresponding concentrations. The combined effect is compared with the effect of each single agent at the concentration used in the combination. Excess over the highest single agent effect indicates cooperativity. HSA does not require the compounds to affect the same target.

Excess Matrix: For each well in the concentration matrix, the measured or fitted values are compared to the predicted non-synergistic values for each concentration pair. The predicted values are determined by the chosen model. Differences between the predicted and observed values may indicate synergy or antagonism, and are shown in the Excess Matrix. Excess Matrix values are summarized by the combination scores Excess Volume and Synergy Score.

As seen from Figures 12A to 12D and Table 2, AZD6738 (AZ13386215) interacted synergistically with DS-8201 and also increased cell death in HER2 + cell lines NCI-N87, KPL4, and HCC1954 at Emax (3 µM AZD6738 and 10 µg/ml (0.064 µM) DS-8201). Combination activity was also observed at lower concentrations where single agent activity was low. The AZD6738 and DS-8201 combination was also active in HER2 low HCC1937, HCC38 and MDA-MB-468

cell lines. Combination benefit was also observed at Emax in HER2 low ER+ cell line T47D.

The results demonstrate that ATR inhibition using AZD6738 enhances the antitumor efficacy of DS-8201 in both high and low HER2-expressing cell lines in vitro. AZD6738 showed synergistic combination activity and increased cell death in HER2 high cell lines. Beneficial combination activity was also observed in HER2 low cancer cell lines.

Example 4: Antitumor test (2)

Combination of antibody-drug conjugate DS-8201 (trastuzumab deruxtecan) with ATR inhibitor AZD6738 (4-{4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine)

DS-8201 or exatecan mesylate was tested alone and in combination with AZD6738 in cancer cell lines with varying HER2 expression levels.

Method:

Cells grown in their respective conditions were plated in 96-well plates at optimal density to allow linear proliferation for the duration of the assay (4 to 8 days; duration of treatment is dependent on the growth rate of each cell line). Immediately after plating, the cells

were dosed with the indicated compounds for a total volume of 200 µL/well and placed in the incubator. Combinations were conducted as a 6 x 8 concentration response matrix for each combination. At the endpoint, the cells were fixed in 2% PFA for 20 minutes at room temperature. In order to obtain the number of cells at the start of treatment, one additional plate was used for each experiment and fixed after cells attached. The cells were then permeabilised in 0.5% Triton-X100 in PBS for 10 minutes. After a PBS wash, the cells were blocked in 5% FBS in PBS 1h at RT and incubated with primary antibodies in 5% FBS + 0.05% triton overnight at 4°C. After 3 washes in PBS cells were incubated with secondary antibodies in 5% FBS + 0.05% triton with Hoechst33258 for 1h at room temperature. After 3 washes in PBS, the cells were scanned with a Cellinsight instrument with a 10x objective and 9 fields/well. Images were analysed using Columbus for cell count based on nuclear Hoechst staining and nuclear intensity of other biomarkers investigated. The total cell count/well was used to calculate the relative growth in each well compared to solvent control. To calculate the synergy scores, the growth inhibition data were analysed using Combenefit software (Di Veroli GY *et al.*, Bioinformatics 2016, 32(18), 2866-8). The mean/well of the sum of the nuclear intensity of the IF biomarkers was also expressed relative to solvent control.

Results:

Results are shown in Figures 13 to 15 and Tables 3 and 4.

Table 3 below shows the monotherapy activities of DS-8201, exatecan and AZD6738 ATR for cell lines used in the *in vitro* studies:

Table 3

Cell line	Tumour type	HER2 expression	DS-8201 GI_{50} (ng/ml)	Exatecan mesylate GI_{50} (nM)	AZD6738 GI_{50} (nM)
NCI-N87	Gastric	High	19	0.503	436
KPL4	Breast	High	44	1.181	>3000
MDA-MB-468	Breast	Low	3367	0.162	2066
SK-OV-3	Ovarian	Moderate	4674	0.914	1012
JIMT-1	Breast	Expressed	22609	0.745	788
DU145	Prostate	Low	18361	0.564	Nd
DU145-SLFN11 KO (CRISPR-Cas9 gene knockout of SLFN11)	Prostate	Low	17080	0.450	Nd

*Nd: not determined

Figure 13 shows synergy matrices for combinations with DS-8201 and AZD6738 (ATR inhibitor) in HER2-high KPL4 cell line.

In Figure 13, (A) shows the relative total cell (nuclear) counts as percentage of DMSO vehicle control (control = 100%, no cells remaining = 0%; dark areas are regions with very low total cell count), and (B) shows synergy

matrices for Loewe, Bliss and HSA scores (higher = more synergy; dark areas are regions with high combination synergy).

Table 4 below shows the overall sum of synergy scores (Loewe, Bliss and HSA) for DS-8201 in combination with AZD6738:

Table 4

Cell line	DS-8201 +AZD6738 Loewe	DS-8201 +AZD6738 Bliss	DS-8201 +AZD6738 HSA
KPL4	134.64	136.77	152.08
MDA-MB-468	3.17	20.81	33.94
SK-OV-3	25.95	28.15	46.21
JIMT-1	23.33	26.03	32.23

Figure 14 shows fold change in total cells remaining after 4-8 days treatment compared to time zero for combinations of DS-8201 with AZD6738 in (A) HER2-high KPL4 cell line and (B) HER2-negative MDA-MB-468 cell line. Positive values indicate growth (fold increase), zero value indicates cytostasis and negative values represent net cell loss and surrogate for cell death. Boxed areas show regions of cytostasis or cell loss of combination compared to monotherapies.

Figure 15 shows induction of ATM-dependent KAP1 pSer824 signalling, DNA double strand break damage (γH2AX) biomarkers or percentage of cell number (vs solvent control) for combinations of DS-8201 with AZD6738 in (A) HER2-high KPL4 cell line or (B) HER2-low MDA-MB-468 cell

line. Boxed areas show regions of increased induction of DNA damage response, DNA damage or cell loss of combination compared to monotherapies.

According to the results above, in the high-HER2 KPL4 breast cancer cell line models, synergistic activity and cell death were observed at clinically relevant concentrations of DS-8201 (and exatecan) in combination with ATR inhibitor AZD6738. In addition DS-8201 (and exatecan) induced in a concentration dependent manner biomarkers of ATM (KAP1 pSer824) activation and DNA strand breaks (γH2AX), which was further augmented in combination with AZD6738. In the HER2-negative MDA-MB-468 breast cancer cell line, weak combination activity and poor DNA damage response pathway activation were observed in combination DS-8201, while exatecan still showed combination activity, supporting the HER2 and tumor targeting dependency of DS-8201 but not with free exatecan. These data show strong potentiation of the activity with DS-8201 when combined with ATR inhibitor AZD6738 which is dependent on tumor HER2 expression and therefore may offer an increased therapeutic index compared to free topoisomerase-I inhibitors.

Example 5: Antitumor test (3) - in vivo

Combination of antibody-drug conjugate DS-8201 (trastuzumab deruxtecan) with ATR inhibitor AZD6738 (4-{4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-((R)-S-

methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine)

Method:

Female Nude mice (Charles River) aged 5-8 weeks were used, following 7 days acclimatisation before entry into the study. $1x10^7$ NCI-N87 tumor cells (1:1 in Matrigel) were implanted subcutaneously onto the flank of the female Nude mice. When tumors reached approximately 150 mm^3, similar-sized tumors were randomly assigned to treatment groups as shown in Table 5:

Table 5

Treatment	Dose	Route of administration	Dosing Schedule (28 days)
Vehicle	----	IV + PO	Single dose + BID
DS-8201	3 mg/kg	IV	Single dose
DS-8201	1 mg/kg	IV	Single dose
AZD6738	25 mg/kg	PO	BID (14d-on/14-d off)
DS-8201 + AZD6738	1 mg/kg or 3mg/kg + 25 mg/kg	IV + PO	Single dose + BID (14d-on/14-d off)

The dose of compound for each animal was calculated based on the individual body weight on the day of dosing. BID (twice daily) dosing was administered 8 hours apart. DS-8201 and AZD6738 were dosed on the same day, with DS-8201 being administered approximately 1 hour post the AM PO dose of AZD6738. Any animals receiving AZD6738 treatment had a wet diet 24 hours prior to dosing until

the end of the dosing period. Duration of dosing was for 28 days (1 cycle) unless otherwise stated.

Formulation of DS-8201 at 3 mg/kg and 1 mg/kg

The dosing solutions for DS-8201 were prepared on the day of dosing by diluting the DS-8201 stock (20.1 mg/ml) in 25 mM histidine buffer, 9% sucrose (pH5.5) to 0.6 mg/ml, and 0.2 mg/ml for the 3mg/kg and 1 mg/kg dosing solutions, respectively. Each dosing solution was mixed well using a pipette before administration via IV injection at a dosing volume of 5 ml/kg.

Formulation of AZD6738 at 25 mg/kg

To formulate for a 25 mg/kg dosing solution, a concentration of 2.5 mg/ml AZD6738 was prepared which resulted in a dosing volume of 10 ml/kg for PO dosing. DMSO (10% of the total vehicle volume) was added to the compound and mixed well with a pellet pestle. Sonication for approximately 5 minutes was required to fully dissolve the compound. Following which, Propylene Glycol (40% of the total vehicle volume) was added and mixed well using a magnetic stirrer. A volume of 10 ml of sterile water was added to the glass wheaton vial to rinse any remaining compound from the vial then transferred to the glass bottle. The remaining volume of sterile water in total (50% of the final vehicle volume) was added to the glass bottle and mixed well using a magnetic stirrer. The dosing solution was protected from

light and kept at room temperature for up to 7 days being continually mixed. The final dosing matrix for 25 mg/kg AZD6738 was a clear solution with a faint yellow hue.

Measurements

Tumor growth inhibition (TGI) from the start of the study to the day of tumor measurements was assessed by comparison of the geometric mean change in tumor volume for the control and treated groups. Tumor regressions were calculated as the percentage reduction in tumor volume from baseline (pre-treatment) value:

% Regression = (1 - RTV)*100%,

where RTV = Geometric Mean Relative Tumor Volume. Statistical significance was evaluated using one-tailed t-test of (log(relative tumor volume) = log(final vol / start vol)) at the day of final measure, comparing to vehicle control.

Results:

Tumor volumes for treatments with DS-8201 and/or AZD6738 are shown in Figure 16. Data represents change in tumor volume over time for treatment groups. The dotted line in Figure 16 represents end of dosing periods. For full dose and schedule information, refer to Table 5 above. Values shown are mean ±SEM; n=10 initially for vehicle-treated mice and n=8 for all other treatment groups.

TGI best responses (maximum TGI/regression) following treatment with DS-8201 or AZD6738 alone or with DS-8201 in combination with AZD6738, in NCI-N87 xenograft, are shown in Table 6:

Table 6

Treatment group	Best response %TGI/regression	Best response Days post treatment	p-value vs vehicle	Significance
DS-8201 3 mg/kg	**84**	**33**	**0.00071**	***
DS-8201 1 mg/kg	**22**	**37**	**0.025**	*
AZD6738 25 mg/kg	**62**	**40**	**<0.0001**	***
DS-8201 1 mg/kg + AZD6738 25 mg/kg	**75**	**30**	**<0.0001**	***
DS-8201 3 mg/kg + AZD6738 25 mg/kg	**120 (regression)**	**33**	**<0.0001**	***

Monotherapy with DS-8201 at 3 mg/kg showed maximum tumor growth inhibition (TGI) of 84% at day 33 post treatment. At 1 mg/kg DS-8201 showed a maximum TGI of 22% at day 37 post treatment. AZD6738 monotherapy achieved a maximum TGI of 62% at day 40 post treatment. Combination treatment with DS-8201 at 1 mg/kg resulted in a significant reduction in NCI-N87 tumor burden compared to vehicle-treated control mice, with significant effect

being observed DS-8201 1 mg/kg + AZD6738 with a maximum TGI at 75% 30 days post treatment.
Combination therapy using higher DS-8201 3 mg/kg dose with AZD6738 achieved tumor regressions with a maximum TGI of 120% at day 33 post treatment and showed better response than either respective monotherapies.
All treatment groups were tolerated and no consistent differences in average bodyweights were observed between vehicle, monotherapy or combination groups.

Example 6: Inhibition of ATR signaling

Combination of DS-8201 with ATR inhibitor AZD6738

Method

Gastric cancer NCI-N87 and breast carcinoma KPL4 cell lines were cultured in RPMI 1640 supplemented with 10 % FCS in a humidified incubator at 37°C with 5% CO_2. Cells were plated in 6-well plates at optimal density to allow linear proliferation for the duration of the assay. Two days after plating, cells were dosed with the indicated compounds (AZD6738 alone, or combined with DS-8201 or exatecan mesylate) and placed back in the incubator. 7h, 24h or 48h after dosing, whole-cell extracts were obtained by lysis in 50mM Tris-HCl pH 7.5, 2% SDS containing protease and phosphatase inhibitors. Lysates were boiled for 5 minutes at 95°C. Protein concentration was measured using a Nanodrop at 240 nm and 50 µg of lysate were loaded in 4-12% Bis Tris gels. Proteins were

transferred using iblot2. Primary antibodies (see Table 7) were incubated overnight at 4°C in 3% milk TBS-tween 0.05% and HRP-conjugated secondary antibodies for 1h at room temperature. Blots were imaged using a G-box.

Table 7

Antibody target name	Host species	Catalog number	Supplier
ATR (P-Thr1989)	rabbit	GTX128145	GeneTex
CHK1 (P-Ser345) (clone 133D3)	rabbit	2348	Cell signalling (NEB)
KAP1 (P-Ser824)	rabbit	ab70369	Abcam
Rad50 (P-Ser635)	rabbit	14223	Cell signalling (NEB)
RPA 32 (P-Ser4/Ser8)	rabbit	A300-245A	Bethyl Laboratories
γH2AX (P-Ser139) (clone JBW301)	mouse	05-636	Merck Millipore
CHK2 (P-Thr68)	rabbit	2661	Cell signalling (NEB)
ATM phosphoSer 1981 monoclonal	mouse	MAB3806	Millipore
p53 (P-Ser15) (clone 16G8)	mouse	9286	Cell signalling (NEB)
CDC2 (P-Tyr15)	rabbit	4539	Cell signalling (NEB)

Results:

Results are shown in Figure 17, in the form of antibody blot images obtained using AZD6738 alone, or combined with DS-8201 (or exatecan mesylate), in (A) NCI-N87 (gastric cancer) and (B) KPL4 (breast carcinoma) cell lines.

In both Her2-high NCI-N87 and KPL4, exposure to DS-8201 at 30 µg/mL or the warhead (exatecan mesylate) induced activation of the ATR pathway, as shown by increase in pATR-T1989 and pChk1-S345, and cell cycle arrest (pCdc2-Y15). Combination with AZD6738 at 1 µM inhibited the activation of pATR and pChk1 and cell cycle arrest, while

exacerbating the DNA damage (pKap1, gH2AX), ultimately leading to increased cell death (cCasp3).

Thus, it is shown that AZD6738 inhibits DS-8201-induced ATR signaling.

Example 7

Combination dosing of antibody-drug conjugate DS-8201 (trastuzumab deruxtecan) with ATR inhibitor AZD6738 in haematopoietic stem and progenitors cells in vitro

Method

Cryopreserved Human Bone Marrow $CD34^+$ Progenitor Cells (Lonza) were defrosted and left to recover overnight in maintenance media (StemSpan SFEM II (Stem Cell Technologies) containing 25 ng/ml SCF, 50 ng/ml TPO, and 50 ng/ml Flt3-L human recombinant protein (all Peprotech)), in a humidified incubator at 37°C with 5% CO_2. The next day cells were resuspended in the presence of drug into media capable of supporting erythroid cell differentiation (Preferred Cell Systems, SEC-BFU1-40H), myeloid cell differentiation (Preferred Cell Systems, SEC-GM1-40H), or megakaryocytic cell differentiation (Stem Cell Technologies, 09707), at a concentration of 5000 cells/ml for erythroid and myeloid cells, or 15000 cells/ml for megakaryocytic cells. Cells (100 µl) were plated into triplicate white walled, clear bottomed 96 well tissue culture plates (Corning) with the addition of

DS-8201 (0.667, 0.222, 0.074, 0.025, 0.008 and 0 µM; equivalent to 100, 33.3, 11.1, 3.7, 1.23 and 0 µg/ml respectively) in combination with ATR inhibitor AZD6738/ceralasertib (1.11, 0.37, 0.123, 0.041, 0.014, 0 µM) in a 6x6 matrix pattern. Cells were cultured for 5 days in a humidified incubator at 37°C, with 5% CO_2. Viability was determined using CellTiter-Glo 2.0 from Promega (using an optimised volume of 10 µl/well), with luminescence detected using an Envision plate reader (Perkin Elmer). Relative Luminescence signal was normalised in Genedata Screener software (Genedata) to percentage of control wells (0 µM of both compounds) with controls equalling 0 and maximum cell death equalling 100. Synergy analysis was assessed using Loewe, Bliss, and Highest Single Agent (HSA) models with synergy scores and excess matrices determined by comparing the difference between the observed viability and that predicted based on a non-synergistic interaction for each combination dose pair.

Results:

Figures 18A and 18B show matrices obtained with the combination dosings of DS-8201 with AZD6738 (ceralasertib) in primary $CD34^+$ bone marrow-derived hematopoietic stem and progenitor cells induced to differentiate into erythroid, myeloid, or megakaryocytic lineages. In Figure 18A, measured cell viability signals are shown, with the X axis representing drug A (DS-8201)

concentrations and the Y axis representing drug B (AZD6738) concentrations. Values in the boxes represent the % growth inhibition of cells treated with drug A + B, normalised to control values which equalled 0, with maximal cell death equalling 100. Figure 18B shows HSA and Loewe excess matrices, in which values in the boxes represent excess values calculated by the HSA and Loewe additivity models respectively.

Table 8 shows HSA additivity and Loewe synergy scores:

Table 8

Cell population	Erythroid	Myeloid	Megakaryocytic
HSA synergy score	0.4	1.0	0.3
Loewe synergy score	-0.3	0.2	-0.5

There was no synergistic toxicity seen with concurrent DS-8201 and AZD6738 treatment in primary $CD34^+$ bone marrow cells differentiated into any lineage, with cell death in combination occuring at monotherapy active doses and following the predicted Loewe synergy interaction.

Thus, AZD6738 did not interact synergistically with DS-8201 in primary $CD34^+$ bone marrow-derived hematopoietic stem and progenitor cells induced to differentiate into erythroid, myeloid, or megakaryocytic

lineages, suggesting that this combination may be associated with a favorable safety profile.

Example 8: Antitumor test (4)

Combination of antibody-drug conjugate DS-8201 (trastuzumab deruxtecan) with ATR inhibitor AZD6738 (4-{4-[(3R)-3-Methylmorpholin-4-yl]-6-[1-((R)-S-methylsulfonimidoyl)cyclopropyl]pyrimidin-2-yl}-1H-pyrrolo[2,3-b]pyridine)

Method:

A high-throughput combination screen was run, in which NCI-H522, a lung cancer cell line with low HER2 expression (Table 9), was treated with combinations of DS-8201 and AZD6738.

Table 9

Cell line	HER2 expression	Cancer type
NCI-H522	Low	NSCLC adenocarcinoma

The readout of the screen was a 7-day cell titer-glo cell viability assay, conducted as a 6 x 6 dose response matrix for each combination (both DS-8201 and AZD6738 were used at half-log serial dilutions).
Combination activity was assessed based on a combination of the ΔEmax and HSA synergy scores.

Results:

Results are shown in Figures 19A and 19B and Table 10.

Figure 19A shows a matrix of measured cell viability signals. X axis represents drug A (DS-8201), and Y axis represents drug B (AZD6738). Values in the box represent the ratio of cells treated with drug A + B compared to DMSO control at day 7. All values are normalised to cell viability values at day 0. Values between 0 and 100 represent % growth inhibition and values above 100 represent cell death.

Figure 19B shows an HSA excess matrix. Values in the box represent excess values calculated by the HSA (Highest Single Agent) model.

Table 10 below shows HSA additivity and Loewe synergy scores:

Table 10

Cell line	NCI-H522
HSA synergy score	13.78
Loewe synergy score	12.87

As seen from Figures 19A and 19B, and Table 10, AZD6738 interacted synergistically with DS-8201 and also increased cell death in a HER2 low lung cell line.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments. The foregoing description and Examples detail certain embodiments and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the embodiments may be practiced in many ways and the claims include any equivalents thereof.

Free Text of Sequence Listing

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

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

SEQ ID NO: 3 – Amino acid sequence of a heavy chain CDRH1 [= amino acid residues 26 to 33 of SEQ ID NO: 1]

SEQ ID NO: 4 – Amino acid sequence of a heavy chain CDRH2 [= amino acid residues 51 to 58 of SEQ ID NO: 1]

SEQ ID NO: 5 – Amino acid sequence of a heavy chain CDRH3 [= amino acid residues 97 to 109 of SEQ ID NO: 1]

SEQ ID NO: 6 – Amino acid sequence of a light chain CDRL1 [= amino acid residues 27 to 32 of SEQ ID NO: 2]

SEQ ID NO: 7 – Amino acid sequence comprising an amino acid sequence of a light chain CDRL2 (SAS) [= amino acid residues 50 to 56 of SEQ ID NO: 2]

SEQ ID NO: 8 – Amino acid sequence of a light chain CDRL3 [= amino acid residues 89 to 97 of SEQ ID NO: 2]

SEQ ID NO: 9 – Amino acid sequence of a heavy chain variable region [= amino acid residues 1 to 120 of SEQ ID NO: 1]

SEQ ID NO: 10 – Amino acid sequence of a light chain variable region [= amino acid residues 1 to 107 of SEQ ID NO: 2]

SEQ ID NO: 11 – Amino acid sequence of a heavy chain [= amino acid residues 1 to 449 of SEQ ID NO: 1]