DUOCARMYCIN ADCS FOR USE IN TREATMENT OF ENDOMETRIAL CANCER

FIELD OF THE INVENTION

The present invention relates to duocarmycin antibody-drug conjugates (ADCs) showing improved in vivo antitumor activity, in particular duocarmycin ADCs for use in the treatment of endometrial cancer. More in particular, the present invention relates to duocarmycin-containing ADCs for use in the treatment of human solid tumours expressing human epidermal growth factor receptor 2 (HER2), wherein the human solid tumour expressing HER2 is endometrial cancer, particularly wherein the human solid tumour expressing HER2 is uterine serous carcinoma (USC).

BACKGROUND OF THE PRESENT INVENTION

Endometrial (uterine) cancer is the most common gynaecological malignancy in Europe and North America. It is the seventh most common cause of death from cancer in women in Western-Europe, accounting for 1%-2% of all deaths from cancer. According to the most recent NCCN guidelines, pathologist review differentiates uterine cancer in three distinct types: i) pure endometrioid carcinoma, ii) serous or clear cell adenocarcinoma, and iii) carcinosarcoma, i.e. a mixed type of carcinoma and sarcoma (NCCN Guidelines®, Version 2.2015). The distinction between endometrioid and serous carcinomas of the endometrium is important for prognostic and therapeutic purposes. Endometrioid carcinomas typically are confined to the uterus and have better prognosis than endometrial serous carcinomas that have frequent peritoneal dissemination and a worse prognosis (K. Garg and R.A. Soslow in Arch. Pathol. Lab. Med., Vol. 138, March 2014, 335-342).

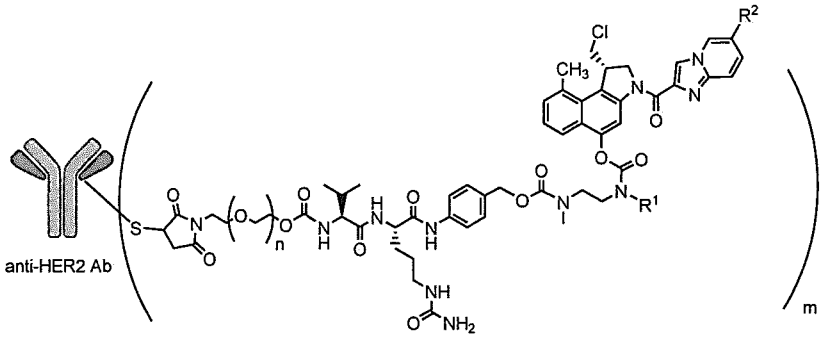
Uterine serous carcinoma (USC), or uterine papillary serous carcinoma, accounts for approx. 10% of endometrial cancer. This subtype of endometrial cancer is biologically highly aggressive and causes the most endometrial cancer deaths. Molecular profiling studies have demonstrated HER2 to be one of the most over-expressed genes in USC. HER2 is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. HER2 over-expression has been reported to range from 18% to 80% in USC due to several factors like tumour type and stage of the tissue sample as well as the immunohistochemistry (IHC) technique used (A.D. Santin et al. in Clin. Cancer Res., 8, 2002, 1271-1279; B.M. Slomovitz et al. in J. Clin. Oncol. 22, 2004, 3126-3132). Up to 35% of USCs may over-express the HER2 oncogene at high level by immunohistochemistry (i.e. HER2 IHC 3+) or harbour HER2 gene amplification by fluorescence in situ hybridization (i.e. FISH positive). An additional 45% of USCs express HER2 at moderate (i.e. HER2 IHC 2+) or low (i.e. HER2 IHC 1+) levels.

Trastuzumab (Herceptin™, Genentech/Roche) is a recombinant humanized IgG1 monoclonal antibody against the extracellular domain of HER2 and is currently approved for the treatment of both metastatic and early-stage breast cancer as well as locally advanced or metastatic gastric cancer over-expressing HER2. Case study reports describe the use of trastuzumab in endometrial cancer. In the Int. J. Gynecol. Cancer 16: 1370-1373, 2006, E. Jewell et al. describe a positive result of the administration of trastuzumab to one patient with metastatic endometrial cancer. In the Int. J. Gynecol. Obstet. 102: 128-131, 2008, A.D. Santin et al. report the results of treatment with trastuzumab in two patients with advanced or recurrent endometrial carcinoma that over-express HER2. In Gynecol. Oncol. 116: 15-20, 2010, Fleming et al. report results of a Phase II trial of 34 patients who had HER2-positive endometrial carcinoma and were treated with trastuzumab. Trastuzumab has not currently been approved for the treatment of any endometrial cancer.

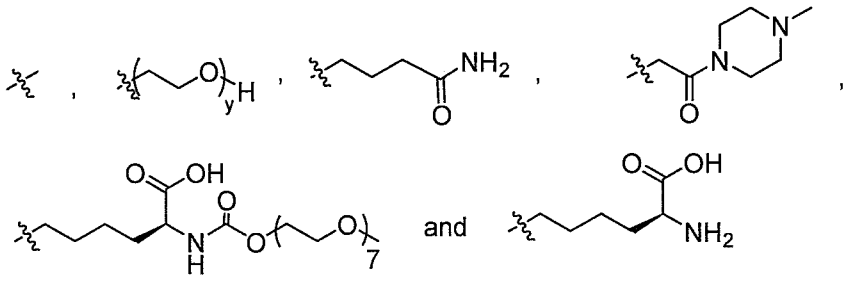
D.P. English et al. report in Cancer Medicine published by John Wiley & Sons Ltd., pp. 1-10, 2014, that T-DM1 is highly effective against primary HER2 over-expressing uterine serous carcinoma (USC) in vitro and in vivo. T-DM1 (Kadcyla™, ado-trastuzumab emtansine, Genentech/Roche) is an antibody-drug conjugate which comprises trastuzumab covalently linked to the anti-microtubule agent DM1. DM1 belongs to the maytansine class of chemotherapeutic agents. On average, 3-4 molecules of DM1 are conjugated to each trastuzumab molecule. T-DM1 is an agent aimed at delivering the highly potent DM1 into HER2 over-expressing cells via receptor-mediated endocytosis. T-DM1 has been approved for the treatment of patients with HER2-positive metastatic breast cancer who received prior treatment with trastuzumab and a taxane. The authors conclude that T-DM1 shows promising antitumor effect in HER2-positive USC cell lines and USC (i.e. HER2 IHC 3+) xenografts in SCID mice (15 mg/kg, i.p. injections once per week) and its activity is significantly higher when compared to trastuzumab, and that T-DM1 may represent a novel treatment option for HER2-positive USC patients with disease refractory to standard chemotherapy. No clinical investigations are currently ongoing with T-DM1 for the treatment of endometrial cancer.

SUMMARY

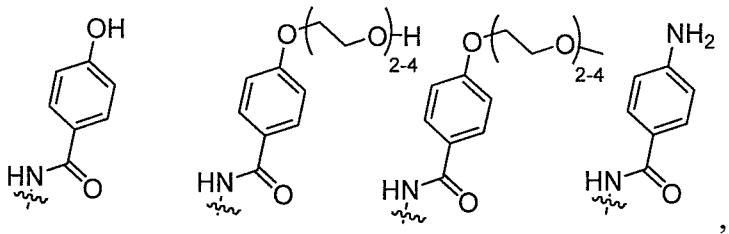
The present invention describes duocarmycin-containing ADCs for use in the treatment of human solid tumours expressing HER2, wherein the human solid tumour expressing HER2 is endometrial cancer, particularly wherein the human solid tumour expressing HER2 is USC. In one aspect, the present invention refers to use of a compound of formula (I)



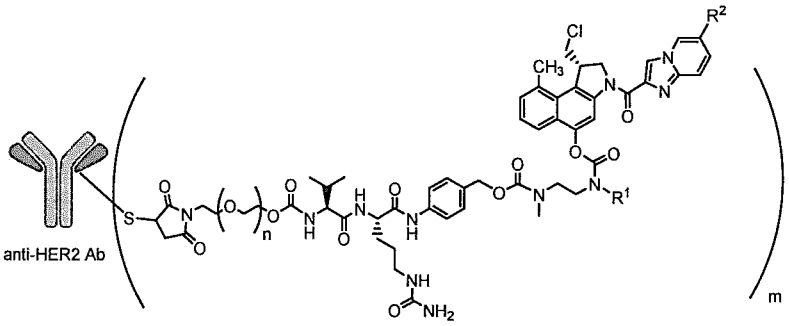
Wherein anti-HER2 Ab is an anti-HER2 antibody, n is 0-3, m represents an average DAR of from 1 to 4, R^1 is selected from



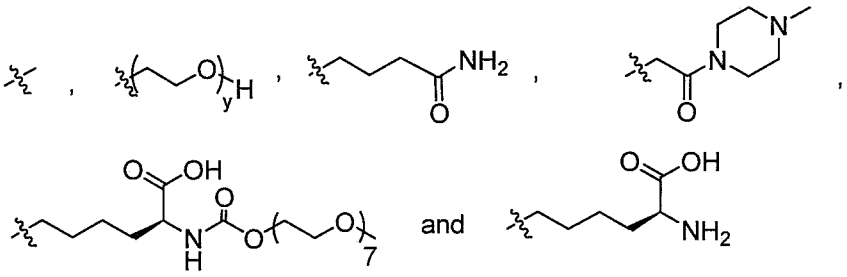
y is 1-16, and R^2 is selected from



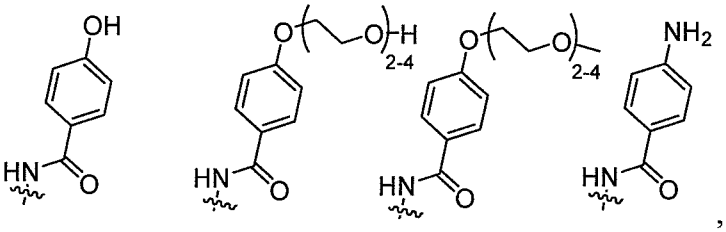
, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour expressing HER2 is endometrial cancer. In one example, there is disclosed the use of a compound of formula (I)



Wherein anti-HER2 Ab is an anti-HER2 antibody or antibody fragment, n is 0-3, m represents an average DAR of from 1 to 4, R^1 is selected from



y is 1-16, and R^2 is selected from



in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour expressing HER2 is endometrial cancer.

In another aspect, there is provided the use of a combination of a compound as disclosed herein with a therapeutic antibody and/or a chemotherapeutic agent, in the manufacture of a medicament for the treatment of endometrial cancer.

In one example, there is disclosed a pharmaceutical composition comprising a compound as disclosed herein or a combination as disclosed herein, and one or more pharmaceutically acceptable excipients.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. In vitro USC cell line cytotoxicity: SYD985 versus T-DM1.

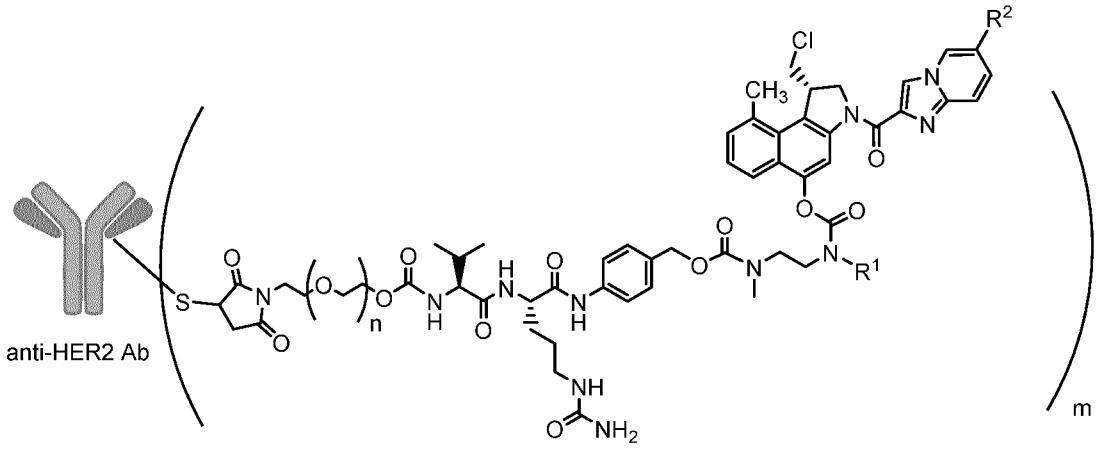
Figure 2. In vivo USC cell line xenograft efficacy in mice: SYD985 versus T-DM1 after multiple injections.

Figure 3. In vivo USC cell line xenograft efficacy in mice: SYD985 versus T-DM1 after single injection at day 0.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention relates to duocarmycin-containing ADCs for use in the treatment of human solid tumours expressing HER2 (i.e. HER2 IHC 3+, 2+ or 1+), wherein the human solid tumour expressing HER2 is endometrial cancer, particularly wherein the human solid tumour expressing HER2 is USC.

In one embodiment, the present invention provides a compound of formula (I)



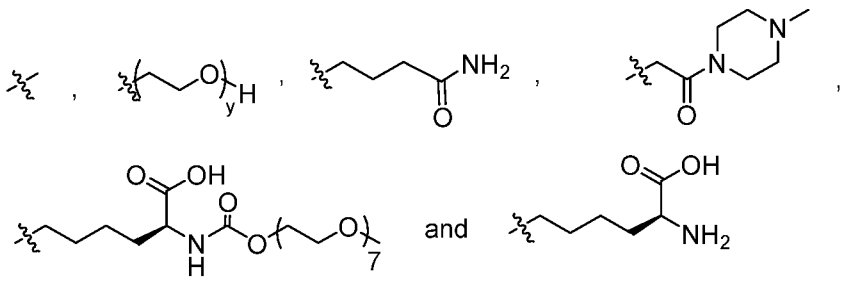
wherein

anti-HER2 Ab is an anti-HER2 antibody or antibody fragment,

n is 0-3, preferably 0-1,

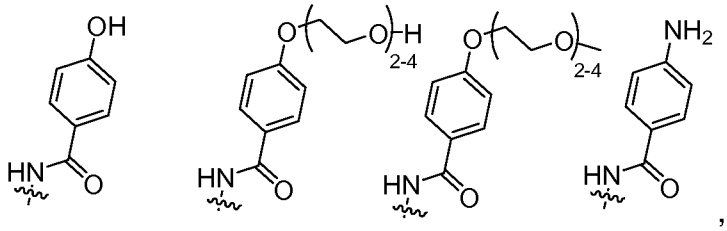
m represents an average DAR of from 1 to 4,

R^1 is selected from



y is 1-16, and

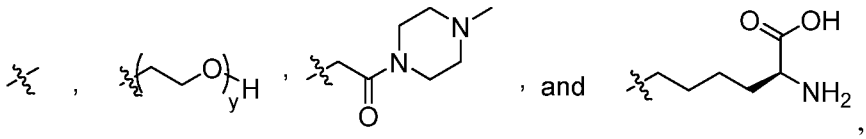
R^2 is selected from



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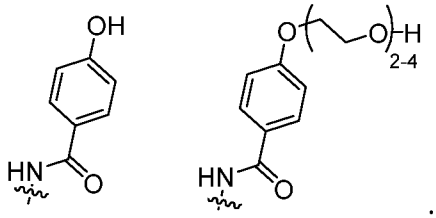
for use in the treatment of human solid tumours expressing HER2, wherein the human solid tumour expressing HER2 is endometrial cancer, particularly wherein the human solid tumour expressing HER2 is USC.

In another embodiment, the present invention relates to a compound of formula (I), wherein anti-HER2 Ab is an anti-HER2 antibody or antibody fragment, n is 0-1, m represents an average DAR of from 1 to 4, preferably from 2 to 3, R^1 is selected from



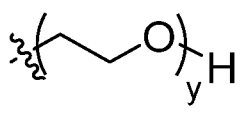
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y is 1-16, preferably 1-4, and R^2 is selected from



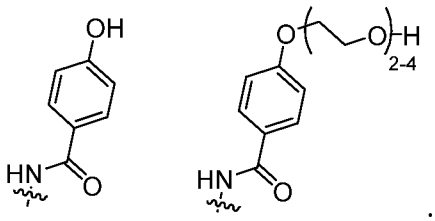
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In a further embodiment, the present invention relates to a compound of formula (I), wherein the anti-HER2 Ab is an anti-HER2 monoclonal antibody, n is 0-1, m represents an average DAR of from 2 to 3, preferably from 2.5 to 2.9, R^1 is selected from

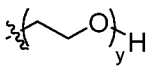


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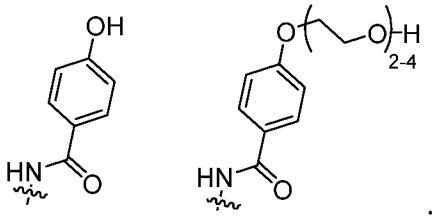
y is 1-4, and R^2 is selected from



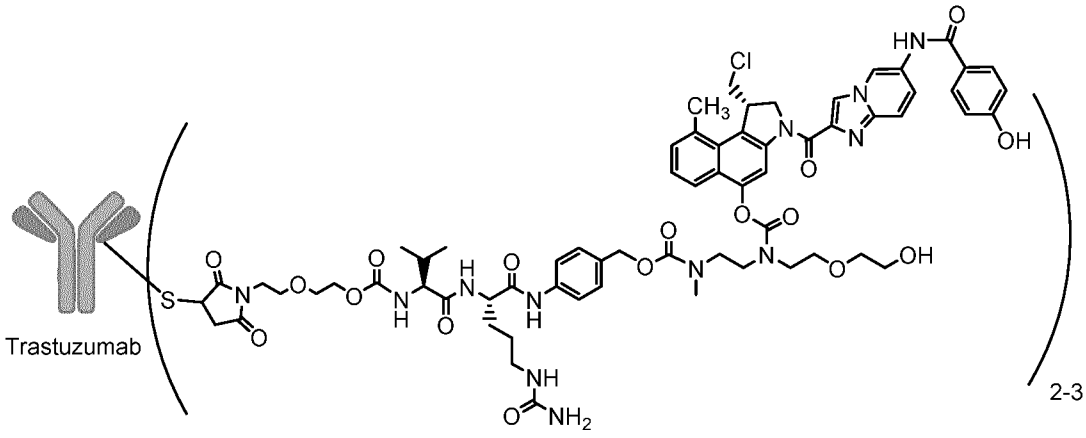
In yet another embodiment, the present invention relates to a compound of formula (I), wherein the anti-HER2 Ab is trastuzumab or a biosimilar thereof, n is 0-1, m represents an average DAR of from 2 to 3, preferably 2.5 to 2.9, R^1 is selected from



y is 1-4, and R^2 is selected from



In a preferred embodiment, the present invention relates to a compound of formula (II), comprising trastuzumab or a biosimilar thereof



The compound of formula (II) that is referred to as SYD985 in the present specification has an average DAR of from 2.6 to 2.9.

In the structural formulae shown in the present specification, n represents an integer from 0 to 3, while m represents an average drug-to-antibody ratio (DAR) of from 1 to 4. As is

well-known in the art, the DAR and drug load distribution can be determined, for example, by using hydrophobic interaction chromatography (HIC) or reversed phase high-performance liquid chromatography (RP-HPLC). HIC is particularly suitable for determining the average DAR.

Duocarmycins, first isolated from a culture broth of *Streptomyces* species, are members of a family of antitumor antibiotics that include duocarmycin A, duocarmycin SA, and CC-1065. These extremely potent agents allegedly derive their biological activity from an ability to sequence-selectively alkylate DNA at the N3 position of adenine in the minor groove, which initiates a cascade of events leading to tumour cell death.

WO2011/133039A discloses a series of analogues of the DNA-alkylating agent CC-1065 and HER2 targeting antibody-drug conjugates (ADCs) thereof. In Example 15, a number of trastuzumab-duocarmycin conjugates were tested against N87 (i.e. HER2 IHC 3+ gastric tumour) xenografts in nude mice.

Typical examples of endometrial (uterine) cancer which can be treated in accordance with the present invention include endometrioid carcinoma, serous or clear cell adenocarcinoma, and carcinosarcoma. Advantageously, the endometrial cancer is USC.

In one embodiment, the present invention provides a compound of formula (I) or (II) for use in the treatment of endometrial cancer showing moderate or low expression of HER2 (i.e. HER2 IHC 2+ or 1+), in particular USC.

In another embodiment, the present invention provides a compound of formula (I) or (II) for use in the treatment of endometrial cancer without HER2 gene amplification (i.e. HER2 FISH negative), in particular USC.

Unexpectedly, the present inventors have found that the compounds of the present invention particularly can be used for the treatment of endometrial cancer, especially USC, with a moderate or low expression of HER2 (i.e. HER2 IHC 2+ or 1+) and/or without HER2

gene amplification (i.e. HER2 FISH negative). Neither trastuzumab nor T-DM1 showed efficacy against such tumours. WO2011/133039A does not teach or suggest the use of duocarmycin-containing ADCs for use in the treatment of endometrial cancer either.

In an advantageous embodiment of the present invention the endometrial cancer is USC showing moderate or low HER2 expression (i.e. HER2 IHC 2+ or 1+) without HER2 gene amplification (i.e. HER2 FISH negative).

Typically, the antitumor activity is evaluated first in (human) tumour cell lines in vitro followed by evaluation in vivo. The antitumor activity of the ADCs falling within the scope of the present invention advantageously is evaluated in animal models, typically nude mice bearing a subcutaneous xenograft. The xenograft can either be a (human) tumour cell line or a patient-derived (primary) tumour.

In accordance with the present invention, the anti-HER2 antibody or antibody fragment can be any antibody or antibody fragment able to bind HER2, e.g. an IgG1 antibody having the complementary determining regions (CDRs) of trastuzumab or an antibody that shows competitive binding with trastuzumab. A preferred antibody is a monoclonal anti-HER2 antibody. A particularly preferred monoclonal antibody is trastuzumab or a biosimilar thereof.

Antibody-drug conjugate (ADC) compounds of formula (I) and (II) in accordance with the present invention have the linker-drug conjugated to the antibody through the S-atom of a cysteine residue, i.e. they are cysteine-linked antibody-drug conjugates. The cysteine residue can either be a natural cysteine residue which is present in the heavy and/or light chain of the antibody (Ab) and forms inter-chain disulfide bonds, or an engineered cysteine residue which is introduced into the Ab at one or more suitable positions in the heavy and/or light chain. The present invention is particularly drawn to ADC compounds wherein the linker-drug is conjugated through inter-chain disulfide bonds of Abs, more particularly monoclonal Abs

(mAbs). Antibodies of different antibody classes contain different numbers of inter-chain disulfide bonds. For example, IgG1 antibodies typically have four inter-chain disulfide bonds, all four located in the hinge region, and after (partial) reduction of the disulfide bonds the linker-drug is randomly attached to free thiol groups.

Compounds of formula (I) and (II) for use in accordance with the present invention can be obtained according to methods and procedures that are well known to a person skilled in the art. Conjugation through inter-chain disulfide bonds can occur after complete or partial reduction of said disulfide bonds. Suitable methods for preparing such compounds can be found in the description and examples of Applicant's WO2011/133039A. In particular, Example 15 of WO2011/133039A describes the partial reduction of trastuzumab to generate 2 free thiol groups per mAb and conjugation with a number of linker-drugs to ADCs having an average DAR of approx. 2. It is easily understood by those skilled in the art how to obtain ADCs having an average DAR of from 1 to 4. Examples 7 and 8 of WO2005/084390A describe partial reduction, partial reduction/partial re-oxidation, and complete reduction strategies for (partial) loading of antibodies (with the linker-drug vcMMAE).

IHC and FISH status of the tumour tissue are determined using known tests, procedures, and equipment. In accordance with the present invention HER2 gene amplication can be measured using either fluorescence (FISH), chromogenic (CISH) or any other in situ hybridization test. Suitable tests for determination of the HER2 membrane expression status of the tumour tissue like the HercepTest™ (Dako Denmark) are commercially available. Further HER2 IHC tests are marketed by Ventana Medical Systems (PATHWAY anti-HER2/neu), Biogenex Laboratories (InSite™ HER2/neu), and Leica Biosystems (Bond Oracle™ HER2 IHC). HER2 FISH/CISH tests can be obtained from Abbott Molecular (PathVysion HER2 DNA Probe Kit), Life Technologies (SPOT-Light® HER2 CISH Kit),

Dako Denmark (HER2 CISH PharmDx™ Kit), Dako Denmark (HER2 FISH PharmDx™ Kit), and Ventana Medical Systems (INFORM HER2 Dual ISH DNA Probe Cocktail).

The present invention also relates to the use of a compound of formula (I) or (II) for the treatment of patients (i.e. women) having endometrial cancer, in particular USC, which is HER2 IHC 2+ or 1+ and/or HER2 FISH negative as described herein above.

The present invention further relates to the use of a combination of a compound of formula (I) or (II) with a therapeutic antibody and/or a chemotherapeutic agent, for the treatment of endometrial cancer, in particular for the treatment of USC.

In one embodiment of the present invention, the therapeutic antibody for use in combination with a compound of formula (I) or (II) in accordance with the present invention is pertuzumab, bevacizumab or trastuzumab, and the chemotherapeutic agent is i) a taxane, particularly docetaxel or paclitaxel, ii) a DNA damaging agent, particularly cisplatin, carboplatin or oxaliplatin, iii) a topoisomerase inhibitor, particularly topotecan or irinotecan, iv) an anthracycline, particularly doxorubicin, liposomal doxorubicin, epirubicin, daunorubicin or valrubicin, more particularly doxorubicin, v) an mTOR inhibitor, particularly temsirolimus, or vi) a tyrosine kinase inhibitor, particularly lapatinib or afatinib.

In another embodiment of the present invention, the therapeutic antibody for use in combination with a compound of formula (I) or (II) in accordance with the present invention is pertuzumab and the chemotherapeutic agent is a taxane, particularly docetaxel or paclitaxel, an anthracycline, particularly doxorubicin, epirubicin, daunorubicin or valrubicin, more particularly doxorubicin, or a tyrosine kinase inhibitor, particularly afatinib.

The present invention further relates to the use of a combination of a compound of formula (I) or (II) with another ADC, such as for example T-DM1, for the treatment of human solid tumours and haematological malignancies expressing HER2, in particular human solid tumours expressing HER2.

The present invention further relates to a pharmaceutical composition comprising a compound of formula (I) or (II) or a combination with a therapeutic antibody and/or a chemotherapeutic agent thereof as described herein above, and one or more pharmaceutically acceptable excipients.

Typical pharmaceutical formulations of therapeutic proteins such as monoclonal antibodies and (monoclonal) antibody-drug conjugates take the form of lyophilized powders or cakes, which require (aqueous) dissolution (i.e. reconstitution) before intravenous infusion, or frozen (aqueous) solutions, which require thawing before use. Particularly, in accordance with the present invention the pharmaceutical composition is provided in the form of a lyophilized cake.

Suitable pharmaceutically acceptable excipients for inclusion into the pharmaceutical composition (before freeze-drying) in accordance with the present invention include buffer solutions (e.g. citrate, histidine or succinate containing salts in water), lyo protectants (e.g. sucrose, trehalose), tonicity modifiers (e.g. sodium chloride), surfactants (e.g. polysorbate), and bulking agents (e.g. mannitol, glycine). Excipients used for freeze-dried protein formulations are selected for their ability to prevent protein denaturation during the freeze-drying process as well as during storage.

The sterile, lyophilized powder multi-dose formulation of Herceptin™ contains 440 mg trastuzumab, 400 mg α,α-trehalose dihydrate, 9.9 mg L-histidine. HCl, 6.4 mg L-histidine, and 1.8 mg polysorbate 20, USP. Reconstitution with 20 ml of Bacteriostatic or Sterile Water for Injection (BWFI or SWFI) yields a multi-dose solution containing 21 mg/ml trastuzumab at a pH of approximately 6. The sterile, lyophilized powder single-use formulation of Kadcyla™ contains upon reconstitution 20 mg/ml ado-trastuzumab emtansine, 0.02% w/v polysorbate 20, 10 mM sodium succinate, and 6% w/v sucrose with a pH of 5.0.

A therapeutically effective amount of the compound of formula (I) or (II) for use in accordance with the present invention lies in the range of about 0.01 to about 15 mg/kg body weight, particularly in the range of about 0.1 to about 10 mg/kg, more particularly in the range of about 0.3 to about 10 mg/kg body weight. This latter range corresponds roughly to a flat dose in the range of 20 to 800 mg of the ADC compound. The compound for use in accordance with the present invention is administered weekly, bi-weekly, three-weekly or monthly, for example weekly for the first 12 weeks and then every three weeks until disease progression. Alternative treatment regimens may be used depending upon the severity of the disease, the age of the patient, the compound being administered, and such other factors as would be considered by the treating physician.

EXAMPLES

In vitro USC cell line cytotoxicity

Nine primary USC cell lines were evaluated for HER2 surface expression by IHC and flow cytometry (FACS) and for HER2 gene amplification by FISH as described by D.P. English et al. in Cancer Medicine published by John Wiley & Sons Ltd., pp. 1-10, 2014.

SYD985 was 50 to 160 times more potent when compared to T-DM1 in primary USC cell lines with HER2 IHC 1+ and IHC 2+ expression. In a set of three HER2 IHC 1+ USC cell lines the average IC_{50}s for SYD985 and T-DM1 were 0.07 µg/ml and 3.58 µg/ml, respectively (p=0.004); in a set of three HER2 IHC 2+ USC cell lines the average IC_{50}s were 0.02 µg/ml and 1.82 µg/ml, respectively (p=0.005); and in a set of three HER2 IHC 3+ USC cell lines the average IC_{50}s were 0.01 µg/ml and 0.04 µg/ml, respectively (p=0.06).

Figure 1 shows representative in vitro dose-response curves of SYD985 versus T-DM1 for HER2 IHC 3+, 2+, and 1+ USC cell lines.

In vivo USC cell line xenograft efficacy in mice

Five- to 8-week old female SCID mice (Harlan, Netherlands) were given a single intra-peritoneal (i.p.) injection of $7.5x10^6$ USC ARK-2 cells (i.e. HER2 IHC 3+, FISH positive) in approx. 400 µl phosphate-buffered saline solution. After a 7-day period to allow for tumour establishment, two groups of 5 mice were treated with either SYD985 (5 mg/kg/wk i.v.) or T-DM1 (5 mg/kg/wk i.v.). No signs of general toxicity were seen in any of the treatment groups. The mice in all treatment groups were given series of five injections after which they were placed in follow-up and observed for overall survival as the primary outcome.

Figure 2 shows the in vivo USC cell line xenograft efficacy in mice of SYD985 versus T-DM1 after multiple injections.

In vivo USC cell line xenograft efficacy study in mice

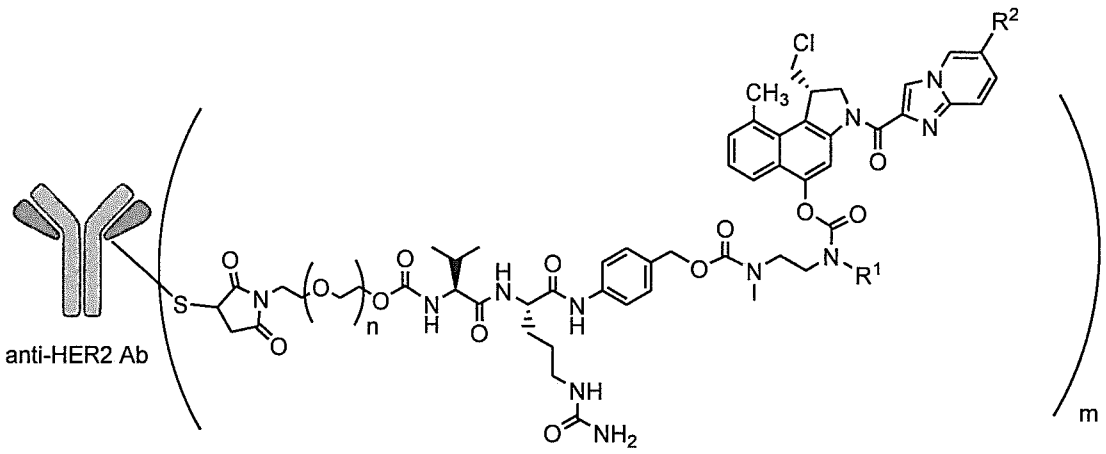
USC ARK-2 cells (i.e. HER2 IHC 3+, FISH positive) were expanded in culture, washed and injected (with Matrigel™) at a concentration of 7 million cells subcutaneously into 5-8 weeks old SCID mice. Once the tumors reached approx. 200 mm^3 in volume they were randomly divided in 5 groups keeping average tumor volume similar between groups. There were a total of 8-10 animals in each group.

The animals were treated with a single, intra-venous injection of vehicle, T-DM1 at 10 mg/kg or SYD985 at 10 mg/kg. Tumor size and weight of the animals were recorded for 21 days. Survival data were recorded for 30 days.

Figure 3 shows the in vivo USC cell line xenograft efficacy in mice of SYD985 versus T-DM1 after single injection at day 0.

CLAIMS

1. Use of a compound of formula (I)



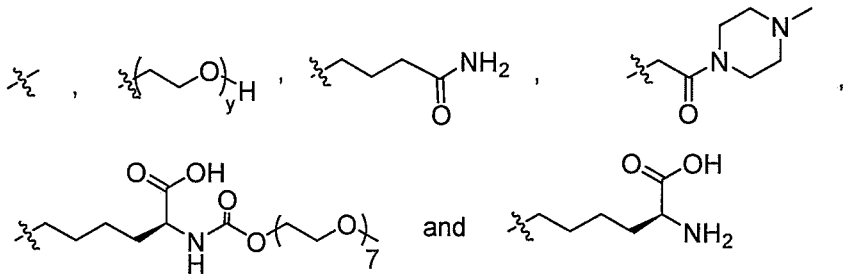
wherein

anti-HER2 Ab is an anti-HER2 antibody,

n is 0-3,

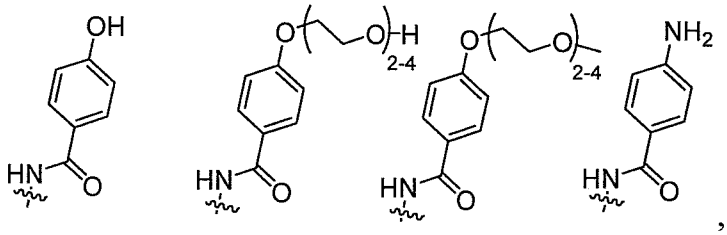
m represents an average DAR of from 1 to 4,

R^1 is selected from



y is 1-16, and

R^2 is selected from



in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour expressing HER2 is endometrial cancer.

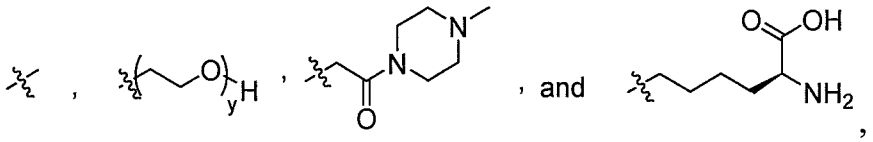
2. The use according to claim 1, wherein in the compound of formula (I)

anti-HER2 Ab is an anti-HER2 antibody,

n is 0-1,

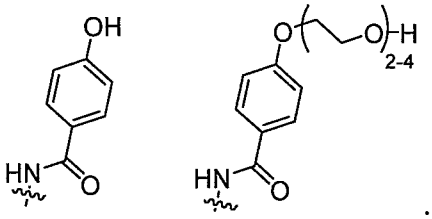
m represents an average DAR of from 1 to 4,

R^1 is selected from

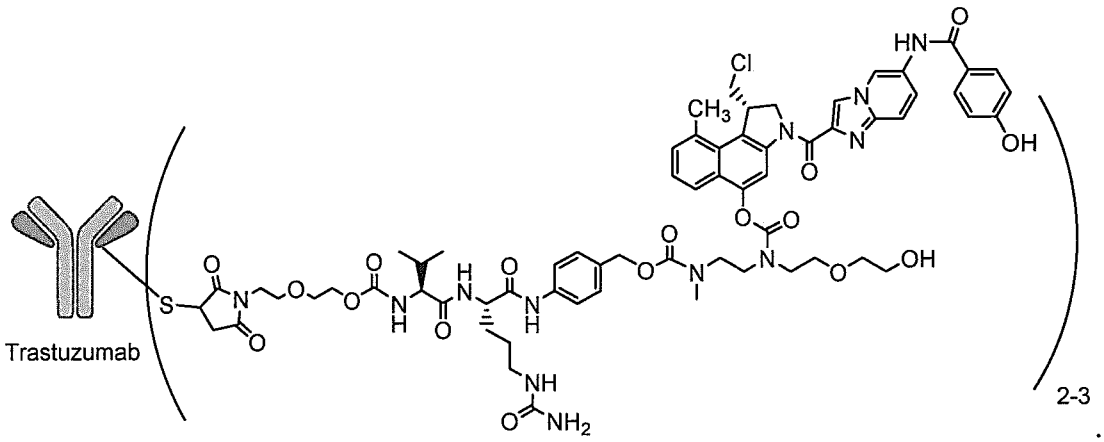


y is 1-16, and

R^2 is selected from



3. The use according to claim 1 or 2, wherein the compound of formula (I) is a compound of formula (II)



4. The use according to claim 3, wherein the compound of formula (II) has an average DAR of from 2.6 to 2.9.

5. The use according to any one of claims 1 to 4, wherein the endometrial cancer is uterine serous carcinoma.

6. The use according to any one of claims 1 to 5, wherein the endometrial cancer is HER2 IHC 2+ or 1+.
7. The use according to any one of claims 1 to 6, wherein the endometrial cancer is HER2 FISH negative.
8. Use of a combination of a compound according to any one of claims 1 to 7 with a therapeutic antibody and/or a chemotherapeutic agent, in the manufacture of a medicament for the treatment of endometrial cancer.
9. The use according to claim 8, wherein the endometrial cancer is uterine serous carcinoma.
10. The use according to claim 8 or 9, wherein the therapeutic antibody is pertuzumab and the chemotherapeutic agent is a taxane, an anthracycline or a tyrosine kinase inhibitor.