DUOCARMYCIN ADCS SHOWING IMPROVED IN VIVO ANTITUMOR ACTIVITY

FIELD OF THE INVENTION

The present invention relates to duocarmycin-containing antibody-drug conjugates (ADCs) for use in the treatment of human solid tumours and haematological malignancies expressing human epidermal growth factor receptor 2 (HER2), in particular breast cancer, gastric cancer, bladder cancer, ovarian cancer, lung cancer, prostate cancer, pancreatic cancer, colorectal cancer, head and neck squamous cell cancer, osteosarcoma, and acute lymphoblastic leukaemia.

BACKGROUND OF THE PRESENT INVENTION

Antibodies have been conjugated to a variety of cytotoxic drugs, including small molecules that alkylate or crosslink DNA (e.g. duocarmycins and calicheamicins or pyrrolo-benzodiazepine dimers, respectively), or disrupt microtubules (e.g. maytansinoids and auristatins) or bind DNA (e.g. anthracyclines). One such ADC comprising a humanized anti-CD33 antibody conjugated to calicheamicin - Mylotarg™ (gemtuzumab ozogamicin, Wyeth) – was approved in 2000 for acute myeloid leukaemia. In 2011, the US Food and Drug Administration (FDA) approved Adcetris™ (brentuximab vedotin, Seattle Genetics), an ADC comprising a chimeric antibody to CD30 conjugated to monomethyl auristatin E (MMAE) for treatment of Hodgkin's lymphoma and anaplastic large cell lymphoma.

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 novel analogues of the DNA-alkylating agent CC-1065 and HER2-targeting ADCs thereof. In Example 15, a number of trastuzumab-duocarmycin conjugates were tested against N87 (i.e. HER2 IHC (immunohistochemistry) 3+ gastric tumour) xenografts in nude mice. The results are shown in Figures 4A, 4B and 4C. After treatment with a single dose of 12 mg/kg i.v., all six ADCs reduced the tumour volume and improved survival compared to the antibody trastuzumab itself and control vehicle, without negatively affecting body weight. It was concluded that conjugates that contain a relatively short linker have a better (antitumor) efficacy than the corresponding conjugate with a relatively long linker, and that both the nature of the linker and the nature of the drug were demonstrated to have an effect on efficacy as well.

Breast cancer remains the most common malignancy among women worldwide. Breast cancer is a heterogeneous disease, which exhibits a wide range of clinical behaviours and prognoses. Breast cancer is an abnormal malignant growth of epithelial cells of the milk lobules or ducts of the mammary gland. The cancer tissue can be exclusively located on the place of origin (cancer in situ) or can have invaded through the basement membrane into the surrounding tissue (invasive cancer). Metastatic cancer occurs as soon as the cancer cells have spread by way of lymph and blood vessels to other organs. Histological differentiation and characterization of the breast cancer cells is performed with use of biomarkers.

Molecular classification of breast cancer for therapeutic decisions mainly consists of the assessment of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression status. This implies that globally three types of breast cancer can be discerned: (1) breast cancer tissue with expression of hormone receptor (ER or PR) without over-expression of HER2, (2) breast cancer tissue with over-

expression of HER2, with or without expression of hormone receptor (HR), and (3) breast cancer tissue that has no therapeutically relevant hormone receptor or HER2 receptor expression, so-called triple negative breast cancer (TNBC).

Breast cancer patients with hormone receptor (HR) positive cancer tissue status (ca. 60-70% of all breast cancer patients) have a better prognosis than those without or with minimal hormone receptor status. On the contrary, patients whose tumour has an IHC 3+ or IHC 2+/FISH (fluorescence in situ hybridization) positive status (occurring in about 20% of all breast cancer cases) have a worse prognosis in comparison with breast cancer patients whose tumour has a lower grade of HER2 membrane expression or a FISH negative amplification rate. Now that patients with hormone receptor positive and HER2 over-expressing breast cancer tissue have the option of targeting therapy, triple negative breast cancer implies the worst prognosis, as only chemotherapy with limited efficacy is available for these patients whose tumour is ER, PR and HER2 negative.

Herceptin™ (trastuzumab), a recombinant humanized IgG1 monoclonal antibody against HER2, was approved in the US by the FDA in 1998 for adjuvant treatment of HER2 over-expressing breast cancer and for the treatment of metastatic HER2 over-expressing breast cancer and gastric cancer, i.e. HER2 IHC 3+ or HER2 IHC 2+/FISH positive. The drug was approved in Europe by the EMA in 2000.

Clinical studies with patients who have metastatic breast cancer disease have demonstrated, that there is only clinical relevant efficacy of trastuzumab treatment if the patient has a tumour with HER2 IHC over-expression or FISH positive gene amplification. For this reason, current HER2 testing algorithms are aimed at identifying those patients most likely to achieve a significant benefit from HER2 targeting. Whereas HER2 membrane expression is biologically a continuum from low to high over-expression, approved IHC tests, like the HercepTest™ (Dako, Glostrup, Denmark), categorize HER2 status on a semi-

quantitative scale ranging from 0 to 3+. An IHC score of 3+ is assigned if there is a strong circumferential membrane staining in >10% of the cancer cells. FISH positive gene amplification is assigned if the amplification rate relative to the centromere is ≥2.0. It identifies patients who might have a benefit of treatment with trastuzumab or other HER2 targeting agents. A review of 6,556 breast cancers revealed that about 92% of tumours with a HER2 score of 3+ had FISH positive gene amplification. Conversely, HER2 amplification was observed at lower rates in tumours with scores of 2+ (23.3%), 1+ (7.4%), and 0 (4.1%). With HER2 amplification as an established predictor of response to HER2 targeting agents, the current algorithm calls for FISH testing of tumours with a HER2 IHC score of 2+.

Ado-trastuzumab emtansine or trastuzumab emtansine (Kadcyla™, T-DM1) is an ADC in which trastuzumab is conjugated to the cytotoxic maytansine anti-tubulin agent DM1. T-DM1 has antitumor activity in tumour xenograft models that are not responding to therapy with trastuzumab as single agent. In the Phase 3 EMILIA trial, patients with HER2 positive advanced breast cancer, previously treated with trastuzumab and a taxane, were randomly assigned to receive T-DM1 or lapatinib plus capecitabine. T-DM1 treatment effectuated significantly longer progression-free and overall survival time in comparison to the treatment of the control group.

Kadcyla™ (T-DM1) was approved in the US by the FDA in February 2013 for the treatment of patients with HER2-positive metastatic breast cancer who received prior treatment with trastuzumab and a taxane. The drug was approved in Japan by the MHLW (Ministry of Health, Labour and Welfare) in September 2013 and in Europe by the EMA in November 2013. The currently approved regimen comprises a dosage of 3.6 mg/kg body weight i.v. every three weeks. A dosage of 2.4 mg/kg body weight i.v. weekly is investigated in an ongoing Phase II study with a combination of T-DM1 and capecitabine for the 2nd line treatment of patients with breast cancer or gastric cancer and in an ongoing Phase III study to

investigate T-DM1 against a taxane as 2nd line treatment of patients with gastric cancer. A Phase III study is also ongoing for the combination of T-DM1 with pertuzumab for the treatment of patients with HER2 positive, locally advanced, or metastatic breast cancer.

Despite the improvement that the introduction of T-DM1 in clinical practice brought over trastuzumab for the treatment of HER2-positive metastatic breast cancer, the use of T-DM1 is associated with a number of serious side-effects, most importantly thrombocytopenia, hepatotoxicity, and neuropathy (irreversible axonal degeneration). Furthermore, neither trastuzumab nor T-DM1 are authorized for the treatment of human solid tumours and haematological malignancies with moderate or low HER2 expression, i.e. IHC 2+ or 1+ and/or FISH negative HER2 amplification status of the cancer tissue.

In analogy to breast cancer, HER2 expression indicates a poor prognosis for patients with ovarian cancer (A. Berchuck et al., 1990, Cancer Res., 50, 4087-4091; H. Meden and W. Kuhn, 1997, Eur. J. Obstet. & Gynecol. Reprod. Biol., 71, 173-179). SKOV3 cells are derived from the ascites fluid of a patient with ovarian adenocarcinoma. This cell line is over-expressing HER2 and is frequently used for in vitro and in vivo explorative investigation of HER2 targeting agents. Trastuzumab and pertuzumab have several anti-cancer effects in this cell line (N. Gaborit et al., 2011, J. Biol. Chem., 286, 13, 11337-11345). Monotherapy with the anti-HER2 antibodies trastuzumab and pertuzumab thus far had modest efficacy (G.M. Mantia-Smaldone et al., 2011, Cancer Management Res. 3, 25–38; S.P. Langdon et al., 2010, Expert Opin. Biol. Ther. 10:7, 1113-1120). The antitumor effect is markedly increased if a HER2 targeting antibody is combined with chemotherapy (S. Makhija et al., 2010, J. Clin. Oncol., 28:7, 1215-1223; I. Ray-Coquard et al., 2008, Clin. Ovarian Cancer, 1:1, 54-59).

Further, a high medical need exists for the treatment of late stage bladder cancer disease. Chemotherapy, e.g. the combination of cisplatin and gemcitabine for advanced or metastatic bladder cancer, has limited efficacy as it effectuates in the mean a response rate

under 50%, whereas patients have an overall survival time of 6 to 12 months. In case of resistance to chemotherapy there is no standard therapy option at all. HER2 positivity was significantly associated with reduced complete response rates (50% versus 81%, p=0.026) after chemo-radiation (A. Chakravarti et al., 2005, Int. J. Radiation Oncology Biol. Phys., 62:2, 309-317). Addition of trastuzumab to a regimen of paclitaxel and carboplatin as first line therapy of HER2 positive advanced bladder cancer showed an overall response rate of 70% and an overall survival time of 14.1 months in a Phase II study (M.H.A. Hussain et al., 2007, J. Clin. Oncol., 25:16, 2218-24). In a casuistic application, a patient with a tumor relapse after standard chemotherapy responded to the combination of trastuzumab, paclitaxel and carboplatin (D. Amsellem-Ouazana et al., 2004, Ann. Oncol., 15, 3, 538).

In case of invasive non-small-cell lung cancer adenocarcinoma, HER2 mutation and amplification are related with unfavorable outcome (M. Suzuki et al., 2014, Lung Cancer, http://dx.doi.org/10.1016/j.lungca.2014.10.014). In lung cancer patients with HER2 mutation, a disease control rate of 93% could be effectuated with trastuzumab-based therapies (J. Mazieres et al., 2013, J. Clin. Oncol., 31:16, 1997-2004). Chemo-resistance of lung cancer often is associated with enhanced HER2 expression (C.-M. Tsai et al., 1993, J. Natl. Cancer Inst., 85:11, 897-901; Z. Calikusu et al., 2009, J. Exp. Clin. Cancer Res., 28:97) and resistance to tyrosine kinase inhibitors is correlated with enhanced HER2 amplification (K. Takezawa et al., 2012, Cancer Discov. 2(10), 922-33).

Patients with early or advanced prostate cancer mostly receive an androgen receptor targeting therapy. There is a cross-talk in the signaling functions of the androgen receptor and HER2 (F.-N. Hsu et al., 2011, Am. J. Physiol. Endocrinol. Metab., 300:E902-E908; L. Chen et al., 2011, Clin. Cancer Res., 17(19), 6218-28). HER2 activation suppresses the expression of the androgen receptor (C. Cai et al., 2009, Cancer Res., 69(12), 5202–5209), increased HER2 expression is associated with PSA progression, rapid proliferation and poor prognosis

(S. Minner et al., 2010, Clin. Cancer Res., 16(5), 1553-60; S.F. Shariat et al., 2007, Clin. Cancer Res., 13(18), 5377-84). Increased expression of HER2 seems to be involved in progression to androgen independence in about a quarter of prostate cancer cases (J.M.S. Bartlett et al., 2005, J. Pathol., 205, 522–529).

Pancreatic cancer is among the most lethal human solid tumors due to its insidious onset and resistance to therapy. Gemcitabine or the combination of 5-FU, leucovorin, irinotecan, and oxaliplatin can help prolong life in patients with advanced disease (H. Burris and A.M. Storniolo, 1997, Eur. J. Cancer 33(1):S18-S22; T. Conroy et al., 2011, N. Engl. J. Med. 364(19):1817-25). More recently, it was reported that HER2 expression is also prevalent in pancreatic cancer with an equal proportion of 10% designated as HER2 2+ and 3+. Based on this fact, HER2-targeted treatment comprising trastuzumab is considered as a viable option in this patient population based on effects observed in pre-clinical models [C. Larbouret et al., 2012, Neoplasia 14(2), 121–130).

Using accepted staining and scoring methods, over-expression of HER2 was observed in approximately 6% of colorectal cancer (CRC) patients (A.N. Seo et al., 2014, PLoS ONE, 9(5): e98528). Based on this, HER2-targeting treatment may be effective in this subset of CRC patients. Two clinical trials have investigated the benefit of trastuzumab-containing combination therapy in advanced or metastatic CRC and clinical responses were observed in these trials providing evidence of treatment efficacy (R.K. Ramanathan et al., 2004, Cancer Invest. 22(6): 858–865; J. Clark et al., 2003, Proc. Am. Soc. Clin. Oncol. 22: abstr 3584). Moreover, one study suggested the inclusion of trastuzumab therapy as part of treatment regimens for (anti-EGFR monoclonal antibody) cetuximab-resistant CRC patients (A. Bertotti et al., 2011, Cancer Discov. 1(6): 508–523).

The management of advanced head and neck squamous cell cancer or carcinoma (HNSCC) consists of multiple-modality therapy with surgery, radiation, and

chemotherapy. Beckhardt et al. reported high HER2 over-expression in 16% of cell line samples, and moderate and low HER2 expression in 31% and 35% of samples, respectively (R.N. Beckhardt et al., 1995, Arch. Otolaryngol. Head Neck Surg. 121:1265-1270). This illustrates the potential therapeutic potential of trastuzumab treatment in HNSCC.

In 1999, Gorlick et al. reported over-expression of HER2 in 20 of 47 osteosarcoma samples, and showed that these patients had a poor response to therapy and a decreased rate of survival compared with patients whose tumors did not over-express this antigen (R. Gorlick et al., 1999, J. Clin. Oncol. 17:2781-8). Hence, HER2 emerged as a promising candidate for targeted biologic therapy in this indication. Recent findings from clinical investigation using trastuzumab indicate that anti-HER2 treatment can be safely delivered in combination with anthracycline-based chemotherapy and dexrazoxane (D. Ebb et al., 2012, J. Clin. Oncol. 30(20), 2545–2551).

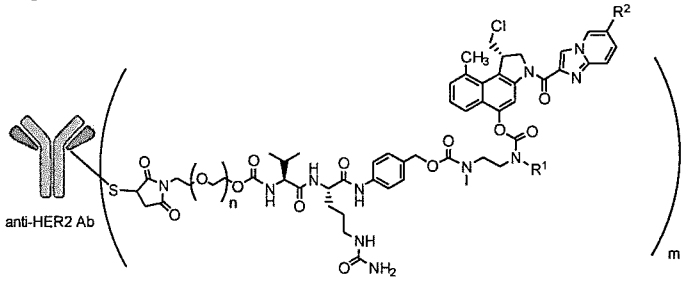
Further, HER2 over-expression is seen in approximately one-third of acute lymphoblastic leukaemia (ALL) patients, even more frequent in the presence of the Philadelphia translocation. Inhibition of HER2 induces apoptosis of the leukemia cells in vitro (M.E. Irwin et al., 2013, PLoS ONE, 8:8, e70608). In a Phase II study, it was demonstrated that trastuzumab treatment of refractory or relapsing adult B-ALL patients with HER2 over-expression in malignant B-cells resulted in an overall response rate of 13%, which shows the response of this disease to a HER2 targeting agent (P. Chevalier et al,, Blood, 2012, DOI 10.1182/blood-2011-11-390781).

Hence, there is a need for new HER2-targeted therapies, notably for treating patients with tumours and malignancies that have (i) a moderate or low IHC status, and/or (ii) a negative FISH status, and/or (iii) a hormone receptor (HR) negative status of the cancer tissue. Particularly, new regulatory approved therapies are needed for the targeted treatment of triple negative breast cancer (TNBC).

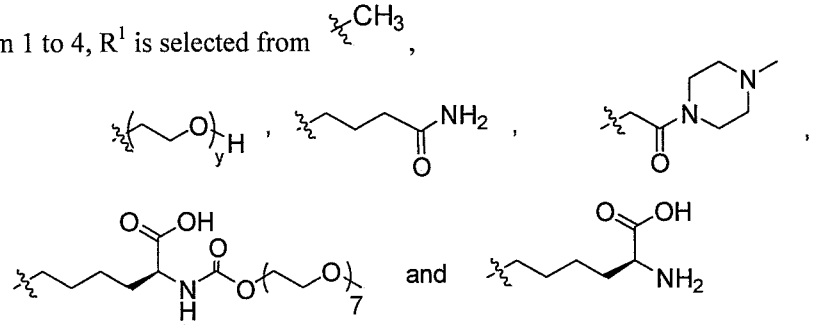
SUMMARY

The present invention relates to duocarmycin-containing antibody-drug conjugates (ADCs) for use in the treatment of human solid tumours and haematological malignancies expressing HER2, in particular breast cancer, gastric cancer, bladder cancer, ovarian cancer, lung cancer, prostate cancer, pancreatic cancer, colorectal cancer, head and neck squamous cell cancer, osteosarcoma, and acute lymphoblastic leukaemia.

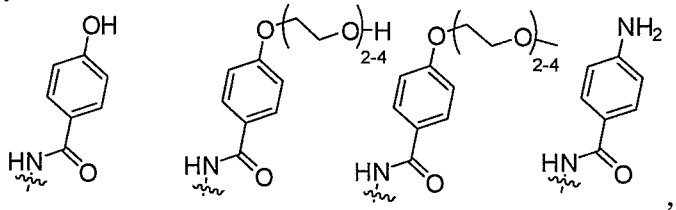
In one aspect, the present invention refers to use of a compound of formula (I)



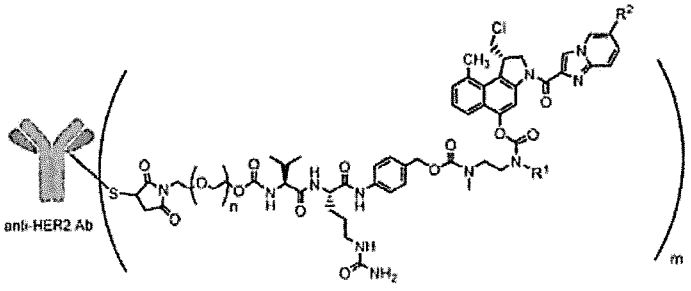
wherein anti-HER2 Ab is an anti-HER2 antibody or an anti-HER2-binding antibody fragment, n is 0-3, m represents an average DAR of from 1 to 4, R^1 is selected from



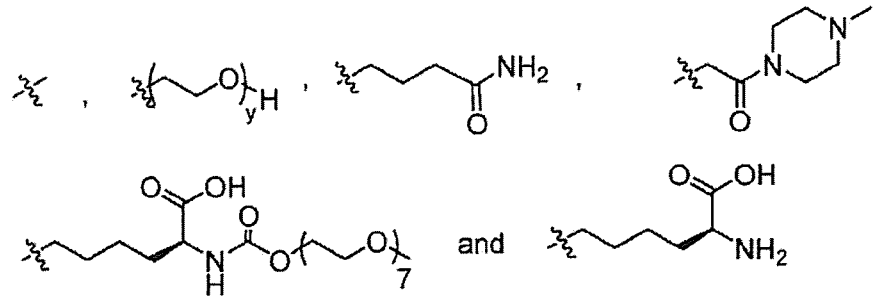
y is 1-16, and R^2 is selected from



, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+. In another aspect, the present invention refers to use of a combination of a compound as defined herein with a therapeutic antibody and/or a chemotherapeutic agent, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+. In yet another aspect, the present invention refers to use of a pharmaceutical composition comprising a compound as defined herein or a combination as defined herein, and one or more pharmaceutically acceptable excipients, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+. In another example, there is disclosed the use of a compound of formula (I)

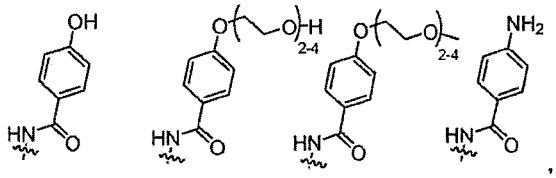


wherein anti-HER2 Ab is an anti-HER2 antibody or an anti-HER2-binding antibody fragment, n is 0-3, m represents an average DAR of from 1 to 4, R^1 is selected from



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 is HER2 IHC 2+ or 1+.

In one 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 human solid tumours expressing HER2.

In one aspect, there is provided a pharmaceutical composition for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+, 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. Antitumor activity of SYD985 compared to T-DM1 in MAXF-1162 PDX model (breast cancer, adenocarcinoma, HER2 IHC 3+, HER2 FISH positive) (CRO: Oncotest).

Figure 2. Antitumor activity of SYD985 compared to T-DM1 in HBCx-34 PDX model (breast cancer, ductal carcinoma, HER2 IHC 2+, HER2 FISH negative, ER and PR positive) (CRO: XenTech).

Figure 3. Antitumor activity of SYD985 compared to T-DM1 in MAXF 449 PDX model (breast cancer, invasive ductal carcinoma, HER2 IHC 1+, HER2 FISH negative, ER and PR negative, i.e. triple negative breast cancer) (CRO: Oncotest).

Figure 4. Antitumor activity of SYD985 compared to T-DM1 in HBCx-10 PDX model (breast cancer, ductal adenocarcinoma, HER2 IHC 1+, HER2 FISH negative, ER and PR negative, i.e. triple negative breast cancer) (CRO: XenTech).

Figure 5. Antitumor activity of SYD985 compared to T-DM1 in MAXF-MX1 PDX model (breast cancer, invasive ductal carcinoma, HER2 IHC 1+, HER2 FISH negative, ER and PR negative, i.e. triple negative breast cancer) (CRO: Oncotest).

Figure 6. Antitumor activity of SYD985 compared to T-DM1 in ST313 PDX model (breast cancer, HER2 IHC 2+, HER2 FISH negative, ER and PR positive) (CRO: Start).

Figure 7. Antitumor activity of SYD985 compared to T-DM1 in GXA3057 PDX model (gastric cancer, HER2 IHC 1+, HER2 FISH negative) (CRO: Oncotest).

Figure 8. Antitumor activity of SYD985 compared to T-DM1 in GXA3067 PDX model (gastric cancer, HER2 IHC 2+, HER2 FISH positive) (CRO: Oncotest).

Figure 9. Antitumor activity of SYD985 compared to T-DM1 in GXA3054 PDX model (gastric cancer, HER2 IHC 3+, HER2 FISH positive) (CRO: Oncotest).

Figure 10. Antitumor activity of SYD985 compared to T-DM1 in GXA3038 PDX model (gastric cancer, HER2 IHC 2+, HER2 FISH negative) (CRO: Oncotest).

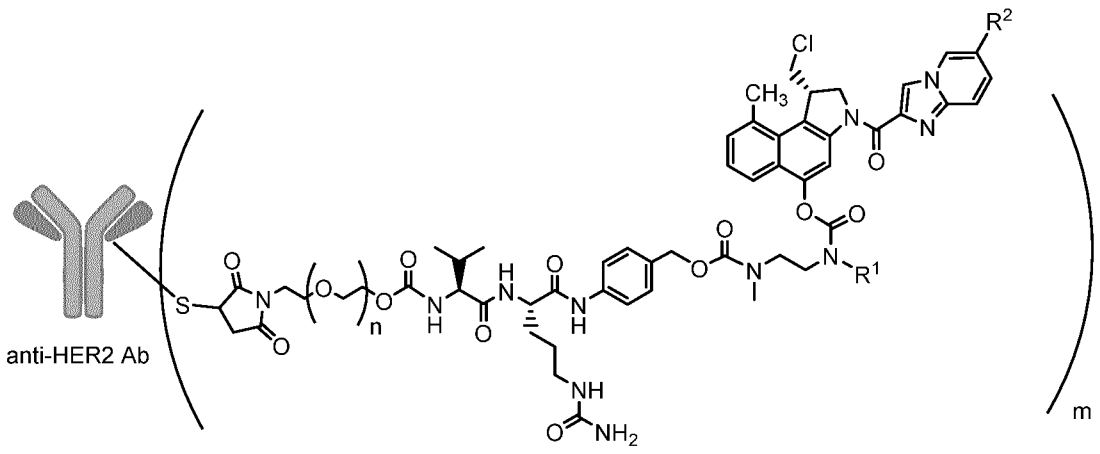
Figure 11. Antitumor activity of SYD985 in BXF439 PDX model (bladder cancer, HER2 IHC 3+, HER2 FISH positive) (CRO: Oncotest).

Figure 12. Antitumor activity of SYD983 in SKOV3 cell line-derived xenograft model (ovarian cancer, HER2 IHC 2+, HER2 FISH positive) (CRO: Piedmont).

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention relates to duocarmycin-containing ADCs for use in the treatment of human solid tumours and haematological malignancies expressing HER2.

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



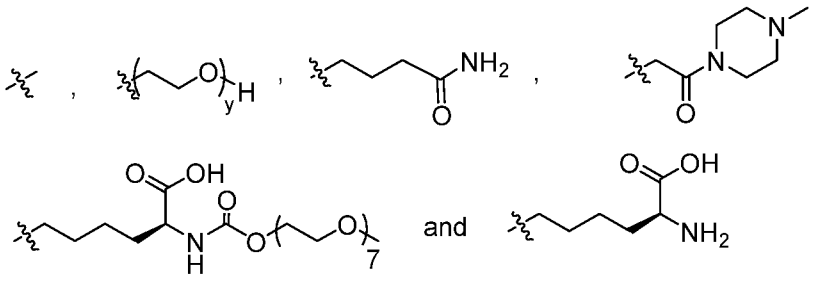
wherein

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

n is 0-3, preferably 0-1,

m represents an average DAR (drug-to-antibody ratio) of from 1 to 4,

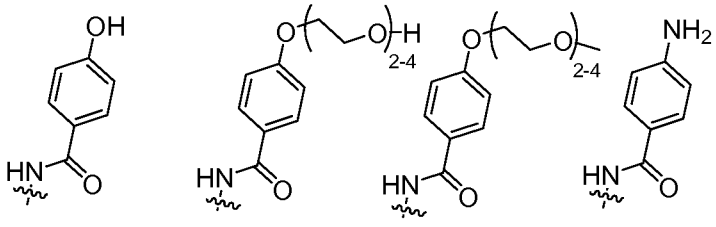
R^1 is selected from



and

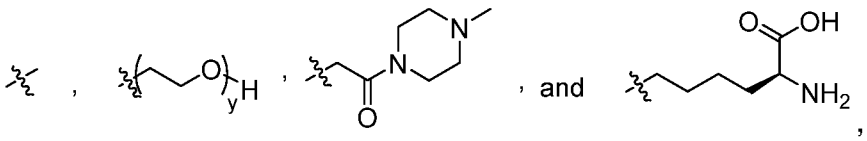
y is 1-16, and

R^2 is selected from

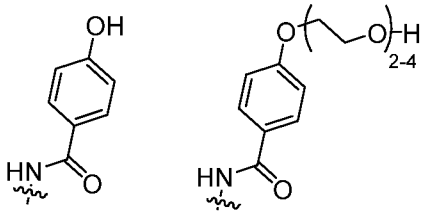


for use in the treatment of human solid tumours and haematological malignancies expressing HER2, in particular for use in the treatment of human solid tumours.

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

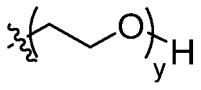


y is 1-16, preferably 1-4, and R^2 is selected from



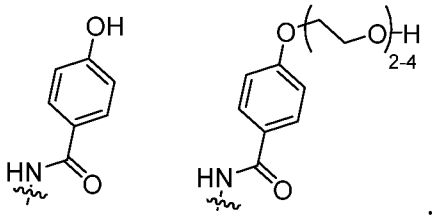
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In a further embodiment, the present invention relates to a compound of formula (I), wherein the anti-HER2 Ab is an anti-HER2 monoclonal antibody, n is 0-1, m represents an average DAR of from 2 to 3, preferably from 2.5 to 2.9, R^1 is selected from



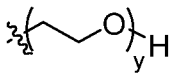
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y is 1-4, and R^2 is selected from



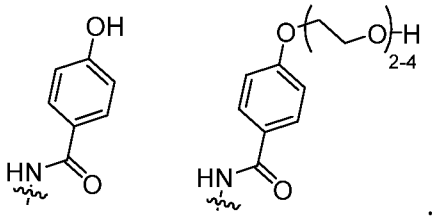
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In yet another embodiment, the present invention relates to a compound of formula (I), wherein the anti-HER2 Ab is trastuzumab or a biosimilar thereof, n is 0-1, m represents an average DAR of from 2 to 3, preferably 2.5 to 2.9, R^1 is selected from



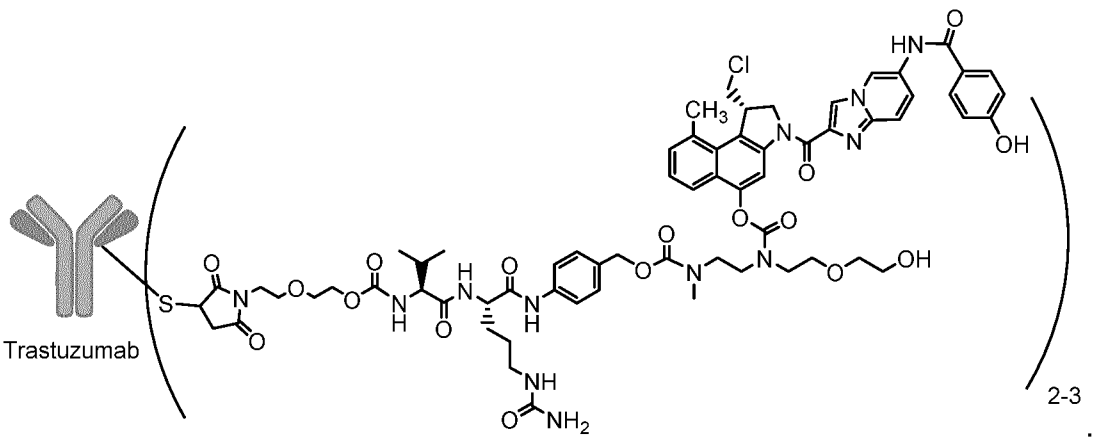
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y is 1-4, and R^2 is selected from



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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. SYD983 of formula (II) has an average DAR of 2.0.

In the structural formulae shown in the present specification, n represent 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.

Examples of human solid tumours which can be treated in accordance with the present invention are breast cancer, gastric cancer, bladder cancer, ovarian cancer, lung cancer, prostate cancer, pancreatic cancer, colorectal cancer, head and neck squamous cell cancer, and osteosarcoma, particularly breast cancer, gastric cancer, bladder cancer, ovarian cancer, lung cancer, and prostate cancer, more particularly breast cancer, gastric cancer, and bladder cancer (see also S. Scholl et al., 2001, Ann. Oncol., 12(1): S81-S87). An example of a haematological malignancy which can be treated in accordance with the present invention is acute lymphoblastic leukaemia (ALL). The scope of the present invention however is not restricted to these specific examples.

In one embodiment, the present invention provides a compound of formula (I) or (II) for use in the treatment of breast cancer, gastric cancer or bladder cancer, particularly breast

cancer or gastric cancer, especially breast cancer. Said breast cancer either is hormone receptor (ER and/or PR) positive or negative, advantageously ER and PR negative.

In another embodiment, the present invention provides a compound of formula (I) or (II) for use in the treatment of human solid tumours showing moderate or low expression of HER2 (i.e. HER2 IHC 2+ or 1+).

In yet another embodiment, the present invention provides a compound of formula (I) or (II) for use in the treatment of human solid tumours without HER2 gene amplification (i.e. HER2 FISH negative).

Unexpectedly, the present inventors have found that the duocarmycin-containing ADC compounds of the present invention particularly can be used for the treatment of human solid tumours, especially breast cancer and gastric cancer, 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 obtained marketing approval for the treatment of patients having such tumours. Furthermore, as shown in the Examples and Figures herein below, T-DM1 lacks efficacy in such tumours. Hence, the duocarmycin-containing ADC compounds of the present invention can be used for the treatment of patient groups for which there is no current HER2-targeted therapy available. The duocarmycin-containing ADC compounds that were tested in mice bearing an N87 (i.e. HER2 IHC 3+ gastric tumour) xenograft in Example 15 of WO2011/133039A indeed showed efficacy after a single i.v. dose of 12 mg/kg. However, there is nothing in this document suggesting the person skilled in the art to test - let alone expect to find efficacy - in lower grade HER2-expressing tumours (i.e. HER2 IHC 2+ or 1+) and/or without HER2 gene amplification (i.e. HER2 FISH negative), already at a dose of 3 mg/kg.

The present inventors surprisingly further found that the duocarmycin-containing ADC compounds of formula (I) or (II) show an improved in vivo antitumor activity in animal

tumour models as compared to T-DM1 (see Examples and Figures) and trastuzumab when administered at the same dose. Notably, it was found that the improvement was the highest in tumour models with the lowest grade of HER2 expression (i.e. IHC HER2 1+), in particular in (triple negative) breast cancer and gastric cancer.

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

In a particularly advantageous embodiment of the present invention, the human solid tumour is triple negative breast cancer (i.e. HER2 IHC 2+ or 1+, HER2 FISH negative, and ER and PR 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 immunodeficient mice bearing a subcutaneous xenograft. The xenograft can either be a (human) tumour cell line or a patient-derived (primary) tumour. Preferably, the animal model is a patient-derived tumour xenograft (PDX) model.

Human tumours in PDX models retain the biological characteristics of the original tumour as assessed by microscopic examination. PDX models are routinely used now in many academic institutions and are offered commercially by a number of Contract Research Organizations (CROs) including Jackson Lab (USA), Oncotest (Germany), Molecular Response (USA), Charles River (USA), Oncodesign (France), XenTech (France), Champions Oncology (USA), and Start (USA). Many have shown the retention of characteristic morphologic and immunohistochemical features of the original human tumour in the xenograft. Besides the close relationship with regards to biological characteristics, PDX models have a very good predictive value for therapeutic clinical outcome. In general, one

could state that reports from different sources indicate at least 90% correct replication of the response to therapy in the PDX compared to that in the patient, both in terms of sensitivity and resistance of the tumour to therapy (Website Champions Oncology, http://www.championsoncology.com/translational-oncology-solutions/predictive-value; Fiebig et al., 1984, Behring Inst. Mitt. 74:343-352; Hidalgo et al., 2011, Mol. Cancer Ther. 10:1311-1316).

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 interchain 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) or 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). 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). FISH positive means a FISH amplification ratio $\geq$2.0 (e.g. by using Dako HER2 FISH PharmDX™ test kit). FISH negative means a FISH amplification ratio <2.0.

HER2 expressing tumours which can be advantageously treated in accordance with the present invention are breast cancer and gastric cancer, particularly breast cancer, most particularly triple negative breast cancer. Unexpectedly, the present inventors have found that the ADC compounds in accordance with the present invention notably were effective in breast cancer PDX models which are HER2 IHC 2+ or 1+ and FISH negative, in triple negative breast cancer PDX models, and in gastric cancer PDX models which are HER2 IHC 2+ or 1+ and FISH negative, as shown in the Examples and Figures herein below. In view of the fact that PDX models have a very good predictive value for therapeutic clinical outcome, these findings particularly offer a new HER2-targeted treatment option for breast and gastric cancers for which there is currently no such approved treatment option available.

The present invention also relates to the use of a compound of formula (I) or (II) for the treatment of patients having human solid tumours or haematological malignancies expressing HER2, in particular of human solid tumours which are HER2 IHC 2+ or 1+ and/or which are 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 human solid tumours and haematological malignancies expressing HER2, in particular human solid tumours, most particularly for the treatment of triple negative breast cancer.

In one embodiment of the present invention, the therapeutic antibody is pertuzumab, bevacizumab, ramucirumab or trastuzumab and the chemotherapeutic agent is i) a taxane, particularly docetaxel, paclitaxel, nab-paclitaxel, or cabazitaxel, ii) a mitotic inhibitor, particularly eribulin, vinorelbine or vinblastine, iii) a DNA damaging agent, particularly 5-fluoro-uracil, capecitabine, gemcitabine, temozolomide, cisplatin, carboplatin, oxaliplain, cyclophosphamide or ifosfamide, iv) an anti-folate, particularly pemetrexed or methotrexate,

v) an anthracycline, particularly mitoxantrone, doxorubicin, liposomal doxorubicin, epirubicin, daunorubicin or valrubicin, more particularly doxorubicin, vi) an mTOR (mammalian target of rapamycin) inhibitor, particularly temsirolimus or everolimus, vii) a topo-isomerase inhibitor, particularly irinotecan or topotecan, viii) a tyrosine kinase inhibitor, particularly gefitinib, erlotinib, pazopanib, crizotinib, lapatinib or afatinib, ix) an androgen receptor modulating agent, particularly enzalutamide or abiraterone acetate, x) a steroid hormone, particularly prednisone, xi) a hormonal therapeutic agent, particularly tamoxifen, xii) an aromatase inhibiting or steroid modifying agent, particularly anastrozole, letrozole, fulvestrant or exemestane, or xiii) a PARP inhibitor, particularly olaparib. The person skilled in the art will have no difficulty in selecting suitable combination therapies for use in the treatment of human solid tumours and haematological malignancies expressing HER2.

In another embodiment of the present invention, the therapeutic antibody is pertuzumab and the chemotherapeutic agent is a taxane, particularly docetaxel or paclitaxel, or an anthracycline, particularly doxorubicin, epirubicin, daunorubicin or valrubicin, more particularly doxorubicin.

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 of 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 appropriate by the treating physician.

EXAMPLES

PDX HER2 gene amplification testing

Amplification of the HER2 gene was determined by in situ hybridization (ISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens using FDA approved tests from Ventana Medical Systems (INFORM HER2 Dual ISH DNA Probe Cocktail) or Abbott Molecular (PathVysion HER2 DNA Probe Kit). Protocols used were as detailed by the suppliers of the tests.

PDX HER2 IHC staining

Tissue sections of formalin-fixed, paraffin-embedded tumour xenograft samples were prepared. HER2 was bound by using a suitable Ab, for instance a polyclonal rabbit anti-human HER2 (DAKO Cat# A0485) antibody and detected by a suitable secondary Ab, for instance biotinylated goat anti-rabbit IgG (JacksonImmuno research, Cat# 111-065-04) and a Biozol (Cat # VEC-PK-4000) ABC kit. Staining was evaluated semi-quantitatively on a suitable microscope, for instance using a Zeiss Axiovert 35 microscope. Staining was interpreted as immunoreactivity, based on the number of stained tumour cells as well as the completeness and intensity of the membrane staining.

0 : <10% of the tumour cells exhibit membranous stain.

1 : >10% of the tumour cells exhibit membranous stain, but incomplete stain of surface.

2 : >10% of the tumour cells exhibit weak or moderate membranous stain distributed all over the surface.

3 : >30% of the tumour cells exhibit strong membranous stain distributed all over the surface.

Known HER2 positive (IHC 3+) and HER2 negative (IHC 0) control tumour slides were included in every HER2 staining procedure.

PDX and cell line-derived xenograft animal studies

All animal studies were approved by local animal ethical committees and were performed according to local ethical guidelines of animal experimentation. Female immunodeficient nu/nu mice (4-6 weeks of age) or SCID mice from a professional animal breeder like Harlan or Charles River were used and randomization was performed according to the detailed protocols of the respective CROs, as described for instance by Fiebig *et al.* in Cancer Genomics & Proteomics 4: 197-210, 1997.

All breast and gastric PDX studies were performed testing SYD985 head-to-head to T-DM1, since the latter ADC was approved for treatment of patients with HER2-positive metastatic breast cancer and approval for T-DM1 in HER2-positive gastric cancer is currently being pursued. Studies in other indications (bladder and ovarian) were done using SYD985 only, since T-DM1 is not an approved drug in those indications. Mice were treated with either vehicle, 3 mg/kg SYD985 or 3 mg/kg T-DM1 in all breast PDX models (Figures 1-6) and with vehicle, 10 mg/kg SYD985 or 10 mg/kg T-DM1 in all gastric PDX models (Figures 7-10). Mice were treated with vehicle or 10 mg/kg SYD985 in the bladder PDX model (Figure 11) and with vehicle or 15 mg/kg SYD983 in the cell line-derived ovarian xenograft model (Figure 12). All treatments were conducted at day 0 by a single dose, i.v. injection into the tail vein. Data, depicted as mean tumour volume ± S.D., consists of 6-8 animals per experimental group. Body weight and tumour size were measured twice weekly. The tumour volume was determined by a two-dimensional measurement with callipers. Termination

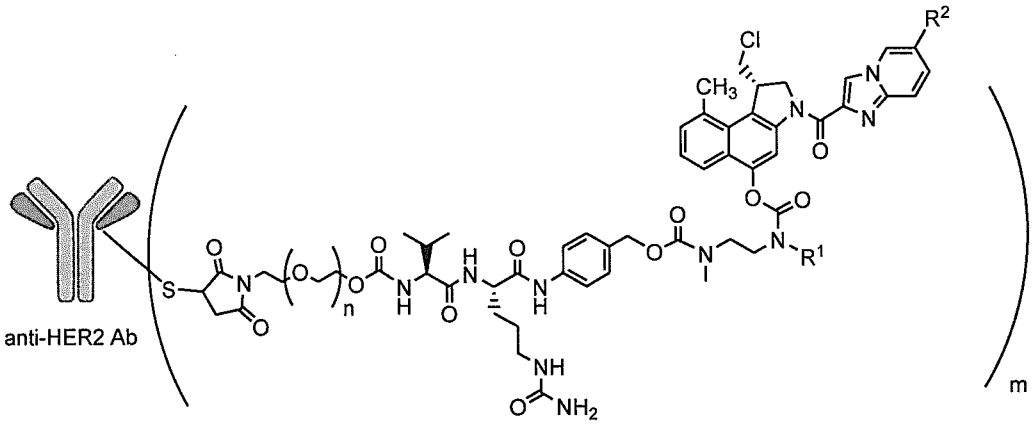
criteria included among others a tumour volume >2000 mm^3 or a body weight loss >30%. Tumour size of individual animals was processed using GraphPad Prism. The results are shown in Figures 1 to 12.

First-in-human clinical study

A two-part first-in-human phase I study (with expanded cohorts) with the antibody-drug conjugate SYD985 (trastuzumab vc-*seco*-DUBA) is being performed to evaluate the safety, pharmacokinetics and efficacy in patients with locally advanced or metastatic solid tumours (i.e. NCT02277717). Part I is the dose-escalation part in which a low dose of SYD985 is given to three cancer patients (females or males having solid tumours of any origin). If it is well tolerated, a higher dose of SYD985 will be given to three other cancer patients. This will continue until it is not safe anymore to increase the dose further. In part II of the study, several groups of patients with a specific type of cancer (including breast and gastric tumours) will receive the SYD985 dose selected for further development. All patients from both parts of the study (it is estimated that a total of 76 patients will be enrolled) will receive SYD985 (intravenous) infusions every three weeks until progression of the cancer or unacceptable toxicity develops.

CLAIMS

1. Use of a compound of formula (I)



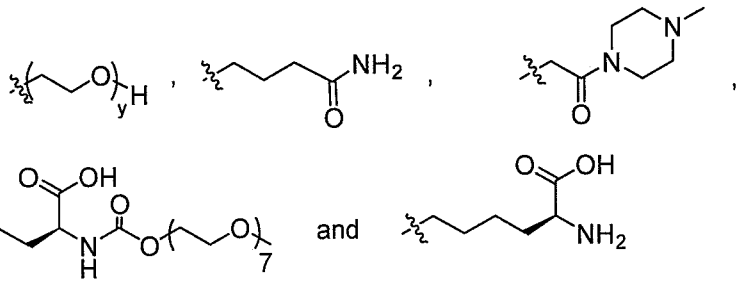
wherein

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

n is 0-3,

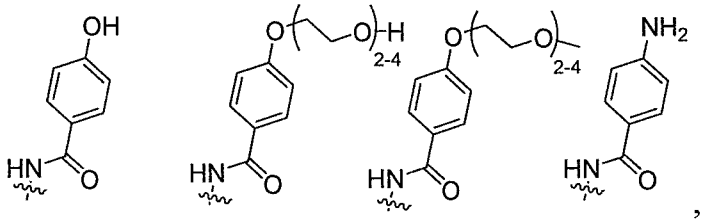
m represents an average DAR of from 1 to 4,

R^1 is selected from ,



y is 1-16, and

R^2 is selected from



,

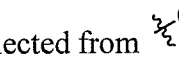
in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+.

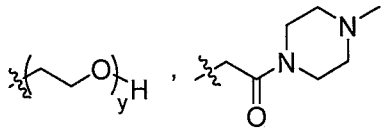
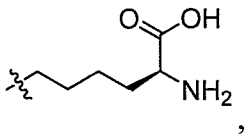
2. The use according to claim 1, wherein

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

n is 0-1,

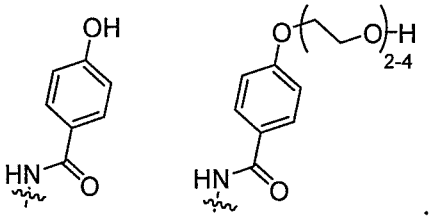
m represents an average DAR of from 1 to 4,

R^1 is selected from ,

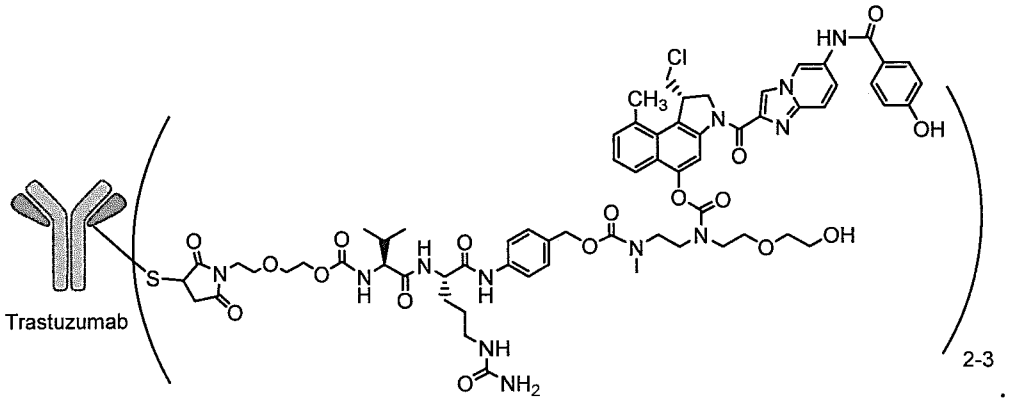
, and ,

y is 1-16, and

R^2 is selected from

.

3. The use according to claim 1 or 2 of formula (II)



4. The use according to any one of claims 1 to 3, wherein the HER2 expressing human solid tumour is breast cancer, gastric cancer, bladder cancer, ovarian cancer, lung cancer, prostate cancer, pancreatic cancer, colorectal cancer, head and neck squamous cell cancer or osteosarcoma.

5. The use according to any one of claims 1 to 4, wherein the HER2 expressing human solid tumour is breast cancer or gastric cancer.

6. The use according to any one of claims 1 to 5, wherein the breast cancer is hormone receptor positive or negative.

7. The use according to any one of claims 1 to 6, wherein the human solid tumour is HER2 FISH negative.

8. The use according to any one of claims 1 to 7, wherein the human solid tumour is triple negative breast cancer.

9. Use of a combination of a compound as defined in any one of claims 1 to 3 with a therapeutic antibody and/or a chemotherapeutic agent, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+.

10. The use according to claim 9, in the treatment of breast cancer, particularly triple negative breast cancer.

11. The use according to claim 9 or 10, wherein the therapeutic antibody is pertuzumab and the chemotherapeutic agent is a taxane, particularly docetaxel or paclitaxel, or an anthracycline, particularly doxorubicin, epirubicin, daunorubicin or valrubicin.

12. Use of a pharmaceutical composition comprising a compound as defined any one of claims 1 to 3 or a combination as defined in any one of claims 9 or 11, and one or more pharmaceutically acceptable excipients, in the manufacture of a medicament for the treatment of human solid tumours expressing HER2, wherein the human solid tumour is HER2 IHC 2+ or 1+.

13. The use according to claim 12, wherein the pharmaceutical composition is in the form of a lyophilized powder or a frozen solution.