(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 August 2024 (22.08.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/173281 A1

(51) International Patent Classification:
A61K 39/44 (2006.01) *A61P 35/00* (2006.01)
A61K 47/68 (2017.01)

(21) International Application Number:
PCT/US2024/015450

(22) International Filing Date:
12 February 2024 (12.02.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/484,699 13 February 2023 (13.02.2023) US
63/590,731 16 October 2023 (16.10.2023) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: ANTIBODY-DRUG CONJUGATE FOR CANCER TREATMENT

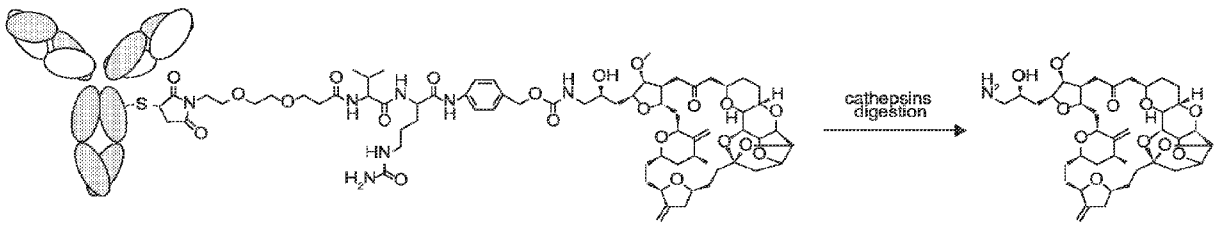

FIG. 1A

(57) Abstract: Methods of using an antibody-drug conjugate (ADC) Ab-(L-D)$_p$ or its compositions to treat various cancers are provided, where Ab is an anti-HER2 antibody moiety (e.g., an antibody having the same structure as trastuzumab), L is a cleavable linker comprising Mal-(PEG)$_2$-Val-Cit-pAB, D is eribulin, and p is an integer from 1 to 8 inclusive.

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ANTIBODY-DRUG CONJUGATE FOR CANCER TREATMENT

Background

HER2, a member of the epidermal growth factor receptor (EGFR/ErbB) family encoded by the ERBB2 gene, is frequently amplified and overexpressed in various types of cancer, including approximately 20-25% of breast cancers, 20-30% of bladder, 10-30% of gastric esophageal, and 20% of lung cancers (1-3). This gene amplification and overexpression is associated with a poor prognosis of the relevant malignancies. Enhanced or constitutive HER2 activity is believed to play a driving role in the malignancy of HER2-expressing human tumor cells. As a clinically validated therapeutic target, a number of therapeutic agents, including antibodies, small molecules, and antibody-drug conjugates (ADCs), have been approved for the treatment of patients with HER2-expressing tumors (4-6).

Unlike anti-HER2 antibodies and small molecule tyrosine kinase inhibitors (TKIs) which inhibit tumor growth through interference with HER2 downstream signaling pathways, HER2-targeting ADCs utilize the high specificity of anti-HER2 antibodies to deliver potent cytotoxic agents to HER2-expressing cells and directly kill the target cancer cells (4-6). Despite the possibility of cancer cells developing resistance to anti-HER2 antibodies or TKI treatment due to mutations in the HER2 gene, downstream signaling molecules, or other genes that may compensate for HER2 signaling pathways, the unique mechanism of action of ADCs may overcome this resistance (7). Kadcyla (T-DM1), which consists of trastuzumab and a tubulin polymerization inhibitor, was the first HER2-targeting ADC approved for the treatment of breast cancer patients whose tumors had become resistant to other HER2-targeting therapies.

Recently, ADCs containing new anti-HER2 antibodies or new toxin payloads such as Dxd or MMAE, Enhertu (Trastuzumab deruxtecan, DS-8201a) and AIDIXI® (RC48), have demonstrated promising responses in HER2-positive breast and gastric cancers, as well as in patient populations that do not typically respond well to other HER2-targeting therapies, such as low HER2-expressing breast cancers and urothelial carcinoma (8-11). These newer generations of ADCs significantly expand HER2-targeting indications, but still face challenges of resistance and/or severe side effects associated with their specific payload toxins. Enhertu has reported an overall response rate of 80% in HER2-positive breast cancer, though the response rate in other indications (gastric, lung, and other cancers) was significantly lower, indicating the presence of

primary resistance in these patient populations. Enhertu also reported approximately 12-18% of interstitial lung disease (ILD) in the breast cancer clinical studies (**DESTINY-Breast03 & 04)** and 7% patients who presented with Grade 3- 4 ILD in **DESTINY-Breast03 study and** 0.5% patients who presented with Grade 5 ILD in **DESTINY-Breast04 study** (10-11). Therefore, ADCs containing toxins with different mechanisms of action and desired safety profiles may have an advantage in overcoming resistance to current treatments.

Eribulin is a synthetic analog of the marine macrolide halichondrin B that has been approved for the treatment of metastatic breast cancer and liposarcoma (12). Its unique mechanism of action involves binding to the interdimer interface or the β-tubulin subunit alone, inhibiting only the growth of microtubules with no effect on shortening. This distinguishes it from taxanes and vinca alkaloids, which suppress both the growth and shortening phases of microtubule dynamic instability. The unique tubulin-based mechanism of eribulin may contribute to its ability to overcome taxane resistance and display a wider range of anti-cancer activity (12, 13).

Summary of the Invention

In one aspect of the present disclosure, therapeutic uses of an antibody-drug conjugate (ADC) is provided. In some embodiments, the ADC takes the form of Ab-(L-D)$_p$, where Ab is an anti-HER2 antibody moiety (e.g., an antibody having the same structure as trastuzumab), L is a cleavable linker comprising maleimido-PEG2-valine-citrulline-p-aminobenzylcarbamyl (or Mal-(PEG)$_2$-Val-Cit-pAB), D is eribulin, and p is an integer from 1 to 8 inclusive.

Also provided herein are therapeutic uses of compositions comprising multiple copies of the described ADCs, wherein the average drug loading (average p) of the ADCs in the composition is between about 2 and 8, or between 3 and 5, or about 3.5 to about 4.5, or about 4.

Further provided herein are therapeutic uses of pharmaceutical compositions comprising the ADC described herein and a pharmaceutically acceptable carrier(s).

The therapeutic uses include treating a proliferative disease, disorder, or condition in a subject or patient (a mammal, or a human being). For example, the present disclosure provides methods of treating a cancer that expresses an antigen targeted by the antibody moiety of the ADC, such as HER2. In various embodiments, methods are provided of killing or inhibiting the proliferation of tumor cells or cancer cells by administering a therapeutically effective amount and/or regimen of any one of the described ADCs or their compositions. In some embodiments,

the cancer is HER2-low expressing breast cancer, HER2-expressing or HER2 mutation non-small cell lung cancer, parotid cancer, urothelial carcinoma, gastric/esophagogastric junction cancer. In some embodiments, the cancer can be HER2 breast cancer, bladder cancer, urothelial carcinoma salivary gland cancer, breast cancer, biliary tract (or bile duct) cancer, ovarian cancer, urologic cancer, endometrial cancer, cervical cancer, lung cancer, stomach cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, tonsil cancer, head and neck tumors, squamous cell carcinoma of the head and neck, liver cancer, mucoepidermoid carcinoma, rectal cancer, oral cavity tumors (or mouth cancer), cholangiocarcinoma, laryngeal tumors, colorectal cancer, urinary system tumors, lip tumors, larynx cancer, palate cancer, mucoepidermoid carcinoma, primary mucinous adenocarcinoma, recurrent cholangiocarcinoma, peritoneum malignant tumors, gastric cancer, tongue tumors, skin tumors, renal cell carcinoma, gastroesophageal junction adenocarcinoma, non-small cell lung cancer, uterine cancer, endometrioid cancer, HER2-mutant non-small cell lung cancer, HER2-positive gastric cancer, HER2-low expression breast cancer, or HER2-positive breast cancer. In some embodiments, the patient is one whose cancer has demonstrated resistance to other HER2-targeting therapeutics including other anti-HER2 antibody drug conjugate treatment.

Brief Description of the Drawings

Figure 1 shows structure and characterization of BB-1701, an ADC of the present disclosure. Figure 1A shows basic structure of BB-1701 and predicted eribulin release upon cathepsins digestion. Figures 1B & 1C shows plasma stability study of BB-1701: Tab (Trastuzumab, ♦), ADC (■) and free eribulin (▲) measurement in human (1B) and monkey (1C) plasma samples where BB-1701 was incubated for up to 21 days.

Figure 2 shows BB-1701 binding to HER2. A: Binding to HER2 (BIAcore). B: Binding to HER2 (ELISA).

Figure 3 shows BB-1701 binding to endogenous HER2 expressed on A: NCI-N87; B: BT-474 or C: JIMT-1 cells.

Figure 4 shows internalization of BB-1701 in BT-474 cancer cells.

Figures 5 shows *in vitro* cytotoxicity. Cytotoxicity curves of cell lines of different HER2 expression levels treated with BB-1701 (T-eribulin) and DS-8201a (T-Dxd). Relative HER2

expression of each cell line was evaluate by flow cytometry, left peak and right peak represented vehicle and anti-HER2 antibody-stained cell populations, respectively.

Figure 6 shows *in vivo* tumor suppression in xenograft models that were insensitive to DS-8201a treatment: Each point represents the mean and standard error.

Figures 7A-7C show the bystander effect of BB-1701. *in vivo* bystander effect with co-inoculation of N87 (HER2+++) and U87-RFP(HER2-null) cells. (A) tumor volume of the mixed tumor; (B) live imaging of the tumor bearing mice from dosing day (day0) to day 9; (C) Average luminescence in (B). Figures 8D-8F shows U87-mCherry only tumors volume and luminescence. D: live imaging of tumor bearing mice in PBS and BB-1701 treatment groups on day 30; E: statistical analysis of mCherry luminescence in D; F: tumor volume of PBS or BB-1701 treated U87-mCherry model. Each point represents the mean and standard error. N.S., not significant.

Figures 8A-8F show immunogenic cell death induced by BB-1701. (A) Calreticulin expression on the cell surface of BT-474 cells treated with eribulin or BB-1701, analyzed with flow cytometry. (B) ATP level was quantified in the culture medium of 2nM eribulin or 0.167nM BB-1701 treated BT-474 cells at indicated time points. (C) IHC analysis of HMGB1 expression in PBS (left box) and BB-1701 (5 mg/kg, right box) treated BT-474 tumors *in vivo*, black arrow heads indicate the tumor cells experiencing ICD where HMGB1 translocate to cytosol from nuclear area. (D) IHC analysis of macrophage infiltration (F4/80 staining) in PBS (left box) and BB-1701 (5 mg/kg, right box) treated BT-474 tumors *in vivo*.

Figure 9 shows mean serum concentration-time curve of BB-1701 ADC and Tab following single *i.v.* administration at 2, 4, 8 mg/kg in cynomolgus monkeys.

Figures 10A is the Waterfall of target lesion changes in patients of HER2 positive breast cancer treated with BB-1701. Figure 10B is the Swimmer plot treatment duration of BB-1701 in patients with HER2 positive breast cancer.

Figure 11A: Waterfall plot of target lesion changes in patients of HER2 low expressing breast cancer treated with BB-1701. Figure 11B: Swimmer plot treatment duration of BB-1701 in patients with HER2 low expressing breast cancer.

Figure 12A: Waterfall plot of target lesion changes in NSCLC patients with Her2 amplification or mutations treated with BB-1701 (data cutoff date Sep, 30th 2023). Figure 12B: Swimmer plot treatment duration of BB-1701 in NSCLC patients with HER2 amplification or mutations (data cutoff date Sep. 30th, 2023)

Detailed Description

In one aspect of the present disclosure, an antibody-drug conjugate (ADC) is provided. In some embodiments, the ADC takes the form of Ab-(L-D)$_p$, where Ab is an anti-HER2 antibody moiety (e.g., an antibody having the same structure as trastuzumab), L is a cleavable linker comprising maleimido-PEG2-valine-citrulline-p-aminobenzylcarbamyl (or Mal-(PEG)$_2$-Val-Cit-pAB), D is eribulin, and p is an integer from 1 to 8 inclusive.

Also provided herein are compositions comprising multiple copies of the described ADCs, wherein the average drug loading (average p) of the ADCs in the composition is between about 2 and 8, or 3 and 5, or about 3.5 to about 4.5, or about 4.

Further provided herein are pharmaceutical compositions comprising an ADC and a pharmaceutically acceptable carrier.

Another aspect of the present disclosure includes therapeutic uses for the described ADC compounds and compositions, e.g., in treating a cancer that expresses an antigen targeted by the antibody moiety of the ADC, such as HER2. In various embodiments, methods are provided of killing or inhibiting the proliferation of tumor cells or cancer cells by administering a therapeutically effective amount and/or regimen of any one of the described ADCs. In some embodiments, the cancer is HER2 breast cancer, HER2-low expressing breast cancer, HER2-expressing or HER2 mutation non-small cell lung cancer, bladder cancer, urothelial carcinoma, or gastric/esophagogastric junction cancer. In some embodiments, the cancer includes salivary gland cancer, breast cancer, biliary tract (or bile duct) cancer, ovarian cancer, urologic cancer, endometrial cancer, cervical cancer, lung cancer, stomach cancer, pancreatic cancer, colorectal cancer, bladder cancer, prostate cancer, tonsil cancer, head and neck tumors, squamous cell carcinoma of the head and neck, liver cancer, mucoepidermoid carcinoma, rectal cancer, oral cavity tumors (or mouth cancer), cholangiocarcinoma, laryngeal tumors, colorectal cancer, urinary system tumors, lip tumors, larynx cancer, palate cancer, mucoepidermoid carcinoma, primary mucinous adenocarcinoma, recurrent cholangiocarcinoma, peritoneum malignant tumors, gastric cancer, tongue tumors, skin tumors, renal cell carcinoma, gastroesophageal junction adenocarcinoma, non-small cell lung cancer, uterine cancer, endometrioid cancer, HER2-mutant non-small cell lung cancer, HER2-positive gastric cancer, HER2-low expression breast cancer, or HER2-positive breast cancer.

In some embodiments, the patient is one whose cancer has demonstrated resistance to other HER2-targeting therapeutics including other anti-HER2 antibody drug conjugate treatment.

Trastuzumab is a recombinant IgG1 kappa, humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of the human epidermal growth factor receptor protein. Under the brand name Herceptin, it was first FDA approved in 1998 to treat HER2+ metastatic breast cancer, and in 2006, it was approved by the FDA to treat Her2+ early breast cancer, and in 2010, it was approved by the FDA to treat HER2+ metastatic stomach cancer.

In some embodiments of the ADC, the drug-linker can be attached to the antibody moiety through cysteine coupling, amine coupling, terminal coupling, enzymatic coupling, or carbohydrate coupling.

In the present disclosure also provide pharmaceutically acceptable salts of the ADCs or compositions described herein, which include acid addition salts of inorganic acids, carboxylic acids and sulfonic acids, for example, salts of the following acids: hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, naphthalene disulfonic acid, acetic acid, trifluoroacetic acid, propionic acid, lactic acid, tartaric acid, malic acid, citric acid, fumaric acid, maleic acid and benzoic acid. The pharmaceutically acceptable salts of the antibody-drug conjugates of the present disclosure also include salts of conventional bases, for example alkali metal salts (e.g., sodium salts and potassium salts), alkaline earth metal salts (e.g., calcium salts and magnesium salts) and ammonium salts derived from ammonia or organic amines containing from 1 to 16 carbon atoms, in which the organic amines are, for example, ethylamine, diethylamine, triethylamine, ethyl diisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzamide, N-methylpiperidine, N-methylmorpholine, arginine, lysine and 1,2-ethylenediamine.

In the present disclosure, "subject" refers to mammal, such as cattle, cats, dogs, and horses, primates, mice and rats. In certain embodiments, the mammal refers to a human.

The term "about" as used herein with reference to a certain given value or quantity herein means a range of up to 25% deviation from (greater or smaller than) the given value.

The term "isolated antibody" as used herein refers to an antibody that is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to an antigen is substantially free of antibodies that do not bind to that antigen. The term "monoclonal antibody" as used herein refer to a preparation of a population of antibody molecules of substantially homogeneous molecular composition, wherein the individual antibodies in the population of the antibody molecules are identical except for possible naturally occurring mutations that may be present in miniscule amounts.

In further aspect, the present disclosure provides a pharmaceutical composition comprising the ADCs herein or the pharmaceutically acceptable salts thereof, together with a pharmaceutically acceptable carrier for the treatment of various HER2-expressing cancers described herein. As used herein, "pharmaceutically acceptable carrier" includes pharmaceutically acceptable diluents, or stabilizers, or other excipients. These include but are not limited solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, surface active agents, thickening or emulsifying agents, solid binders, dispersion or suspension aids, solubilizers, colorants, flavoring agents, coatings, disintegrating agents, lubricants, sweeteners, preservatives, isotonic agents, and the like that are physiologically compatible. The selection of a suitable carrier is within the knowledge of an artisan skilled in the art. The composition may comprise one or more additional pharmaceutically active ingredients, such as another antibody, a drug, e.g., a cytotoxic or anti-tumor agent.

Pharmaceutical composition can be suitable for intravenous, intramuscular, subcutaneous, parenteral, epidermal, and other routes of administration. Depending on the route of administration, the active ingredient can be coated with a material or otherwise loaded in a material or structure to protect it from the action of acids and other natural conditions that may inactivate it. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, the composition of the invention can be administered via a non-parenteral route, such as a topical,

epidermal or mucosal route of administration, e.g., intranasally, orally, vaginally, rectally, sublingually or topically.

In a further aspect, the present invention provides a method of treating cancer in a human subject, comprising administering an effective amount of the ADC (such as BB-1701) or the pharmaceutical composition herein containing the ADC. The cancer can be a cancer associated with expression of HER2. Her2 positive breast cancer, HER2-low expressing breast cancer, HER2-expressing or HER mutation non-small cell lung cancer, bladder cancer, urothelial carcinoma, gastric/esophagogastric junction cancer, biliary tract cancer, ovarian cancer, endometrial cancer, cervical cancer. In some embodiments, the patient is one whose cancer has demonstrated resistance to other HER2-targeting therapeutics including other anti-HER2 antibody drug conjugate treatment. The route of administration can be intravenous, intramuscular, subcutaneous, parenteral, epidermal, and other routes of administration.

In the administration of the composition to the subject, dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). Single bolus or divided doses can be administered based on the subject, the disease to be treated, etc. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated. Each unit contains a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Sustained release formulation can be used in which case less frequent administration is required.

For administration of an antibody or ADC pharmaceutical salts thereof of the present disclosure, the dosage may range from about 0.0001 to 100 mg/kg, and more usually 0.01 to 10 mg/kg, of the body weight of the subject. For example, dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. A suitable treatment regime can be once per week, once every two weeks, once every three weeks, once every four weeks, once a month, etc. Example dosage regimens for an anti-HER2 antibody of the invention can include 1 mg/kg body weight or 10 mg/kg body weight via intravenous administration.

A "therapeutically effective amount" or "effective amount" of the ADCs or compositions herein preferably results in a decrease in severity of disease symptoms, an increase in frequency and/or duration of disease symptom-free periods, prevention or reduction of likelihood of impairment or disability due to the disease affliction, or inhibition or delaying of the progression

of disease. For example, for the treatment of tumor-bearing subjects, a "therapeutically effective amount" of the ADC or compositions herein may inhibit tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects.

Examples

Part A: Pre-clinical Evaluation

Generation and characterization of an example of the anti-HER2 eribulin containing ADC, BB-1701

As shown in Fig. 1A, BB-1701 is composed of an anti-HER2 antibody linked to a cytotoxic payload of eribulin, a synthetic analog of the marine macrolide halichondrin B. The sequence of the anti-HER2 antibody used in BB-1701 is identical to that of the well-known trastuzumab which was produced by mammalian cell culture in CHO cells and purified with a process that includes three chromatography steps and a dedicated viral clearance step. The conjugation of the eribulin payload to the antibody is achieved through a maleimido-PEG2-valine-citrulline-p-aminobenzylcarbamyl linker. Each of BB-1701 molecule can include 2, 4, 6, 8 linked drug portion, and in this study the drug distribution as determined by hydrophobic interaction chromatography (HIC) had an average Drug to Antibody Ratio (DAR) of about 4.

The linker peptide (valine-citrulline, VC) can be decomposed by lysosomal cathepsins which are highly expressed in tumor cells. After binding to HER2 molecules on the cell membrane and internalized into tumor cells, BB-1701 is cleaved by lysosomal enzymes (14), the p-aminobenzycarbamyl (pAB) spacer undergoes self-immolation upon cleavage of VC linker, and free eribulin, not the eribulin-linker construct, is the primary form of free toxin after being released from the ADC (Fig. 1A). Study of *in vitro* plasma stability where BB-1701 was incubated in human or cynomolgus monkey plasma for up to 21 days revealed that both ADC and TAb appeared to be stable during the 21-day incubation (Fig. 1B and 1C,). There was a trend of time-dependent increase of free eribulin released in plasma, however, there was no more than 0.2% of conjugated eribulin in BB-1701 that was released even at the end of incubation time (day 21), (Fig. 1B, and 1C), suggesting that BB-1701 would be stable in circulation.

To determine the potential impact of the eribulin payload on the binding and internalization properties of the anti-HER2 antibody, *in vitro* studies were conducted comparing BB-1701 with the naked antibody. The results of these studies, which included binding to recombinant human HER2, binding to HER2 expressed on the cell membrane, and internalization into cancer cells, indicated that there were no significant differences between BB-1701 and the naked antibody in any of these measured properties (Figs. 2-4). This suggests that the conjugation of the eribulin payload did not negatively affect the ability of the anti-HER2 antibody to engage and internalize within target cancer cells.

Cytotoxicity activity of BB-1701 in comparison with DS-8201a

The cytotoxic activity of BB-1701 was evaluated and compared with DS-8201a which had the same anti-HER2 antibody linked to a different toxin payload Dxd (exatecan derivative for ADC) in four cell lines: human breast cancer cell line BT-474, gastric cancer cell line NCI-N87, lung cancer cell line NCI-H1975 and A549, where HER2 expression of these cell lines were analyzed by flow cytometry and histogram graphs were shown in the corresponding kill curves graphs in Fig. 5. BT474 and NCI-N87 have previously been found to be sensitive to DS-8201a. Results showed that BB-1701 had higher potencies against NCI-N87 and BT-474 cells than DS-8201a, with IC_{50} values that were 5-10-fold lower than the IC_{50} values of DS-8201a-treated cells (Fig. 5 upper panels). Under the same assay conditions, DS-8201a was unable to completely suppress cell growth, as indicated by the higher low-plateau in the kill curves compared with BB-1701. In contrast, while BB-1701 retained some cytotoxicity against NCI-1975 and A549 cells, these two cell lines were largely resistant to DS-8201a treatment. These findings suggest that BB-1701 may be a more effective treatment option for certain cancer types compared with DS-8201a (Fig. 5 lower panels).

Potent anti-tumor activity in tumors insensitive to DS-8201a

The *in vivo* antitumor activity of BB-1701 was also evaluated in several animal models that had developed resistance to DS-8201a treatment. In a model derived from a non-small cell lung cancer line NCI-H1975 cells, when given a single dose of 5 mg/kg, DS-8201a was unable to suppress tumor growth (Fig. 6). In contrast, BB-1701 greatly suppressed tumor growth, with a TGI of over 90%, and caused 40% tumor regression (2/5 CR) (Fig. 6). No gross toxicity (bodyweight

loss, food consumption or any other behavior changes) was noted with the treatment of BB-1701 in the tested model (data not shown). Overall, these results suggest that BB-1701 may be a potentially effective treatment option for tumors that are resistant to the best known HER2-targeting ADC on the market.

Bystander effect of BB-1701

The bystander effect of BB-1701 was investigated.

In an *in vivo* study, Her2-null U87-RFP cells (which have stable expression of the red fluorescent protein mCherry) were co-inoculated with NCI-N87 (HER2+++) cells into mice. When given a single low-dose (3 mg/kg) treatment, BB-1701 caused significant tumor growth suppression compared with PBS treatment (Fig. 7A). Additionally, while the mean fluorescence intensity in the PBS-treated group increased steadily over the course of the study, the mean fluorescence intensity in the BB-1701-treated group remained largely unchanged (Fig. 7B-C). This suggests that the bystander effect of BB-1701 may have contributed to the suppression of HER2-null U87-RFP tumor cell growth in mix-cell tumors. In a separate experiment with mice bearing tumors of U87-RFP cells only, BB-1701 treatment at the same dose did not slow down tumor growth or reduce the increase in mean fluorescence intensity (Fig. 7D-F). The strong bystander effect observed in these results suggests that BB-1701 may be a promising treatment option for HER-low breast cancer and other indications with heterogeneous HER2 expression.

BB-1701 induced ICD and the activation of the immune system

Certain chemotherapy drugs, oncolytic viruses, and radiotherapy can stimulate the production of neoantigens and promote immunogenic cell death (ICD) in cancer cells, which is characterized by the release of molecules called damage-associated molecular patterns (DAMPs) that can stimulate the immune system (15). To determine if the treatment BB-1701 can induce ICD, BT-474 cells were exposed to increasing concentrations of either BB-1701 or eribulin. Expression of calreticulin, a type of DAMP, was found increased in a dose-dependent manner in BT-474 cells treated with either BB-1701 or eribulin (Fig. 8A). The EC_{50} values (the concentration at which half-maximum calreticulin was induced) for BB-1701 and eribulin were similar to their cytotoxic IC_{50} values (the concentration at which 50% of the cells were killed) for this cell line (Fig. 8A). Additionally, the release of ATP, another type of DAMP, was transiently increased in

BT-474 cells treated with either BB-1701 or eribulin (Fig. 8B). HMGB1 (yet another type of DAMP) were also observed in a BT-474 xenograft model by analyzing formalin-fixed paraffin-embedded tissue sections with immunohistochemistry (Fig. 8C). Finally, treatment with BB-1701 was found to increase the number of immune cells called macrophages in the tumor compared to treatment with a control substance (Fig. 8D).

Pharmacokinetics and toxicity studies of BB-1701 in cynomolgus monkey

To evaluate the pharmacokinetics and potential toxic effects of BB-1701 when given repeatedly at different doses, five male and five female cynomolgus monkeys (Aujun Biological Technology Co., Ltd., Guangzhou, China) were divided into groups and received four repeated doses (intravenous, every 3 weeks × 4) of BB-1701 at doses of 0, 2, 4, and 8 mg/kg. The animals were observed for clinical signs, and various laboratory tests were conducted to evaluate hematology, clinical chemistry, coagulation, and immunophenotype. They were euthanized one week after the last dose or at the end of the 6-week recovery period, after which gross pathology and histopathology evaluations were performed. Toxicokinetic (TK) studies were incorporated into the repeated dose toxicity study. Intensive blood sampling was performed in the first and third dose cycles, while sparse sampling was performed in the second and fourth dose cycles. In addition, cardiovascular and respiratory function were evaluated as part of the safety pharmacology assessment during the repeated dose toxicity study. The results showed that BB-1701 was stable in circulation and that its concentration in the blood was roughly proportional to the dose given (Fig. 9, Table 1). System exposure of BB-1701 was achieved at all three dose levels. The half-life of BB-1701 was around 111-192h (Table 1), and there was no accumulation of the drug or development of neutralizing antibodies at any of the doses tested.

Table 1. Serum pharmacokinetic parameters of ADC and Tab after administration of BB-1701 in cynomolgus monkeys.

Dose Levels (mg/kg)	Gender	ADC				TAb			
		$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-504h} (h×μg/mL)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-504h} (h×μg/mL)
2	Male	119.272	0.266	65.485	12406.315	117.171	0.450	53.088	9564.147
	Female	132.225	1.833	63.910	12827.924	126.120	0.450	52.326	10045.080
4	Male	166.717	0.450	97.010	24699.408	133.141	5.650	102.739	25600.546
	Female	111.512	12.000	101.952	20658.105	113.598	20.050	89.159	20699.889
8	Male	192.134	0.542	265.021	55919.672	170.630	0.542	216.175	46008.008
	Female	160.989	25.042	248.328	59691.963	151.260	0.312	220.489	48190.899

At a high dose of 8 mg/kg, one female monkey had to be euthanized for humane reasons due to a systemic bacterial infection on the 11th day of treatment, and one male monkey was found dead unexpectedly on the 13th day of treatment. The infection and death were likely caused by a weakened immune system and were accompanied by significant decreases in certain types of white blood cells, including neutrophils (NEUT) and lymphocytes (LYMP).

Remarkable hematological toxicity was observed in all animals in this dose group, with WBC, NEUT, LYMP, and RET significantly reduced on day 7 after administration and then rebounded in each dosing cycle. Microscopic examination of the affected organs revealed immune organ toxicity, lymphoid depletion in the thymus, spleen, and lymph nodes (mandibular, mesenteric, inguinal), as well as Peyer's patches. The changes in the immune system were also accompanied by toxicity in the bone marrow, including decreases in the degree of cell proliferation and reduced numbers of myeloid cells. There was also an imbalance in the ratio of myeloid to erythroid cells (M:E ratio) in the bone marrow. These effects tended to recover or improve by the end of a six-week recovery period.

At lower doses of 4 and 2 mg/kg, the monkeys experienced similar, but less severe, changes. These changes were considered not toxicologically important because they were within the normal range for the test facility and did not produce significant harmful effects.

Based on the mortality and severity of the effects seen at the different doses, the highest non-severely toxic dose (HNSTD) for monkeys was determined to be 4 mg/kg. This means that BB-1701 was well-tolerated at doses up to 4 mg/kg in monkeys when given repeatedly, and the nonclinical safety profile was acceptable for testing in humans.

The safety profile of ADCs is largely determined by their toxin payload. DS-8201a has been associated with approximately 10% interstitial lung disease (ILD) in breast cancer clinical studies, with 2.2% of deaths resulting from ILD (8, 10, 17). A repeated dose of DS-8201a to monkeys caused pulmonary toxicity at 30 mg/kg (given every 3 weeks for 3 doses) (16). T-DM1 has had low incidences of ILD in human patients and a preclinical toxicity study showed that T-DM1 treatment caused infiltration of mononuclear cells into the interstitium of the lung in cynomolgus monkeys at 10 mg/kg (given every 3 weeks for 4 doses). In the repeated dose toxicity study of BB-1701 in monkeys, there were no toxicities suggestive of ILD even at the highest dose groups. Therefore, despite the expression of HER2 on the cell membranes of epithelial cells in the human lung, it seems that the pulmonary effects of DS-8201a and T-DM1 may be toxin-specific and may be mediated by HER2 expression in the lung (18). The main adverse events of T-DM1 are thrombocytopenia and peripheral neuropathy, which are typical toxicities of tubulin inhibitor-conjugated ADCs (22). In the repeat-dose toxicology study of BB-1701 in cynomolgus monkeys, the main toxicities included histopathological changes in the thymus, spleen, lymph nodes, bone marrow, and male reproductive system at high doses, including two unscheduled deaths at the 8 mg/kg dose level due to immune system deficiency. This toxicity profile is consistent with that of eribulin in human patients and similar to other tubulin inhibitor-conjugated ADCs, such as MMAE-containing ADCs.

Both primary and acquired resistance to current cancer therapies present challenges and opportunities for a new drug in development. The EMILIA study of T-DM1 in late stage HER2 positive breast cancer patients found an overall response rate of 43.6% and a progression-free survival of 9.6 months, indicating more than 50% primary resistance and an additional 50% acquired resistance within 10 months (20). The DESTINY-Breast003 studies of DS-8201a in the third line treatment of late stage HER2 positive breast cancer patients found impressive overall response rates of 69%, respectively, and progression-free survivals of 18.5 months, suggesting that primary resistance to DS-8201 may be relatively infrequent, but around 30% of DS-8201a-treated patients acquired resistance within one year and more than 50% acquired resistance within two years (10). DS-8201 has had lower response rates and shorter progression-free survival in other indications, presenting more severe resistance challenges (8). There are dozens of potential mechanisms of resistance to HER2-targeting ADCs, and as long as HER2 expression persists, ADCs with different mechanisms of action and/or different drug metabolism mechanisms may

offer the potential to overcome resistance challenges (21). BB-1701 treatment showed significant anti-tumor activity in DS-8201a-resistant model. In addition to its unique antimitotic and non-antimitotic mode of action, eribulin uses a completely different set of ATP-binding Cassette (ABC) transporters from those used by topotecan and diflomotecan (of the same drug family as DXd) for cytoplasmic membrane trafficking (22). Therefore, it is hopeful that BB-1701 may use nonoverlapping mechanisms to overcome drug resistance and may be effective against tumors with acquired resistance to other HER2-targeting ADCs.

Part B: Clinical Evaluation

Example 1: BB-1701 is effective in suppressing tumor growth in HER2 positive breast cancer patients

HER2 positive breast cancer patients who had positive HER2 diagnosis (HER2 3+ by IHC, or 2+ by IHC plus FISH positive), and had progressed on, or were intolerant to, or developed resistance to prior standard treatment, received BB-1701 treatment once every three weeks (Q3W) at 0.8 (n=3), 1.2 (n=2), 1.6 (n=6), 2.0 (n=8) mg/kg. At the tumor evaluation after the treatment, the target lesions of 66.7% patients showed ≥30% reduction, of 33.3% patients largely remain unchanged (<30% changes in size); no patient with target lesion grew >30% changes in size.

Figures 10A and 10B are waterfall and swimmer plots of HER2 positive breast cancer patients. The study showed a best overall response rate of 66.7% (12/18 patients) and disease control rate of 100% (18/18 patients). The longest treatment duration was 60 weeks and 40.7% patients were on treatment for over 6 months. All patients received BB-1701 after they had failed treatment of standard therapies or experimental treatments, with prior therapy lines of treatment ranging from 1 to 6 lines. All patients had progressive diseases from their previous treatments when they were enrolled in the study with BB-1701. The tumor shrinkage and tumor growth suppression observed with the study treatment of BB-1701, was likely the results of the anti-tumor activity of BB-1701 in these HER2 positive breast cancer patients.

Example 2: BB-1701 is effective in suppressing tumor growth in patients bearing HER2 low-expressing breast cancer

HER2 low-expressing breast cancer patients who had diagnosis of HER2 1+ by IHC, or 2+ by IHC plus FISH negative, and had progressed on at least two lines of prior standard therapies, received BB-1701 treatment intravenously once every three weeks (Q3W) at 1.0 (n=5), 1.2 (n=18), 1.4 (n=5), 1.6 (n=10) mg/kg.

Figures 11A and 11B are waterfall and swimmer plots of HER2-low breast cancer patients. Tumor evaluation after the treatment showed that the target lesions of 34.2% patients showed ≥30% reduction, of 55.3% patients largely remain unchanged (<30% changes in size); in contrast the target lesions of only 10.5% patients grew >30% changes in size. The study showed a best overall response rate of 34.2% (13/38 patients) and disease control rate of 89.5% (34/38 patients). The longest treatment duration was 64 weeks and 31.6% patients were on treatment for over 6 months at the cutoff date of Sep 30th 2023 (study ongoing). Among these patients, some had failed other anti-HER2 ADC treatment. At 1.2 mg/kg dose level, one patient who achieved SD and the treatment duration is 62 weeks and is still ongoing was previously progressed on Disitamab Vedotin®. At 1.4 mg/kg dose level, one patient who achieved PR was previously progressed on TRODELVY®. At 1.6 mg/kg dose level, one patient who achieved CR was previously progressed on Disitamab Vedotin®, and one patient who achieved PR was previously progressed on ENHERTU®. All patients had failed standard therapies with prior lines of therapy ranging from 2 to 9 lines. All patients had progressive diseases from their previous treatments when they were enrolled in the study with BB-1701. The tumor shrinkage and tumor growth suppression observed with the study treatment of BB-1701, was likely the result of the anti-tumor activity of BB-1701 in these HER2 low expressing breast cancer patients.

Example 3: BB-1701 is effective in suppressing tumor growth in patients bearing HER2 expressing Lung cancer

HER2 expressing lung cancer patients who had HER2 amplification or mutation by NGS, and had progressed on at least two lines of prior standard therapies, received BB-1701 treatment intravenously once every three weeks (Q3W) at 1.0 (n=2), 1.2 (n=9), 1.4 (n=3), 1.6 (n=1) mg/kg.

Figures 12A and 12B are waterfall and swimmer plots of HER2 expressing lung cancer patients. Tumor evaluation after the treatment has shown that the target lesions of 33.3% patients showed ≥30% reduction, 60.6% patients largely remain unchanged (<30% changes in size); in contrast the target lesions of only 6.7% patients grew >30% changes in size. At 1.2 mg/kg, the

study showed a best overall response rate of 66.7% (4/6 patients) and disease control rate of 100% (6/6 patients) for HER2 mutation NSCLC. The longest treatment duration was 52 weeks and 46.7% patients were on treatment for over 6 months at the cut-off date of Sep 30th 2023 (study ongoing). All patients had progressive diseases from their previous treatments when they were enrolled in the study with BB-1701. The tumor shrinkage and tumor growth suppression observed with the study treatment of BB-1701, was likely the result of the anti-tumor activity of BB-1701 in NSCLC patients with Her2 amplification or mutations.

Example 4: BB-1701 is effective in suppressing tumor growth in patients with other HER2 expressing cancer types

HER2 expressing cancer patients of gastric/esophagogastric junction cancer (n=6), colorectal cancer (n=4), urothelial carcinoma (n=1) who had HER2 expression status (HER2 3+ by IHC, or 2+ by IHC plus FISH positive, or HER2 amplification by NGS), and had progressed on at least two lines of prior standard therapies, received BB-1701 treatment intravenously once every three weeks (Q3W) at 0.4 (n=1), 1.2 (n=6), 1.6 (n=1), 2.0 (n=1), 2.6 (n=2) mg/kg. Tumor evaluation after the treatment has shown that, the target lesions of the only one urothelial carcinoma patient had 65.7% reduction; the target lesions of the only one parotid cancer patient had 48.6% reduction; the target lesions of 5/6 gastric/esophagogastric junction cancer patients remained largely unchanged (<30% changes in size), only 1/6 patients with the target lesions grew >30% changes in size. The longest treatment duration of gastric/esophagogastric junction cancer patients was 63 weeks. All patients had progressive diseases from their previous treatments when they were enrolled in the study with BB-1701. The tumor shrinkage and tumor growth suppression observed with the study treatment of BB-1701, was likely the result of the anti-tumor activity of BB-1701 in the patients with different Her2 positive cancers.

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The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been described above in connection with one or more embodiments, it should be understood that the invention is not limited to those embodiments, and the description is intended to cover all alternatives, modifications, and equivalents, as may be included within the spirit and scope of the appended claims.

CLAIMS

1. A method of treating a patient bearing a HER2-expressing caner, comprising:

administering to a patient a therapeutically effective amount of an antibody-drug conjugate (ADC) having the Formula I:

$$Ab\text{-}(L\text{-}D)_p \quad (I)$$

wherein Ab is an anti-HER2 antibody moiety having the same structure as trastuzumab, L is a cleavable linker comprising Mal-$(PEG)_2$-Val-Cit-pAB, D is eribulin, and p is an integer from 1 to 8 inclusive, wherein the HER2-expressing cancer is selected from the group consisting of non-small cell lung cancer, gastric/esophagogastric junction cancer, urothelial carcinoma, parotid cancer, and HER2-low expressing breast cancer.

2. The method of claim 1, the patient is one whose cancer has demonstrated resistance to other HER2-targeting therapeutics including other anti-HER2 antibody drug conjugate treatment.

3. The method of claim 1, wherein the cancer is HER2-low expressing breast cancer, and where HER2-expression has been determined using HER2 1+ by IHC, or 2+ by IHC plus FISH negative diagnosis.

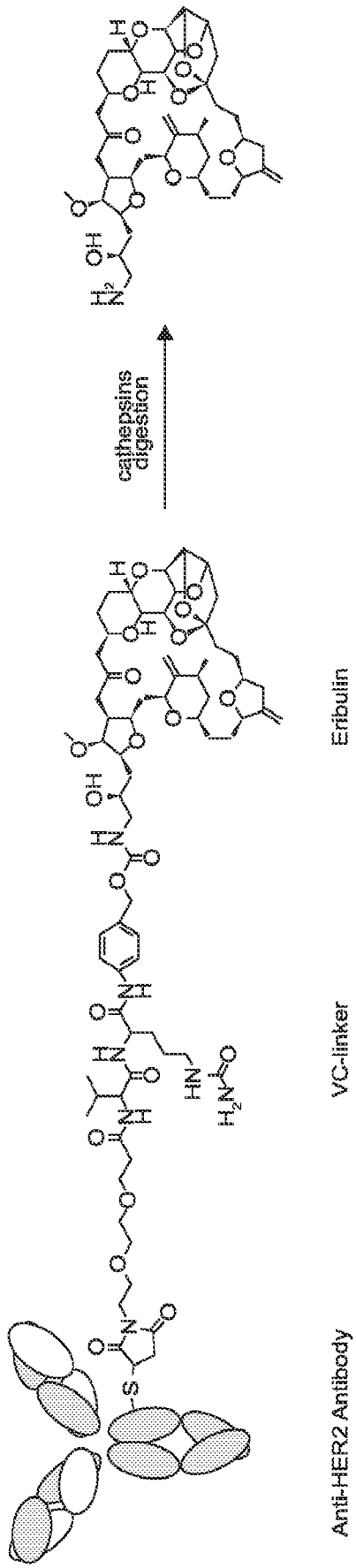

cathepsins digestion
Eribulin
VC-linker
Anti-HER2 Antibody

FIG. 1A

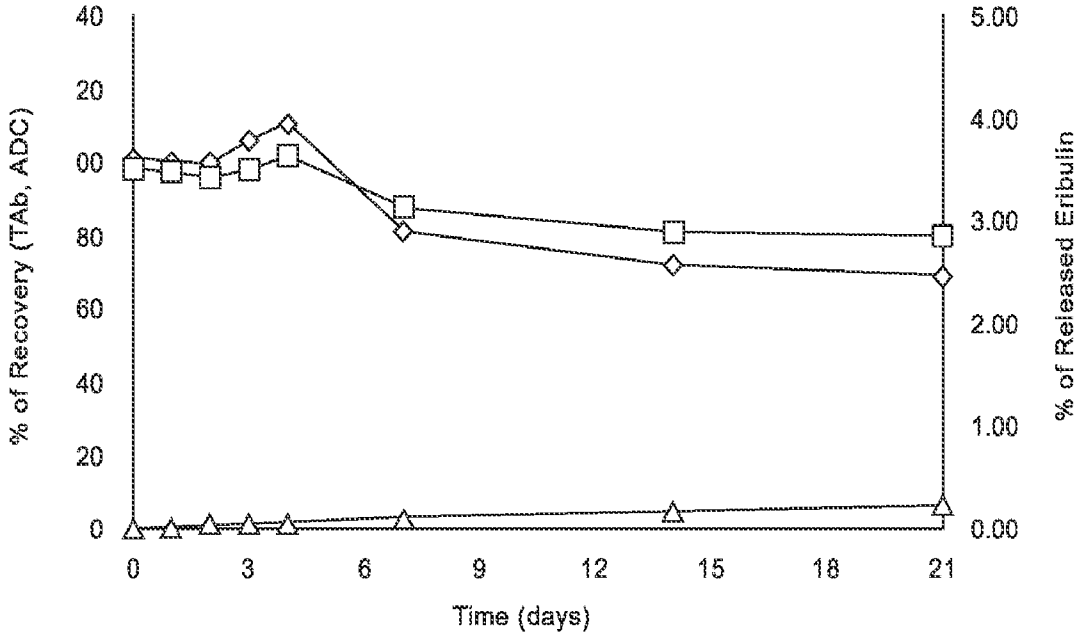

% of Recovery (TAb, ADC)
% of Released Eribulin
Time (days)
40
20
00
80
60
40
20
0
5.00
4.00
3.00
2.00
1.00
0.00
0
3
6
9
12
15
18
21

FIG. 1B

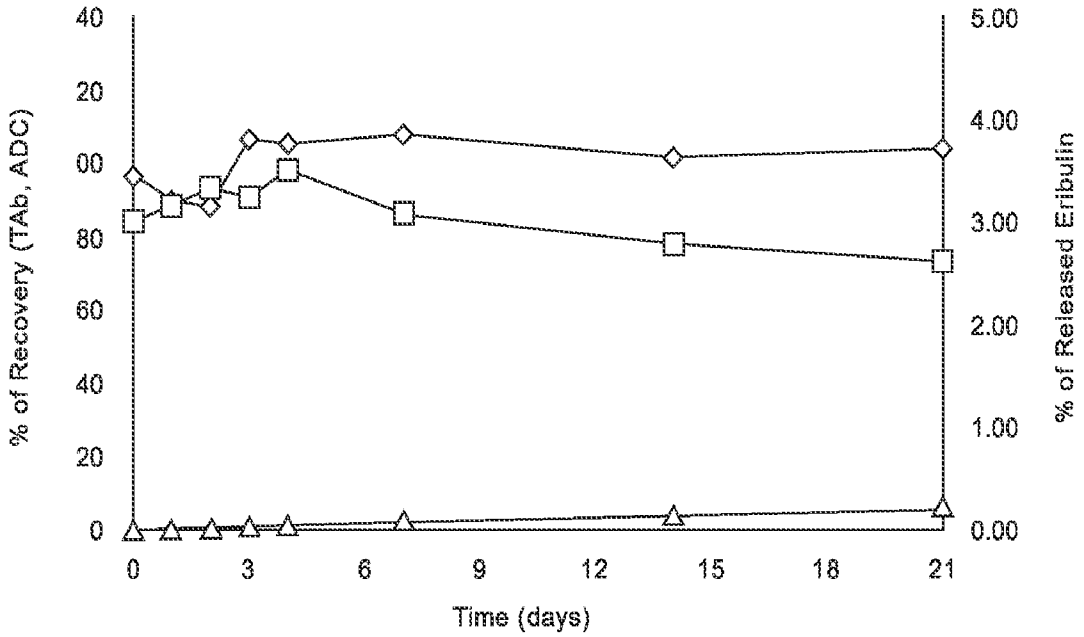

% of Recovery (TAb, ADC)
% of Released Eribulin
Time (days)
40
20
00
80
60
40
20
0
5.00
4.00
3.00
2.00
1.00
0.00
0
3
6
9
12
15
18
21

FIG. 1C

Samples	Ka ($M^{-1}s^{-1}$)	Kd (s^{-1})	K_D (M)
anti-HER2 antibody	1.17E+05	3.54E-05	3.03E-10
BB-1701	1.66E+05	3.08E-05	2.93E-10

FIG. 2A

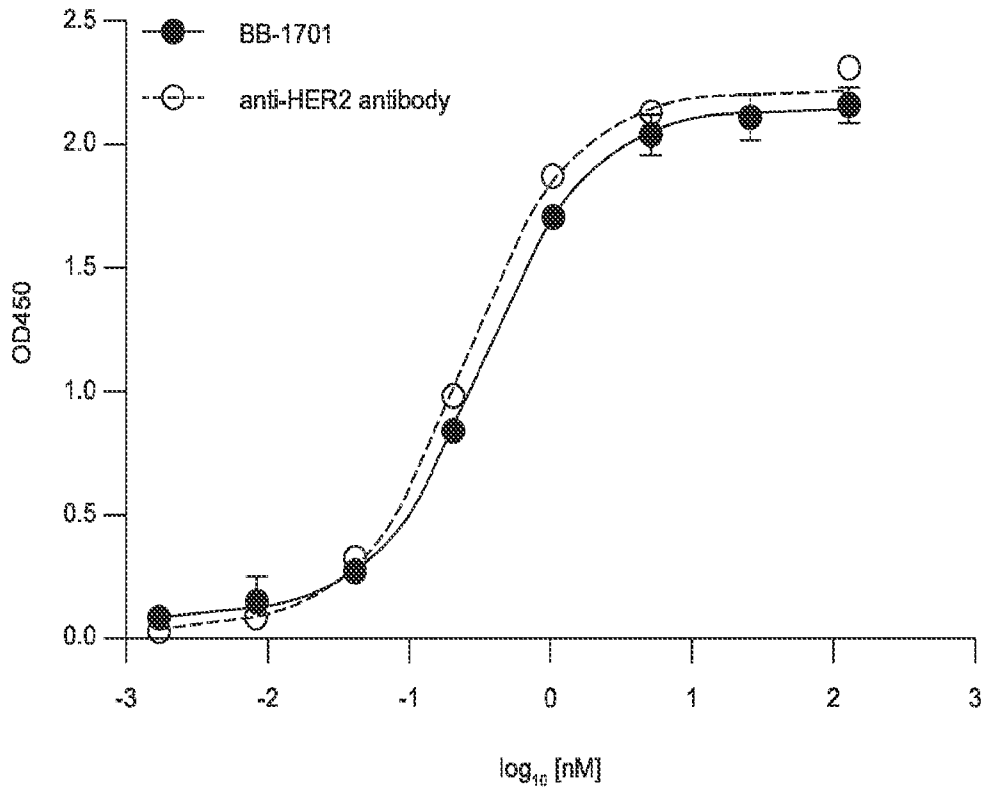


FIG. 2B

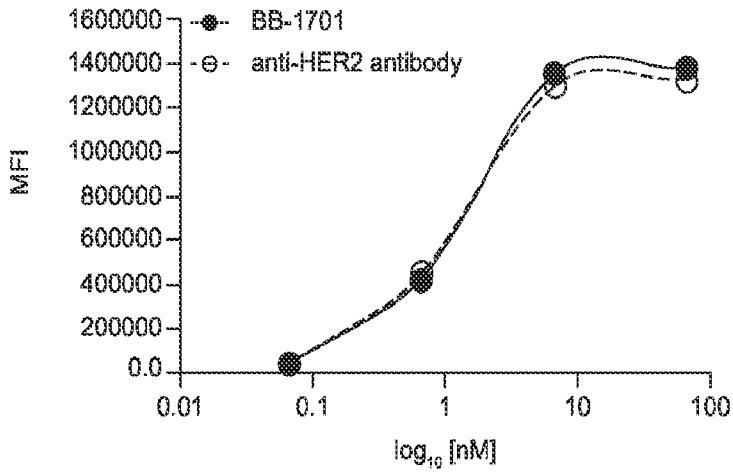


FIG. 3A

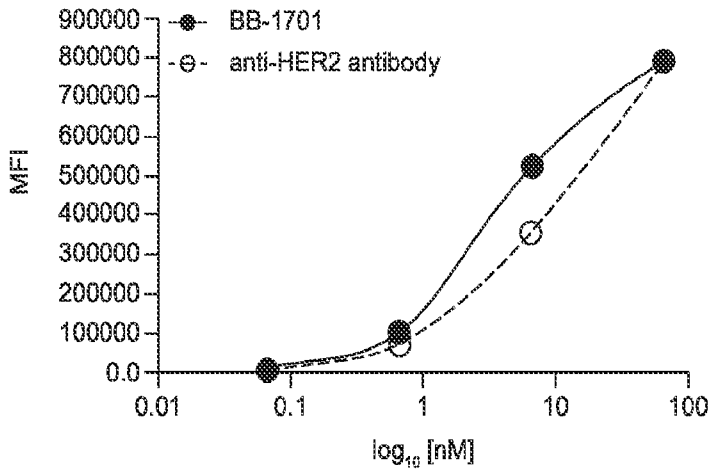


FIG. 3B

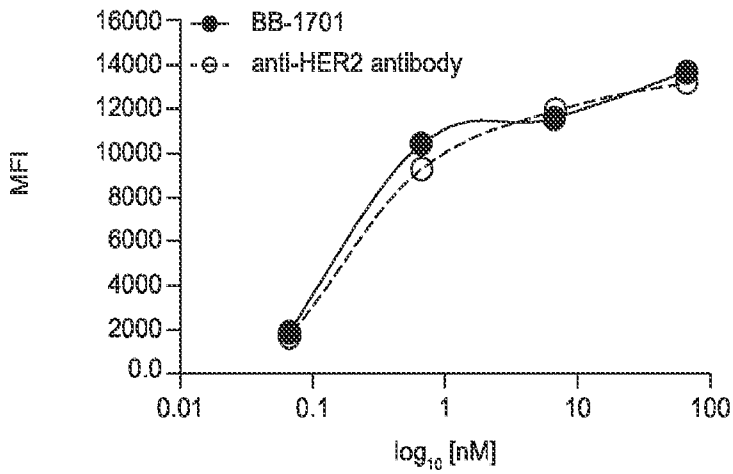


FIG. 3C

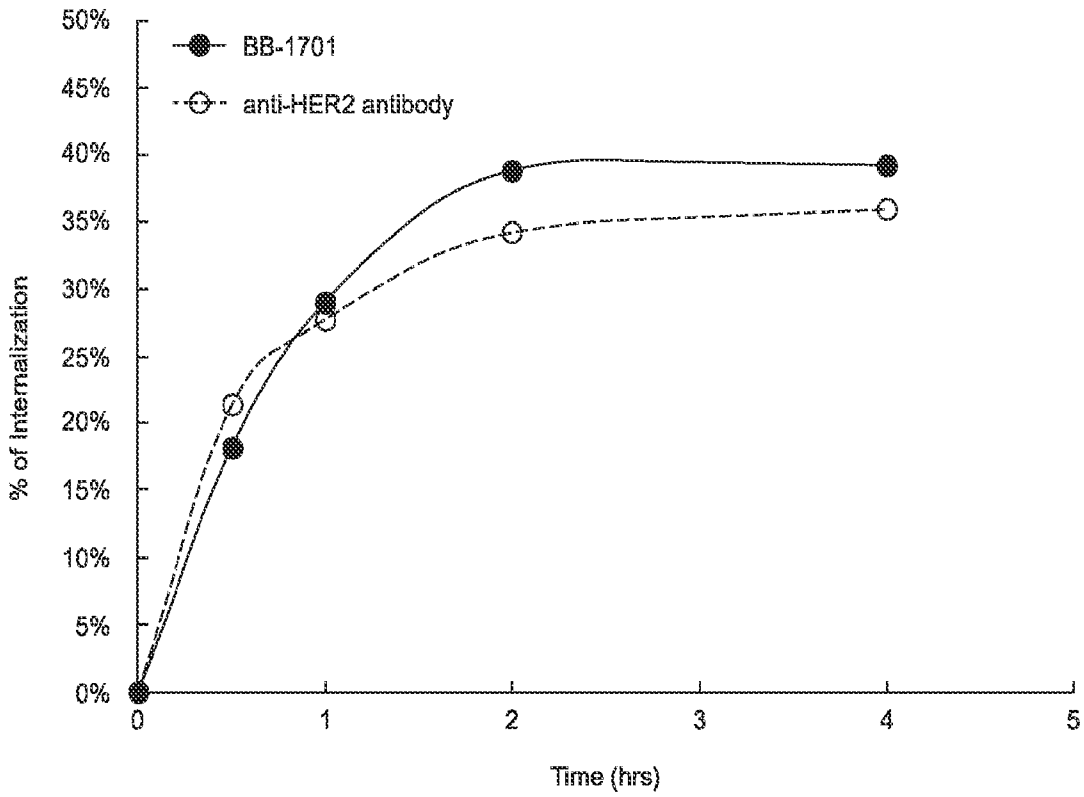

50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
% of Internalization
BB-1701
anti-HER2 antibody
0
1
2
3
4
5
Time (hrs)

FIG. 4

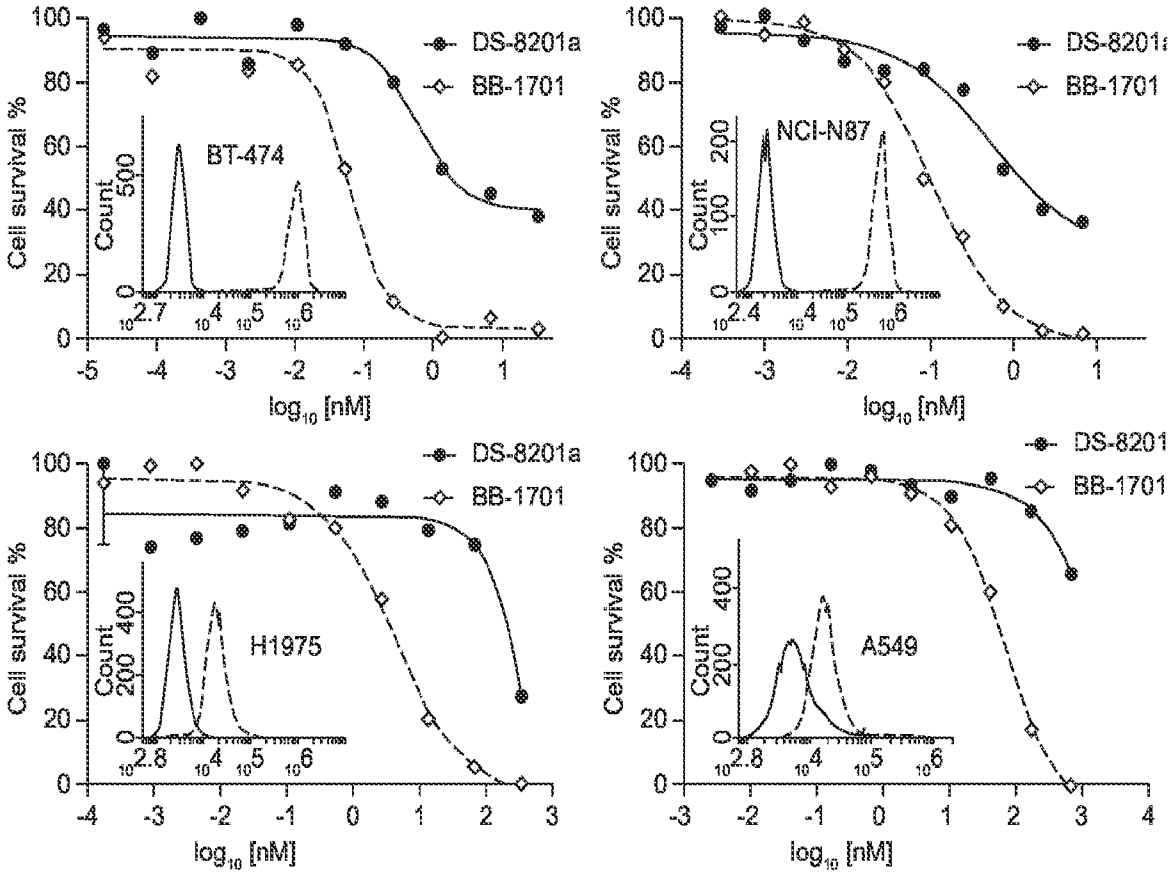

Cell survival %
log10 [nM]
DS-8201a
BB-1701
BT-474
Count
NCI-N87
H1975
A549

FIG. 5

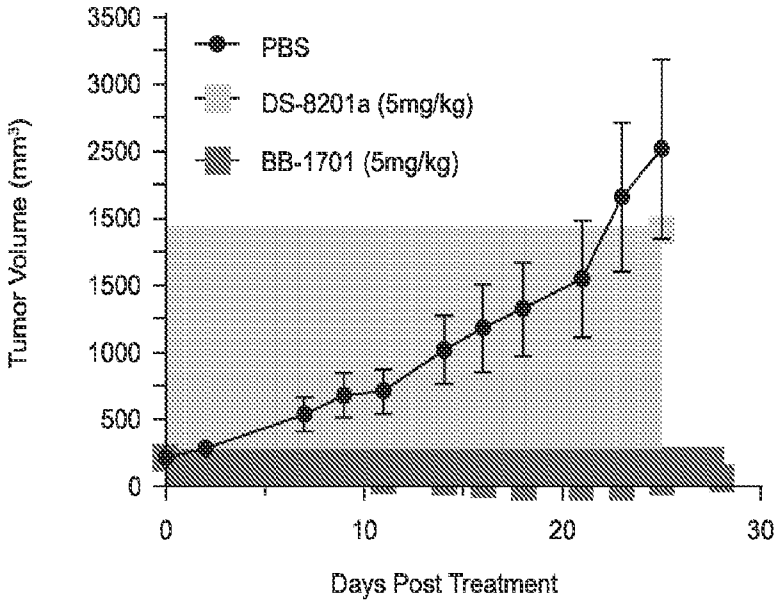

PBS
DS-8201a (5mg/kg)
BB-1701 (5mg/kg)
Tumor Volume (mm³)
3500
3000
2500
1500
1500
1000
500
0
0
10
20
30
Days Post Treatment

FIG. 6

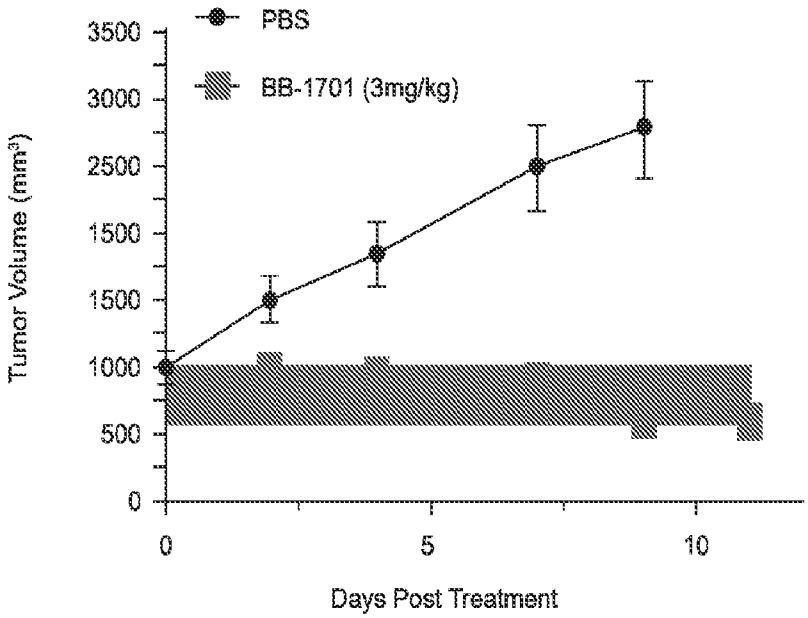

PBS
BB-1701 (3mg/kg)
Tumor Volume (mm³)
3500
3000
2500
1500
1500
1000
500
0
0
5
10
Days Post Treatment

FIG. 7A

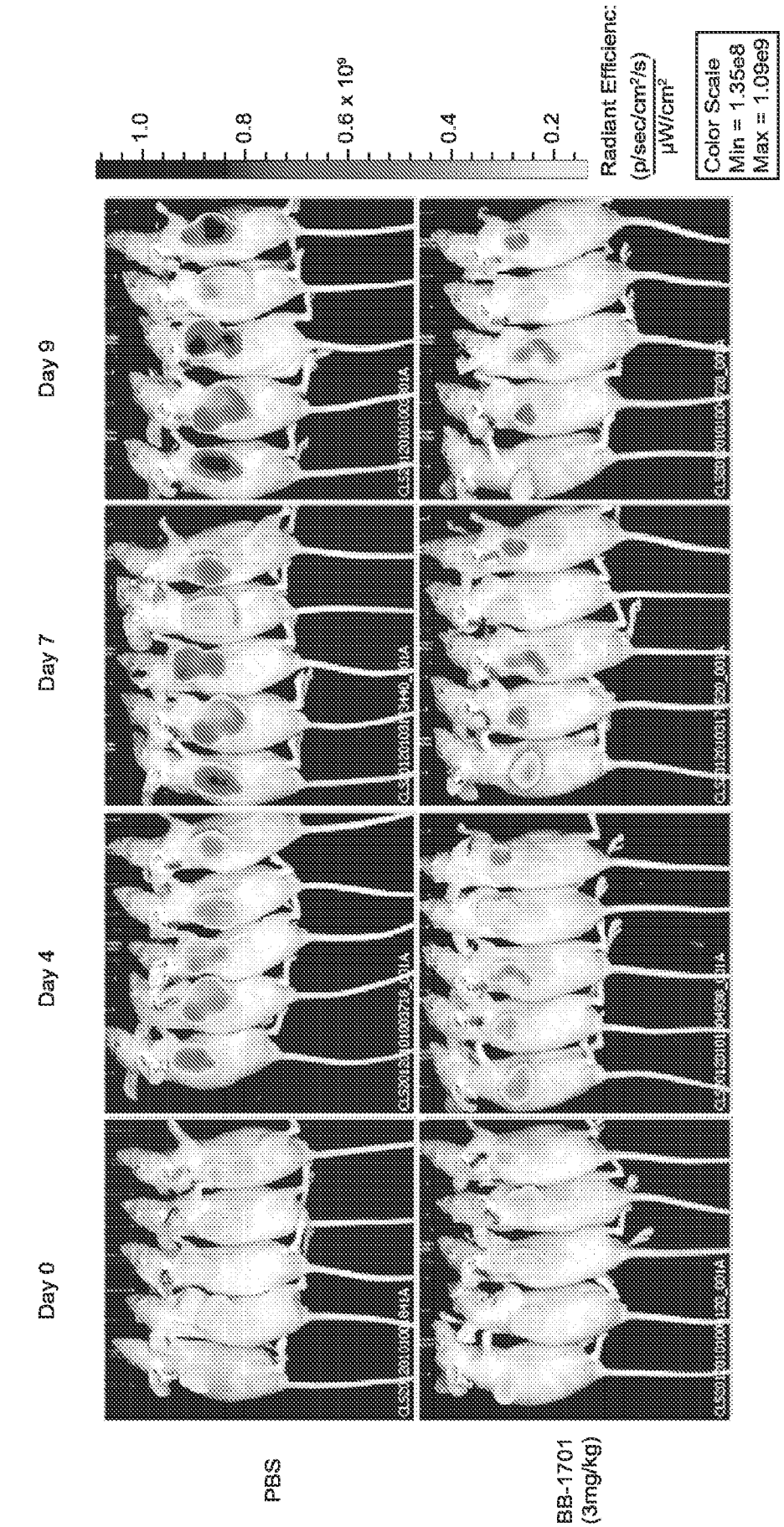

Day 0
Day 4
Day 7
Day 9
PBS
BB-1701 (3mg/kg)
1.0
0.8
0.6 x 10⁹
0.4
0.2
Radiant Efficienc: (p/sec/cm²/s) / µW/cm²
Color Scale
Min = 1.35e8
Max = 1.09e9

FIG. 7B

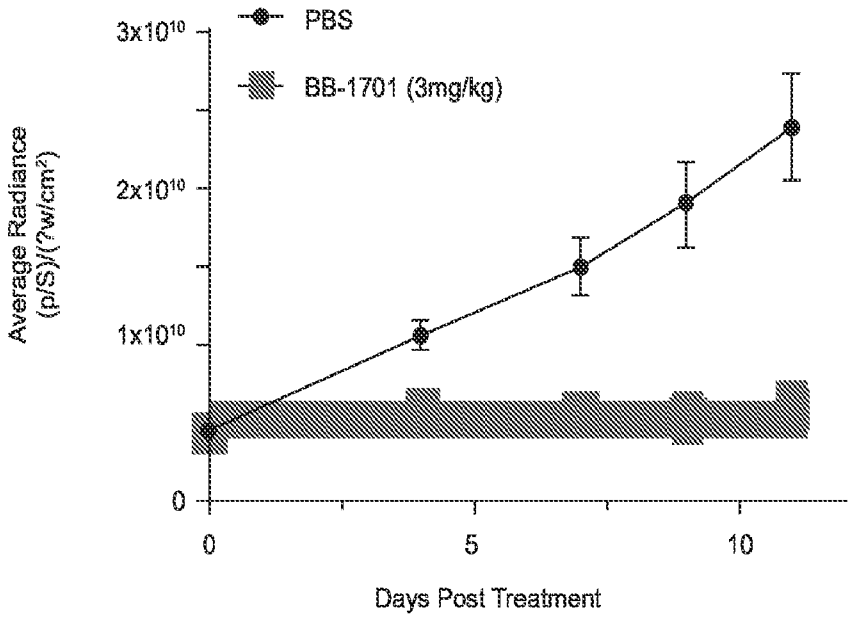

PBS
BB-1701 (3mg/kg)
3x10^10
2x10^10
1x10^10
0
Average Radiance (p/S)/(?w/cm²)
0
5
10
Days Post Treatment

FIG. 7C

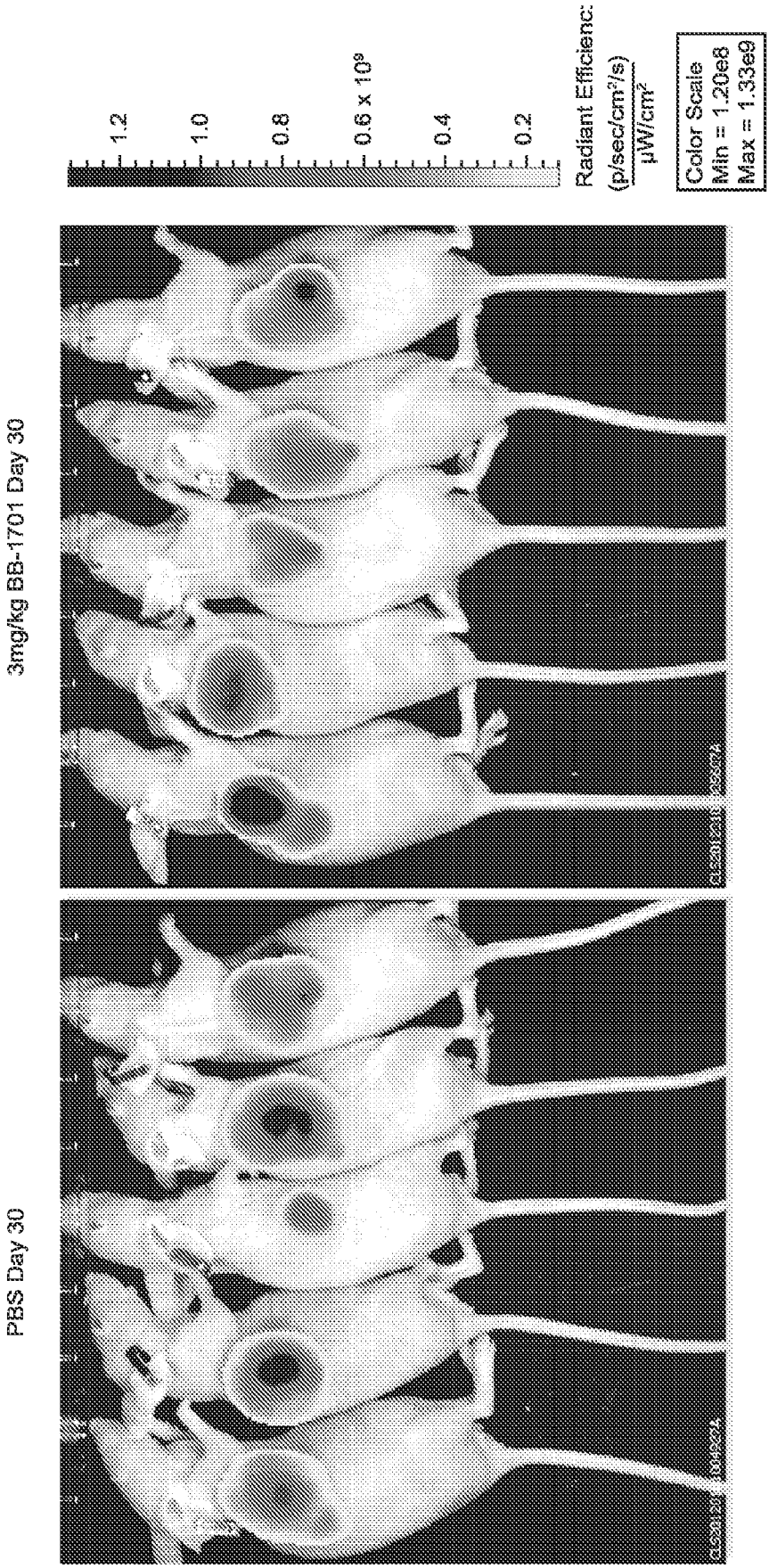

PBS Day 30
3mg/kg BB-1701 Day 30
1.2
1.0
0.8
0.6 x 10⁹
0.4
0.2
Radiant Efficienc:
(p/sec/cm²/s)
µW/cm²
Color Scale
Min = 1.20e8
Max = 1.33e9

FIG. 7D

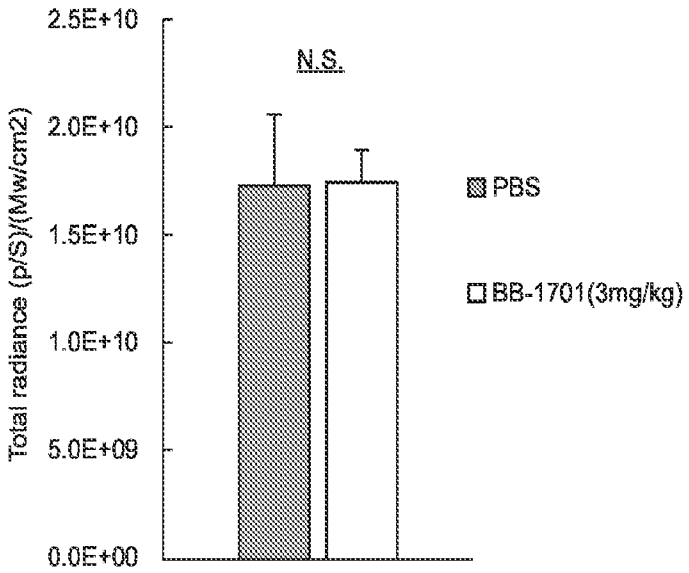

2.5E+10
2.0E+10
1.5E+10
1.0E+10
5.0E+09
0.0E+00
Total radiance (p/S)/(Mw/cm2)
N.S.
PBS
BB-1701(3mg/kg)

FIG. 7E

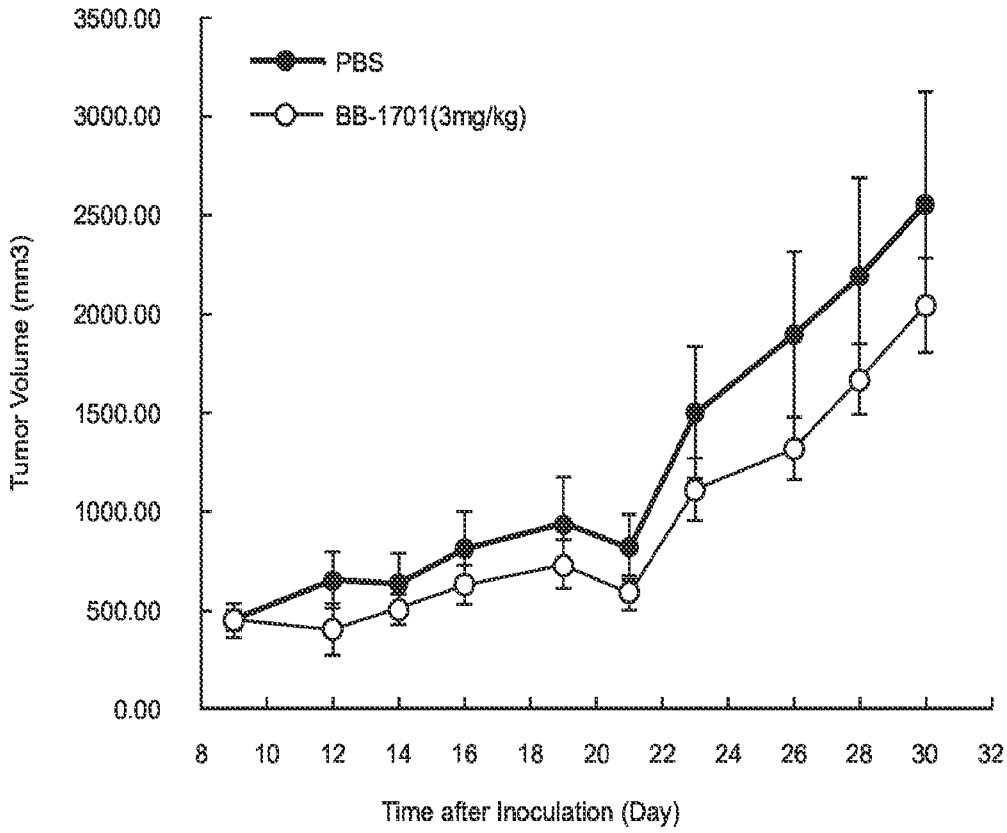
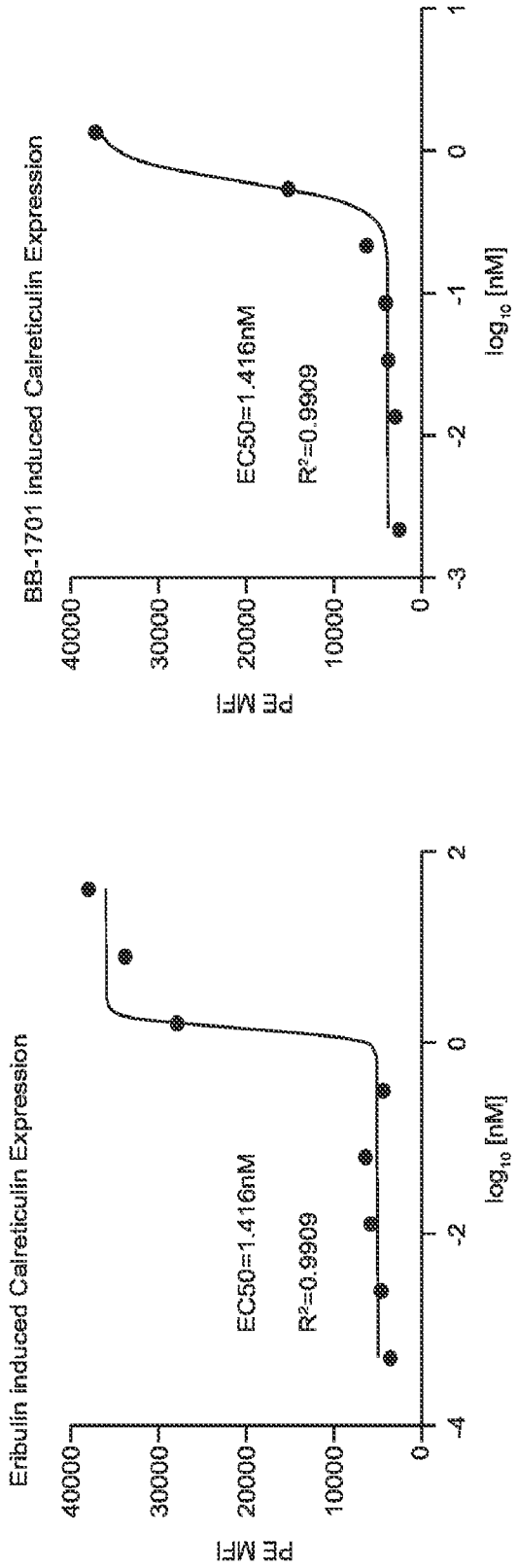

3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
Tumor Volume (mm3)
PBS
BB-1701(3mg/kg)
8
10
12
14
16
18
20
22
24
26
28
30
32
Time after Inoculation (Day)

FIG. 7F



Treatment	Cytotoxic IC_{50} nM	ICD EC_{50} nM
Eribulin	0.8677	1.416
BB-1701	0.352	0.6054

FIG. 8A

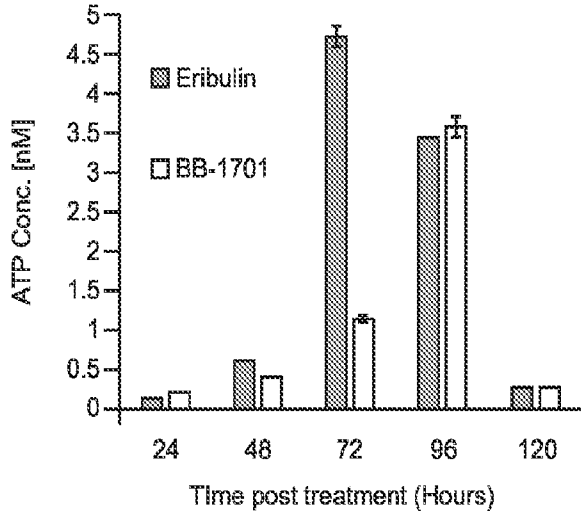


FIG. 8B

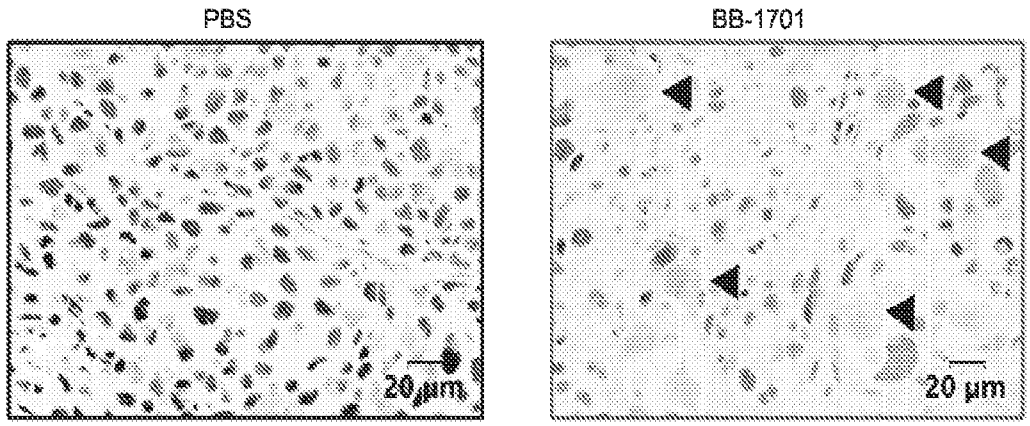


FIG. 8C

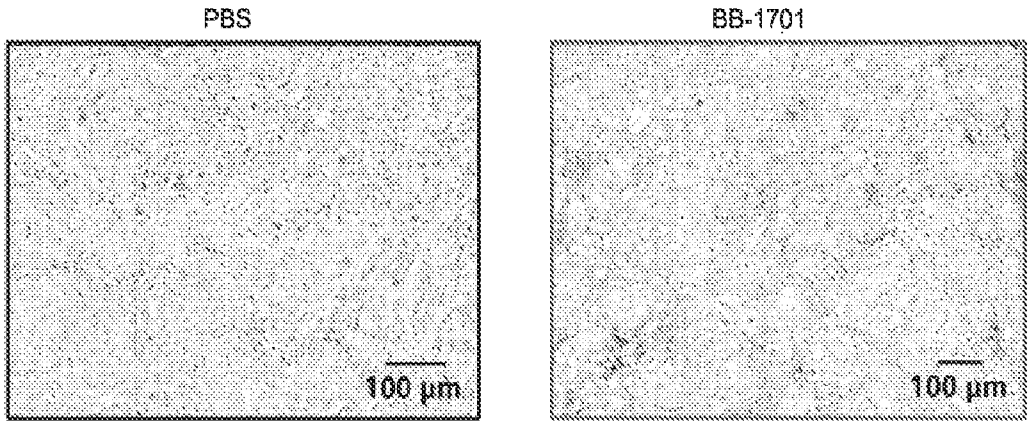


FIG. 8D

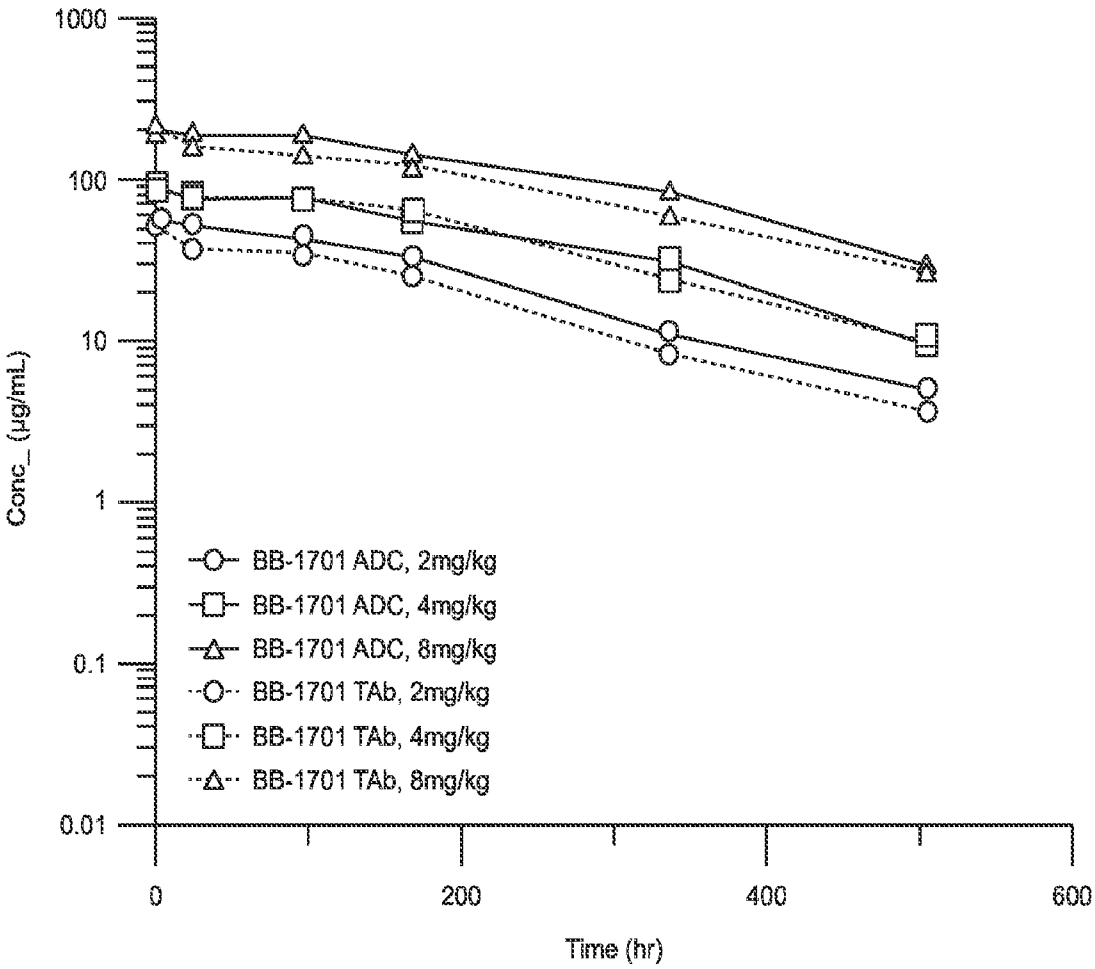

1000
100
10
1
0.1
0.01
Conc_ (µg/mL)
0
200
400
600
Time (hr)
BB-1701 ADC, 2mg/kg
BB-1701 ADC, 4mg/kg
BB-1701 ADC, 8mg/kg
BB-1701 TAb, 2mg/kg
BB-1701 TAb, 4mg/kg
BB-1701 TAb, 8mg/kg

FIG. 9

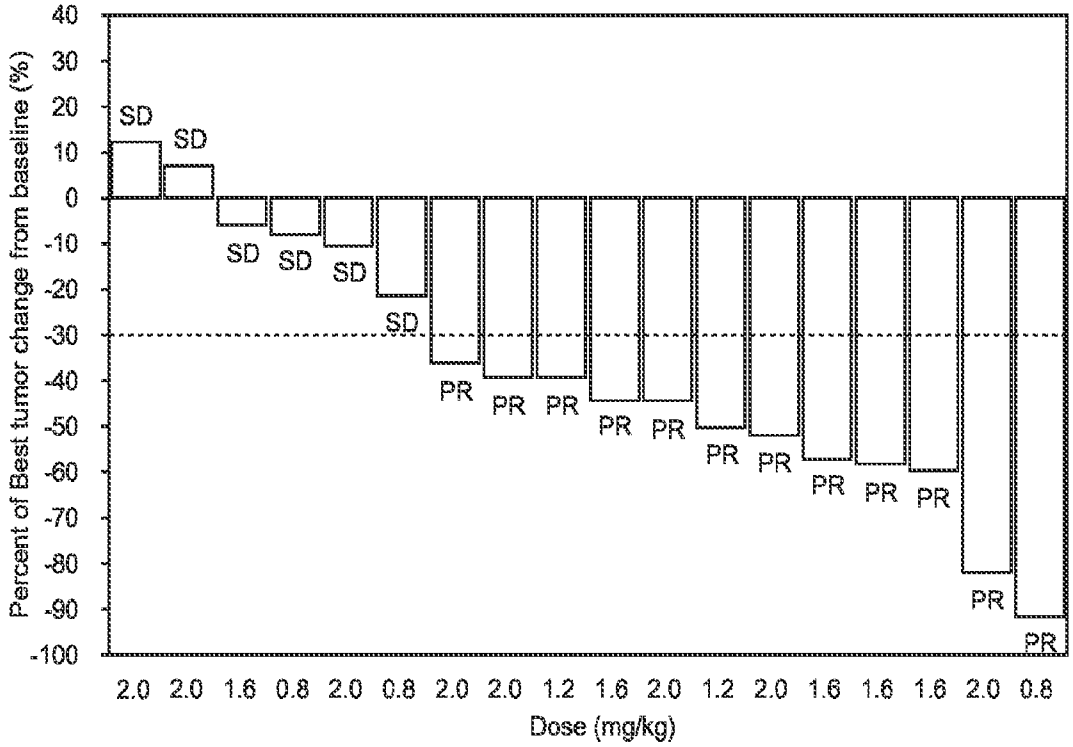


FIG. 10A

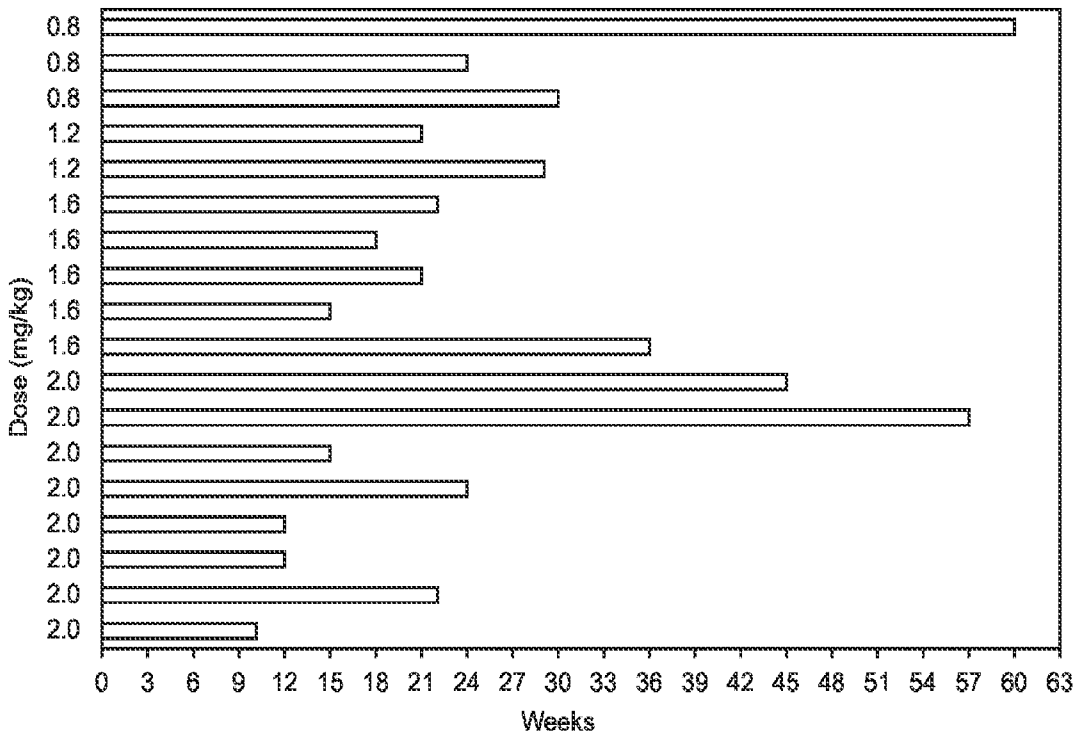


FIG. 10B

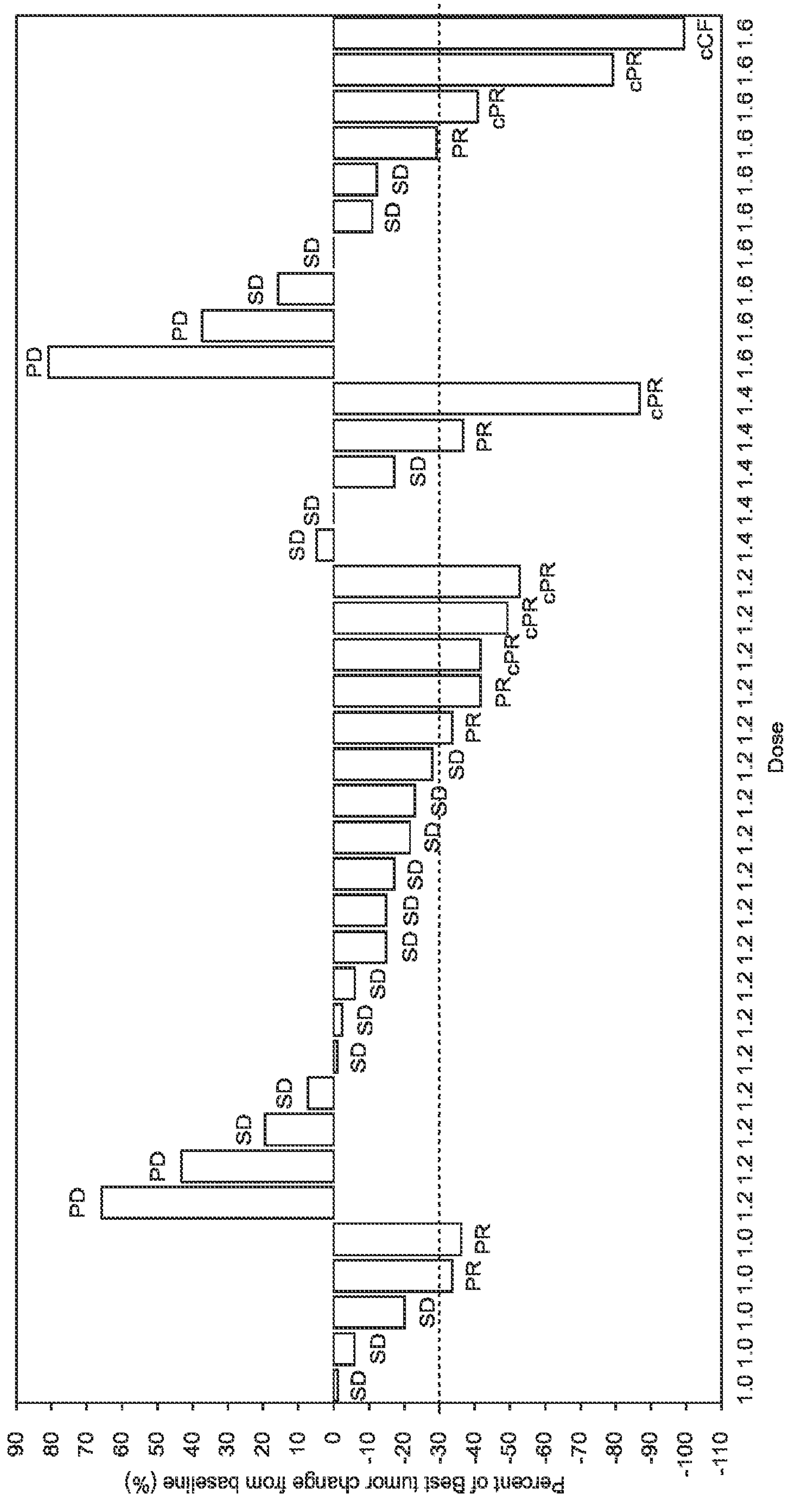

Percent of Best tumor change from baseline (%)
Dose
90 80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110
1.0 1.0 1.0 1.0 1.0 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.4 1.4 1.4 1.4 1.4 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6
SD SD SD PR PR PD PD SD SD SD SD SD SD SD SD SD SD SD PR PR cPR cPR cPR SD SD SD PR cPR PD PD SD SD SD SD PR cPR cPR cCF

FIG. 11A

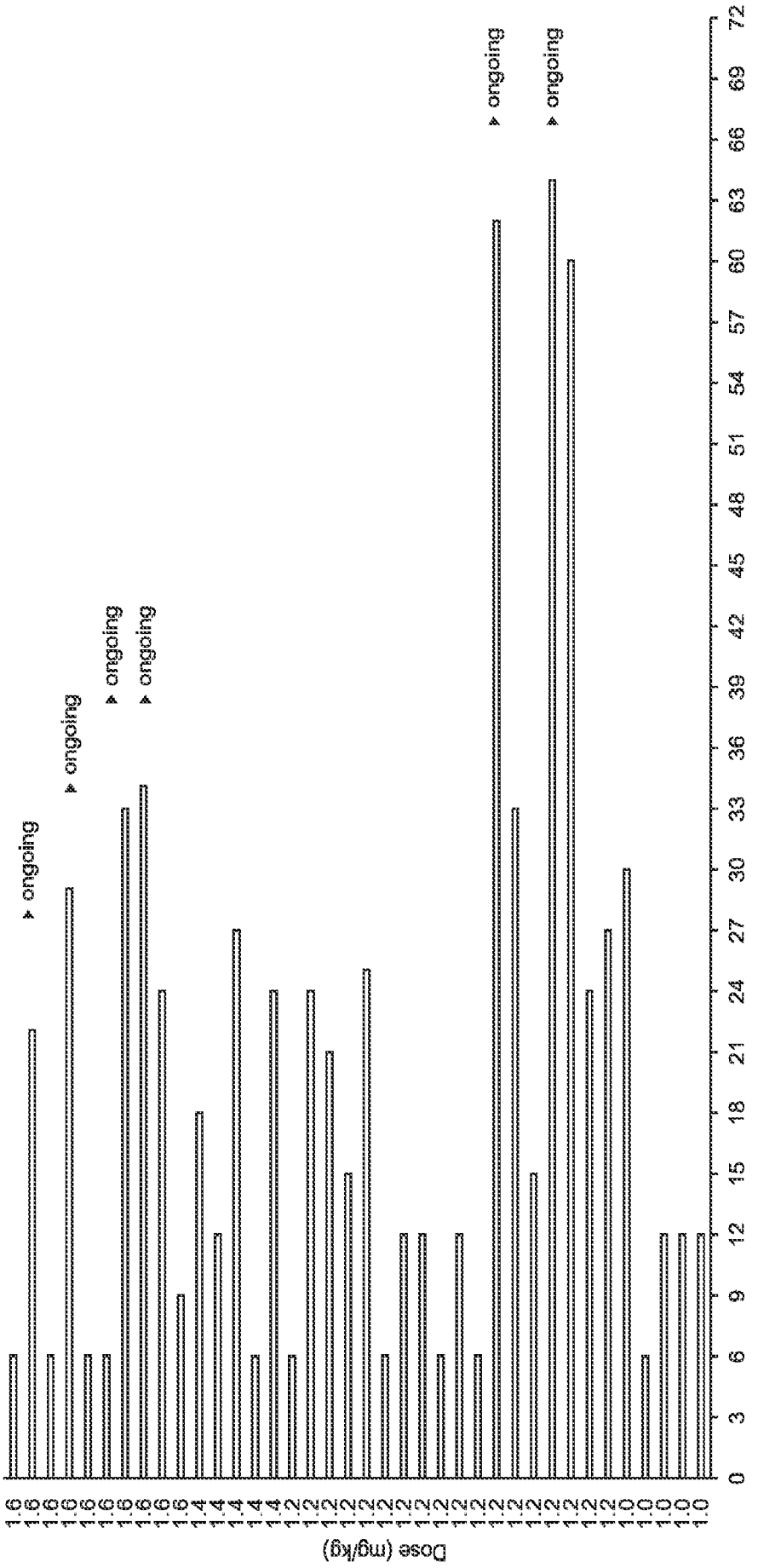

Dose (mg/kg)
1.6
1.6
1.6
1.6
1.6
1.6
1.6
1.6
1.6
1.6
1.4
1.4
1.4
1.4
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.2
1.0
1.0
1.0
1.0
0 3 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72
Weeks
ongoing
ongoing
ongoing
ongoing
ongoing
ongoing

FIG. 11B

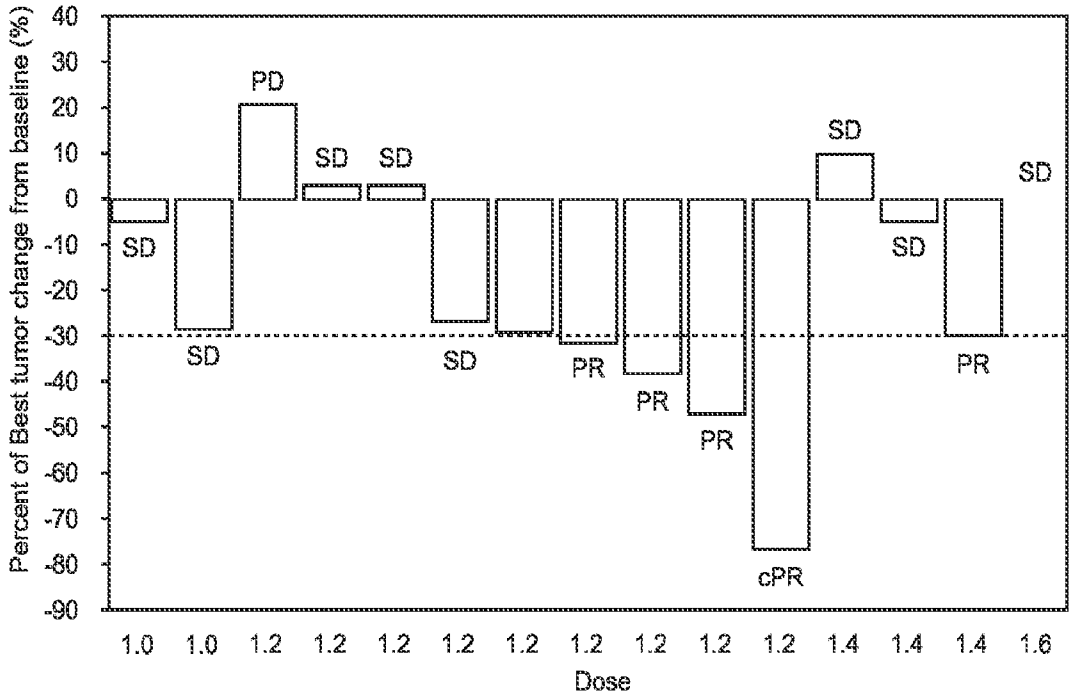


FIG. 12A

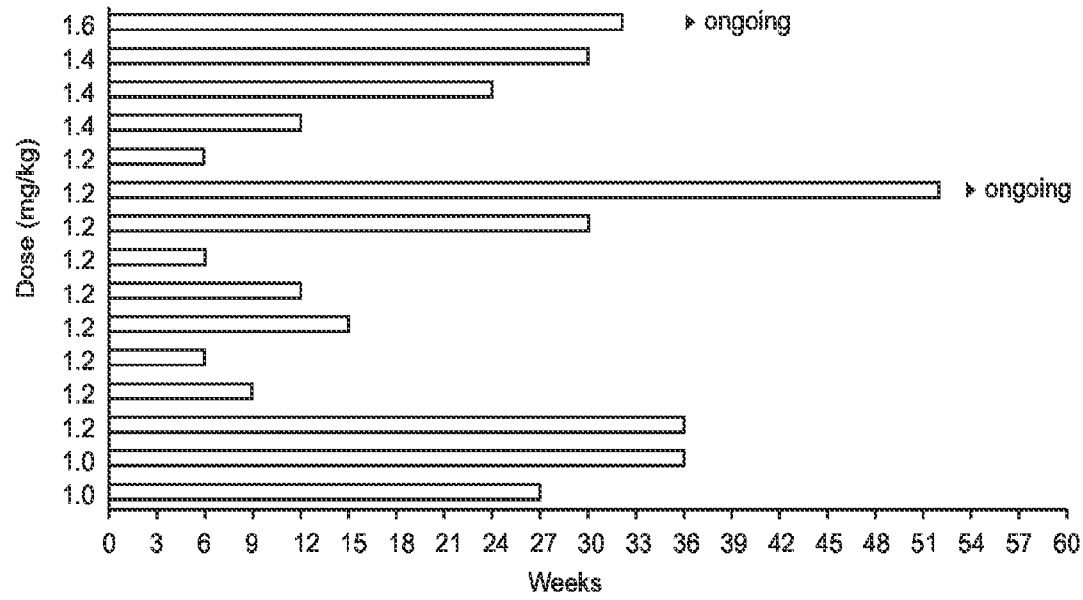


FIG. 12B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/015450

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 39/44*** (2024.01); ***A61K 47/68*** (2024.01); ***A61P 35/00*** (2024.01)

CPC: ***A61K 39/44; A61K 47/6803; A61P 35/00;*** *A61K 2039/505*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 2017/0252458 A1 (EISAI CO LTD et al.) 07 September 2017 (07.09.2017)	
X	entire document	1
Y	entire document	2, 3
	GUIDI et al., Resistance to Antibody-Drug Conjugates Targeting HER2 in Breast Cancer: Molecular Landscape and Future Challenges, Cancers, Vol. 15, No. 1130, 10 February 2023 [retrieved on 10 April 2024]. Retrieved from the Internet: <URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9954056/>. Pgs. 1-14	
Y		2, 3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 2024 (10.04.2024)	**19 April 2024 (19.04.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**