Preclinical evaluation of MRG002, a novel HER2-targeting antibody-drug conjugate with potent antitumor activity against HER2-positive solid tumors

Hu Li*, Xiao Zhang†, Zhenyi Xu, Lingrui Li, Wenchao Liu, Zhenyu Dai, Zhongrun Zhao, Lili Xiao, Hongfeng Li and Chaohong Hu

Research and Development, Shanghai Miracogen, Suite 4E, Bldg. 3, No. 1238 Zhangjiang Road, Pudong District, Shanghai 201203, China

Received: July 5, 2021; Revised: August 18, 2021; Accepted: August 19, 2021

ABSTRACT

Background: ERBB2 is a proto-oncogene of multiple cancers including breast and gastric cancers with HER2 protein overexpression or gene amplification and has been proven clinically as a valid target for these cancers. HER2-targeting agents such as Herceptin®, Kadcyla® and ENHERTU® have been approved by the FDA for the treatment of breast cancer, but these drugs still face the challenge of acquired resistance and/or severe adverse reactions in clinical use. Therefore, there is significant unmet medical need for developing new agents that are more effective and safer for patients with advanced HER2-positive solid tumors including breast and gastric cancers.

Methods: We report here the making of MRG002, a novel HER2-targeted antibody drug conjugate (ADC), and preclinical characterization including pharmacology, pharmacodynamics and toxicology and discuss its potential as a novel agent for treating patients with HER2-positive solid tumors.

Results: MRG002 exhibited similar antigen binding affinity but much reduced antibody-dependent cellular cytotoxicity (ADCC) activity compared to trastuzumab. In addition to potent in vitro cytotoxicity, MRG002 showed tumor regression in both high- and medium-to-low HER2 expressing in vivo xenograft models. Furthermore, MRG002 showed enhanced antitumor activity when used in combination with an anti-PD-1 antibody. Main findings from toxicology studies are related to the payload and are consistent with literature report of other ADCs with monomethyl auristatin E.

Conclusion: MRG002 has demonstrated a favorable toxicity profile and potent antitumor activities in the breast and gastric PDX models with varying levels of HER2 expression, and/or resistance to trastuzumab or T-DM1. A phase I clinical study of MRG002 in patients with HER2-positive solid tumors is ongoing (CTR20181778).

Statement of Significance: Featuring hyper-fucosylation of trastuzumab by design and clinically proven linker-payload, MRG002 exhibited reduced ADCC activity yet strong antitumor activity in trastuzumab- or T-DM1-resistant HER2-expressing PDX models. MRG002 has the potential to become one of the best HER2-targeting agents to address significant unmet medical needs in high- or medium-to-low HER2 expressing solid tumors.

KEYWORDS: T-DM1; MMAE; MRG002; HER2; antibody drug conjugate

*To whom correspondence should be addressed: Hu Li, Shanghai Miracogen, Suite 4E, Bldg. 3, No. 1238 Zhangjiang Road, Pudong District, Shanghai 201203, China. Tel: +86-21-61637960; Fax: +86-21-6163-7962; Email: li_hu@miracogen.com.cn
†Current affiliation and address: Sumitomo Pharmaceuticals (Suzhou) Co., Ltd, 3rd Floor, Building A, The New Bund World Trade Center (phase I), No.5, Lane 255, Dongyu Road, Pudong New Area District, Shanghai, China
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INTRODUCTION

The human epidermal growth factor receptor (HER/ErbB) family comprises four cell surface receptors—HER1 (epidermal growth factor receptor), HER2, HER3 and HER4. HER1, HER3 and HER4 have several ligands, including transforming growth factor-α, epithelial growth factor and heregulins. HER2 has no natural ligand, but is capable of forming heterodimers with other HER family members to activate downstream signaling transduction pathways, and thereafter regulates a series of physiological processes such as cell migration, differentiation, adhesion, apoptosis and angiogenesis [1].

ERBB2 is a proto-oncogene of multiple cancers including breast and gastric cancers with HER2 protein overexpression or gene amplification [2]. About 15–20% breast cancer patients overexpress HER2. This overexpression is closely related to increased malignancy and poor prognosis [3], and targeting HER2 for treating breast cancer is well validated by significant clinical data. For gastric and gastroesophageal junction (GEJ) cancer, the rate of HER2 overexpression is approximately 17.9%. Although the relationship between HER2 overexpression and the prognosis of gastric cancer and GEJ cancers are still debatable [4–7], HER2 has already been proven clinically as a valid target for these cancers.

Trastuzumab is the first marketed anti-HER2 humanized monoclonal antibody. It was first approved by the U.S. Food and Drug Administration (FDA) in 1998 for the treatment of HER2-positive metastatic breast cancer after the completion of chemotherapy, and then approved in combination with taxol as first-line therapy for the same indication. Trastuzumab was also approved in combination with chemotherapy as first-line therapy for treating HER2-positive progressive gastric cancer [8–12]. Additionally, synergistic antitumor activity was observed when combining trastuzumab with another HER2-targeted monoclonal antibody 1E11 in HER2-positive gastric cancer [13]. Thus, targeting HER2 is a validated strategy in treating breast cancer and gastric cancer. However, a large proportion of patients with HER2-positive breast cancer or gastric cancer have shown primary resistance or acquired resistance to trastuzumab [9, 14]. It is reported that less than 35% of naïve patients with HER2-positive breast cancer respond to trastuzumab, suggesting primary resistance in about 65% patients; furthermore, approximately 70% of patients initially responded to trastuzumab progress to metastatic breast cancer within 1 year [15].

Among numerous efforts to overcome resistance issues, antibody drug conjugates (ADCs) are a novel class of agent with unique mechanism of action that combines the antitumor potency of highly cytotoxic small-molecule drugs with highly selective, stable and favorable pharmacokinetic profile of mAbs [16]. The mAbs can specifically deliver highly potent cytotoxic agents to target cells while leaving normal tissues largely unaffected [17], thereby enabling the use of agents with ~1000-fold higher cytotoxicity than standard chemotherapy agents [18] to enhance tumor killing.

Kadcyla® (T-DM1), an ADC that links trastuzumab through a non-reducible thioether linker SMCC (N-succinimidyl-4- (N-maleimidomethyl) cyclohexane-1-carboxylate) to the small molecule toxin (Maytansine, DM1), has been approved for the treatment of patients with advanced HER2-positive metastatic breast cancer. Compared with existing HER2-targeted therapies, Kadcyla® has demonstrated therapeutic advantages, specifically with good efficacy in trastuzumab- or lapatinib-resistant breast cancer, and in HER2-positive trastuzumab-resistant gastric cancer [19]. However, Kadcyla® still suffers from the challenge of acquired resistance in breast cancer [20], and it also failed to meet primary endpoint in an effort to expand its indication to gastric cancer [21]. More recently, Trastuzumab deruxtecan (Enhertu) has gained accelerated approval by FDA for HER2-positive unresectable metastatic breast cancer following two or more prior anti-HER2 based regimens but it is reported to have approximately 10% interstitial lung disease (ILD) in the clinical study and 2.2% death resulted from ILD [22]. Therefore, a drug of improved efficacy and safety profile is needed for patients with HER2-positive advanced breast and gastric cancers.

We report here the preclinical characterization of a novel HER2-targeted ADC, MRG002, in pharmacology, pharmacodynamics and toxicology, which shows superior efficacy than trastuzumab and Kadcyla® in mouse xenograft models of breast cancer and gastric cancer as well as enhanced antitumor activity in combination with anti-PD-1 antibody.

MATERIALS AND METHODS

Antibodies and ADCs

MRG002 and MAB802 were produced by Shanghai Miracogen Inc. Herceptin® (Roche), Kadcyla® (Roche) and Rituxan® (Roche) were purchased. In brief, the anti-HER2 monoclonal antibody MAB802 was produced in Chinese hamster ovary (CHO) cells grown in CD Opti CHO AGT (Gibco) medium supplemented with appropriate feeding materials in fed-batch mode. Cells were harvested after 13–14 days of cultivation, and MAB802 was purified by a classic purification process for therapeutic monoclonal antibodies including Protein A affinity chromatography, low pH inactivation, cation and anion exchange chromatography, followed by virus nanofiltration and formulation. Anti-PD1 antibody, HX008, used in STO#410 model was a gift from Lepu Biopharma.

The ADC, MRG002, was prepared by reducing MAB802 with addition of TCEP in molar excess in a reduction buffer (25 mM sodium borate, pH 8.0, 25 mM NaCl, 1 mM EDTA) at ambient temperature. The partially reduced MAB802 was then alkylated with 1.1 molar equivalent of vcMMAE/mAb-cysteine thiol followed by quenching with excess of N-acetyl cysteine. The conjugate mixture was purified using Pellicon3 cassettes (Merk Millipore) followed by compounding with excipients and filtration through a 0.22 µm filter (Sartorius).

Cell lines

NCI-N87 and SKBR3 cell lines were originally purchased from American Type Culture Collection (ATCC); BT-474 and MDA-MB-453 were purchased from the Cell Bank of...
Serum stability

MRG002 at a final concentration of 150 μg/mL was added into normal human serum (NHS). After incubation at 37°C for indicated time, an aliquot of sample was taken out and the concentration of MRG002 (conjugated antibody, intact MRG002) in the sample was measured.

Binding of MRG002 and MAB802 to human HER2 and CD16a

To test the binding affinity of MRG002 and MAB802 to human HER2 antigen, recombinant human HER2-hFc was diluted to 0.1 μg/mL in sodium acetate (pH 4.5) and immobilized on Series S Sensor Chip CM5 (GE Healthcare) by amine coupling, after which serially diluted MRG002 or MAB802 at varying concentrations ranging from 0 to 10 nM was injected into the flow cells at a flow rate of 30 μL/min.

To test the binding affinity of MRG002 and MAB802 to human CD16a variants (176Val and 176Phe), anti-His human IgG (GE Healthcare) was diluted to 20 μg/mL in sodium acetate (pH 4.5) and immobilized on Series S Sensor Chip CM5 (GE Healthcare) by amine coupling, then 0.5 μg/mL human CD16a (176Val, His Tag) or human CD16a (176Phe, His Tag) was injected at 10 μg/mL to be captured by the immobilized anti-human His IgG, after which serially diluted MRG002 or MAB802 (Sino Biological Inc.) was injected into the flow cells at a flow rate of 30 μL/min.

The above binding experiments were carried out by surface plasma resonance (SPR) on Biacore T200. The association rate (Kₐ), the dissociation rate (Kₐ) and the dissociation equilibrium constant (KD) values were calculated using the BIAevaluation software (BIAcore) by fitting the data with bivalent binding model.

Competitive binding assay for HER2 of different species

In total, 100 μL of recombinant human HER2-hFc at 2 μg/mL was coated on 96-well enzyme linked immunosorbent assay (ELISA) plates (Nunc, Cat. No. 442404) overnight. The plate was then blocked with 3% Bovine serum albumin (BSA) and washed with phosphate-buffered saline with Tween 20 (PBST, 1 × PBS/0.05% Tween-20). A mixture of 100 μL of serially diluted MRG002-Biotin (biontin-labeled MRG002) of concentrations from 1 000 ng/mL to 0.0128 ng/mL and 10 μg/mL of HER2 (human HER2 was custom-made from Destiny Biotech, HER2 of other species were purchased from Sino Biological Inc.) from different species were added to the plates and incubated for 60 min at room temperature. After washing the plates with PBST, 100 μL of 1:10 000 diluted HRP-labeled streptavidin (Thermo scientific, product Cat. No.21130) was added and incubated for 30 min. Then the plates were washed with PBST followed by addition of 100 μL of tetramethylbenzidine (TMB) substrate. Absorbance (OD₄₅₀/OD₆₅₀) was read using M5 spectrometer (molecular devices), and data were analyzed.

Assessment of antibody-dependent cellular cytotoxicity activity

Antibody-dependent cellular cytotoxicity (ADCC) assays were performed using NK92/CD16a as effector cells and SKBR3 as target cells with an effector to target (E:T) ratio of 5:1. Herceptin® was used as the reference drug. Target cells (20 000 target cells/well) were added into 96-well plates containing Herceptin® or varying concentrations of MRG002 or MAB802, effector cells were added, and the mixture was incubated at 37°C for approximately 3 ~ 4 hrs. After which, cell viability was measured using the Cytotoxicity LDH Assay Kit-WST (DOJIINDO) according to the manufacturer’s instruction. Data (OD=OD₄₉₀nm-OD₆₅₀nm) were collected using FlexStation3 (molecular devices) and analyzed using a 4-PL nonlinear regression model within SoftMax Pro 6.5.1 (molecular devices). Cytotoxicity was calculated using the following formulas: % Target Cell Lysis = (ODexperimental − ODspontaneous effector cell release + spontaneous target cell release)/(ODmaximum release − ODspontaneous release) × 100%, %EC₅₀ = EC₅₀ of Herceptin® group/EC₅₀ of experimental group.

Assessment of complement dependent cytotoxicity activity

Complement dependent cytotoxicity (CDC) assays were performed using SKBR3 as target cells and 10% NHS as complement source. Rituxan®’s CDC activity on Ramos cells was used as positive control, and Herceptin® was used as a reference control. Target cells (8 000 target cells/well in a 96-well plate) were mixed with varying concentrations of MRG002, MAB802, Herceptin® or Rituxan®, 10% NHS was added, and then the mixture was incubated for 4 hrs. Cell viability was measured using CellTiter-Glo® Assay (Promega, Cat. No. G7571). Data (fluorescence intensity, RLU) were collected using PHERAStar Plus (BMG Labtech) and analyzed using SoftMax Pro 6.5. Curves were fitted using a 4-PL nonlinear regression model within SoftMax Pro 6.5.1 software (molecular devices). Cytotoxicity was calculated using the following formula: % target cell Lysis = (RLUtarget cell release − RLUexperimental release)/(RLUtarget cell release − RLUbackground)) × 100%.

Flow cytometry for internalization study

To monitor change of membrane-bound MRG002 with incubation time, 2 000 μL of HER2-expressing SKBR3 cells (cell density 3E+5 cells/mL) were co-incubated with 100 μL of 4 μg/mL MAB802 (antibody component of MRG002, included as a reference control) or MRG002 in a 6 Well Cell Culture Plate (Costar cat. Cat. No. 3516) for 30 min on ice, washed with PBS to remove unbound MAB802 or MRG002, and cultured at 37°C for varying time length. After which, cells were incubated with FITC-conjugated goat anti-human IgG Fc (Jackson Immuno Research, Cat. No. 109–095-098) for another 30 min; washed one more time with PBS, digested with trypsin, and subjected to flow cytometry analysis on FACS Calibur (Becton Dickinson).
Confocal microscopy for ADC internalization and trafficking

Confocal microscopy was used to study internalization of MRG002. Briefly, 2,000 μL of BT-474 cells (cell density 5E+5 cells/mL) were seeded onto Cellvis glass bottom dishes (Cellvis, Cat. No. D35-20-1-N) and were allowed to grow for 24 hrs. Cells were then incubated with 100 μL of 4 μg/mL MRG002 for 2 ~ 30 hrs at 37°C. After the incubation period, cells were fixed with BD Cytofix/Cytoperm (Becton Dickinson) for 30 min and then cell surface-bound and internalized MRG002 were visualized using Alexa Fluor 488-labeled goat anti-human IgG (Invitrogen, Cat. No. A-11029), whereas lysosomes were visualized using Lyso-Tracker-Red (Beyotime Biotechnology, Cat. No. C1046). Nuclei were counterstained with DAPI (Roche, Cat. No. 10236276001). Images were acquired using a Zeiss LSM710 confocal microscope (Zeiss).

In vitro cytotoxicity in HER2-expressing cell lines

Three breast cancer cell lines (SKBR3, BT-474 and MDA-MB-453) and one gastric cancer cell line (NCI-N87) were plated on 96-well flat-bottomed plates (Costar, Cat. No. 3595) respectively, and 100 μL of 200 μg/mL serially-diluted MRG002, Kadcyla® of concentration from 200 μg/mL to 0.02 ng/mL in appropriate culture medium or drug-free media (as control) were added to the wells. The plates were incubated for 96 ± 2 hrs, thereafter cell viability was determined by the CCK-8 assay (Dojindo, Cat. No. CK04) in accordance with the manufacturer’s instruction, and IC50 values were calculated using a 4-PL nonlinear regression model within Softmax Pro 6.5.1 (molecular devices).

In vitro antitumor activity in tumor xenograft models

Cell line-derived xenograft (CDX) models were established by subcutaneous inoculation of ∼ 5 × 10^6 BT-474 cells or NCI-N87 cells into BALB/c nude mice (Beijing Vital River Laboratory Animal Technology Co., Ltd). Patient-derived xenograft (PDX) models were established by inoculating blocks of breast cancer or gastric cancer tissues subcutaneously in BALB/c nude mice except the combination study. Subdermal estradiol pellets were applied to support the growth of BT-474 xenografts and breast cancer PDX xenografts.

In the efficacy study of single agent, when tumors grew to the size of approximately 150 mm^3 ~ 250 mm^3, animals were randomized and grouped (8 animals per group), and then dosed intravenously. Non-binding control ADC (non-binding antibody conjugated with monomethyl auristatin E (MMAE) at a drug load similar to MRG002), vehicle control and Kadcyla® were used as reference compounds. Tumor size was measured twice a week throughout the study.

The antitumor activity of MRG002 and HX008 (a humanized anti-HER2 monoclonal antibody) as a single agent or combo was assessed in a gastric PDX model STO#410 in humanized mice. The PDX model was established by subcutaneous (s.c.) inoculation of tumor fragments into Hu-HSC-NPG mice (NPG mice were humanized by transplantation of human CD34+ hematopoietic stem cells). When tumors grew to the size of approximately 151 mm^3 ~ 289 mm^3, 24 tumor-bearing mice were divided into four groups (six animals per group), and administered with vehicle, MRG002, HX008 or MRG002 + HX008 combo intravenously. Tumor size was measured three times a week throughout the study. On Day 21, Bonferroni multiple comparison method was used to test the significant difference (MRG002 vs. MRG002 + HX008 combo groups).

The following formula was used for tumor size calculation: tumor volume (TV) = tumor length × tumor width^2/2. The use of animals was in compliance with the requirements by the Institutional Animal Care and Use Committee established by the Contract Research Organizations (CRGO) who performed the study.

Toxicology studies

The toxicity studies were carried out in a good laboratory practice (GLP)-compliant facility.

Repeated dose toxicity study in cynomolgus monkeys

Cynomolgus monkeys (♂ 5♂/group, Nanning Deheng Biotechnology Co., Ltd) received four repeated doses (i.e., q3w × 4) of vehicle, 0.06 mg/kg MMAE or 1, 3, 6 mg/kg MRG002. Animals were observed for clinical signs, hematology, clinical chemistry, coagulation and immunophenotype, and were euthanized at 1 week after the last dose or at the end of the 6-week recovery period before gross pathologic and histopathologic evaluation were performed.

Toxicokinetic (TK) studies were incorporated into the aforementioned GLP repeated dose toxicity study. Intensive blood sampling was performed in the first and the third dose cycles, whereas sparse sampling was performed in the second and the fourth dose cycle. Safety pharmacology of cardiovascular and respiratory function was incorporated into the repeated dose toxicity study. Telemetry (JET Devices, Data Science International, USA) was used for recording ECG, body surface temperature and respiration. Non-invasive blood pressure meter (BP-98E, Softron) was used for measuring blood pressure.

RESULTS

Characterization of MRG002

MRG002 was generated by conjugating MMAE to the interchain cysteines of MAB802 via a protease cleavable valine-citrulline linker (vc linker) with an average drug–antibody ratio of ∼3.8 (Fig. 1A, Supplementary Figure 1). MAB802 is a recombinant humanized monoclonal antibody that shares the same amino acid sequence as trastuzumab (Herceptin®) but is hyper-fucosylated, which was designed to have lower ADCC activity and therefore potentially has less impact on the immune cells.

The in vitro binding properties of MRG002 to human HER2, as well as to HER2 of different species were studied using SPR and a competitive ELISA assay, respectively.
Figure 1. Basic characteristics of MRG002. (A) Structure of MRG002. (B) Binding affinity of MRG002, MAB802 and Herceptin® to human HER2 as determined by SPR. (C) Internalization of MRG002 as determined by flow cytometry. (D) Representative figures of MRG002 (green) internalization and transportation to lysosomes (red). Colocalization is indicated in orange. Note that abundant orange signal can be observed at 20 and 40 hr in the MRG002-treated group.

SPR results showed that MRG002 binds to HER2-hFc with sub-nanomolar affinity similar to that of MAB802 and Herceptin® (Fig. 1B). Competitive ELISA results revealed that MRG002 specifically binds to human or monkey HER2, but does not bind to rat or mouse HER2 (data not shown), suggesting that cynomolgus monkey is the relevant species for MRG002 toxicity evaluation.

The serum stability of MRG002 was evaluated by incubating MRG002 in NHS at 37°C for different length of time. The results showed that, with the increase of incubation time, the percentage of intact MRG002 decreased slowly and remained at approximately 76% after 96 h.

Internalization of MRG002 was studied using flow cytometry and confocal microscopy in HER2 expressing SKBR3 cells and BT-474 cells, respectively. In the flow cytometry study (Fig. 1C), the "Surface Localized" signal of MRG002 was abundant initially, but reduced progressively upon incubation at 37°C. The internalization
rate of MRG002 was similar to the unconjugated mAb, MAB802, indicating that the conjugation with vcMMAE did not have significant impact on the antibody’s binding to the cell surface antigen and trafficking. Similarly, in the confocal microscopy study (Fig. 1D), MRG002 (shown in green color) predominantly localized on cell surface initially, but gradually became manifest inside cells, where it overlapped (shown in orange color) with the lysosomal marker Lyso-Tracker-Red (shown in red color). These results demonstrated that MRG002 was able to bind to HER2 on cell surface, and was efficiently internalized and translocated into lysosomes.

Monoclonal antibody of the IgG1 isotype tends to elicit Fc-mediated effector functions such as ADCC and CDC. Here, the ADCC activity of MRG002 and MAB802 was found to be similar, but significantly lower than that of Herceptin® (Supplementary Figure 2). On the one hand, the comparable Fc effector function between MAB802 and MRG002 showed that this function of MAB802 was preserved after conjugation. On the other hand, the reduced ADCC activity of MRG002 and MAB802 compared with Herceptin® was just as designed. ADCC activity of antibody is positively correlated with its affinity to CD16 [23], and the hyper-fucosylation of the Fc region of MRG002 may reduce its affinity to CD16a receptors on NK cells [24].

None of MRG002, MAB802 or Herceptin® showed significant CDC activity, which is consistent with literature reports for Herceptin [25].

### In vitro cytotoxicity of MRG002

The cytotoxic activity of MRG002 was tested in a battery of HER2-expressing tumor cells, including the breast cancer cell lines SKBR3, BT-474 and MDA-MB-453, as well as the gastric cancer cell line NCI-N87 [26]. The marked HER2-targeted ADC Kadcyla® was purchased and used as the reference drug (Fig. 2 and Table 1). The results showed that MRG002 displayed significant cytotoxicity at sub-nanomolar concentrations. And it is worth mentioning that the cytotoxicity of MRG002 was stronger than that of Kadcyla® in all the tested HER2-high expressing cell lines.

### In vivo anti-tumor activity of MRG002

To evaluate the in vivo efficacy of MRG002, studies were first performed in CDX models established with HER2-overexpressed breast cancer cell line BT-474 and gastric cancer cell line NCI-N87. MRG002 (0.3, 1 and 3 mg/kg), the reference article Kadcyla® (3 mg/kg) and a non-binding control ADC (3 mg/kg) were intravenously administered to tumor bearing mice on a qw (once a week) × 3 regime (Fig. 3A and B). In both CDX models, MRG002 significantly inhibited the tumor growth at as low as 0.3 mg/kg. The reference drug Kadcyla® was also effective at 3 mg/kg in both models. The non-binding control ADC, however, did not show tumor growth inhibition in either model.

PDX models, which are xenograft models established in immunodeficient mice using primary tumor tissues, could to the greater degree preserve the heterogeneity, molecular diversity and histological characteristics of original tumors, thus is of higher value in the prediction of efficacy in clinical indications [27]. In order to more effectively predict the efficacy of MRG002 in intended clinical indications, the antitumor effects of MRG002 on 11 PDX models with various levels of HER2 expression were then studied. Of the 11 PDX models, 8 were human gastric cancer models and 3 were human breast cancer models, and 8 out of the 11 were Herceptin®-resistant. In all these studies, Kadcyla® (T-DM1) was used as the reference drug. In order to assess the potential synergistic effect of MRG002 with PD-1 inhibition, STO#410, one of the eight gastric PDX models, was used.

Results of all 11 PDX models including combination treatment are summarized in Fig. 3C–G and Table 2. As a single agent, MRG002 significantly inhibited tumor growth in all 11 PDX models (8 of which were Herceptin®-resistant), and its antitumor activity was always greater than that of Kadcyla® at the same dose. Particularly, MRG002 showed significant tumor-inhibition activity in those Kadcyla®-resistant gastric PDX models. The combination of MRG002 and HX008, anti-PD-1 antibody, showed enhanced antitumor activity than MRG002 or HX008 alone at the same dose. All tumor-bearing mice showed good tolerability to MRG002, Kadcyla® or anti-PD-1 antibody (data not shown).

### Toxicity

IND-enabling repeated-dose (q3w × 4) GLP toxicity study of MRG002 was conducted in cynomolgus monkeys. When MRG002 was administered repeatedly (1, 3 and 6 mg/kg, Q3W × 4) for 9 weeks, the major toxicity, which could be observed at 3 and 6 mg/kg, were reversible dose-dependent hematotoxicity and myelotoxicity that mainly manifested as decrease in erythrocyte series, decrease and subsequent

### Table 1. Cytotoxic activity of MRG002 and Kadcyla® in various cell lines

| Cell Line   | Tissue Origin      | HER2 expression level [25] | IC₅₀ (nM) | MRG002 | Kadcyla® |
|-------------|--------------------|---------------------------|----------|--------|---------|
| SKBR3       | Breast cancer      | High                      |          | 0.012  | 0.031   |
| BT-474      | Ductal breast cancer | High                    |          | 0.035  | 0.907   |
| MDA-MB-453  | Metastatic breast cancer | Medium                |          | 0.398  | 0.438   |
| NCI-N87     | Gastric cancer     | High                      |          | 0.151  | 0.806   |

Toxicity

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increase in reticulocytes and white blood cells, as well as hypocellularity of the bone marrow. The no-observed-adverse-effect level for MRG002 was determined at 1 mg/kg and the highest non-severely toxic dose was 6 mg/kg. No toxicologically significant changes in cardiovascular, respiratory, or neurologic assessment were noted.

The TK analysis in the repeated dose toxicity study confirmed exposure to the conjugated antibody (MRG002), total antibody (TAb, including unconjugated and conjugated antibody), and MMAE after q3w × 4 repeated intravenous infusion of 1, 3 and 6 mg/kg MRG002. Generally, the exposures of MRG002, TAb and MMAE were proportional to the dose levels. No accumulation of MRG002 was observed after three cycles of q3w × 4 repeated administration in any dose group. Clearance of MRG002 was comparable to that of TAb, and serum MMAE concentration always remained at very low level, indicative of good stability of MRG002 in circulation. In addition, exposures of MRG002, TAb and MMAE were similar between genders at all dose levels (data not shown).

**DISCUSSION**

Here, we describe preclinical evaluation of MRG002, a novel vcMMAE-based anti-HER2 ADC. MRG002 demonstrated potent *in vitro* and *in vivo* anti-tumor activity in HER2-expressing breast cancer and gastric cancer CDX and PDX models, and displayed manageable toxicity in GLP toxicology studies. The antitumor activity of MRG002 was shown to be superior than that of Kadcyla® at the same dose in the models tested. Strikingly, MRG002 showed significant tumor-inhibition activity in trastuzumab- and Kadcyla®-resistant gastric PDX models (Fig. 3E and F). Additionally, in a gastric cancer STO#410 model where MRG002 and HX008 were tested alone or in combination, MRG002 significantly sensitized the tumor to PD-1 inhibitor. For instance, HX008 alone at 5 mg/kg did not exhibit significant antitumor activity, however, combination of MRG002 and HX008 showed synergistic activity in the tumor growth inhibition compared with the MRG002 group (Fig. 3G). This observation is consistent with literature reports that ADCs bearing
Figure 3. *In vivo* antitumor activity of MRG002. (A) and (B) Mice bearing BT-474 and NCI-N87 tumors were treated with MRG002 (0.3, 1, 3 mg/kg) or the reference drug Kadcyla® (T-DM1, 3 mg/kg) on a qw × 3 regime; a nonbinding control ADC using the same linker-drug was used to confirm target-specific tumor inhibition. The TVs are presented as means from \( N = 3 \) animals for each time point. (C–F) Representative results of MRG002 tumor growth inhibition activity in PDX models of breast cancer and gastric cancer. The reference drug Kadcyla® (T-DM1) was used at the dose identical to the highest dose used in a specific model. The TVs are presented as means from \( N = 8 \) animals for each time point. (G) Tumor growth curve of MRG002, anti-PD-1 antibody (HX008) or MRG002 + HX008 combination in a gastric PDX model. The humanized mice (Hu-HSC-NPG) were treated with HX008 (5 mg/kg, q3d (once every 3 days) × 8), MRG002 (3 mg/kg, q3w × 2) or HX008 + MRG002. The TVs are presented as means from \( N = 6 \) animals for each time point. \( *p<0.05: D21, \text{MRG002 3 mg/kg vs MRG002 + HX008 combination.} \)

A microtubule-disrupting agent can reactivate suppressed tumor microenvironment, such as promoting PD-L1 expression in tumor-associated macrophages, and sensitize the tumor to immune checkpoint inhibitors [28–30]. As an ADC, MRG002 functions via HER2-mediated endocytosis and subsequent lysosomal release of the cytotoxic payload MMAE from the internalized ADC, and thereby to certain extent circumvents several...
Table 2. Summary of the in vivo efficacy of MRG002 in PDX models

| Tumor type   | Model number | HER2 IHC/ Herceptin® resistance | Kadcyla® T/C (%) | Anti-tumor activity | MRG002 T/C (%) | Anti-tumor activity |
|--------------|--------------|---------------------------------|----------------|--------------------|----------------|--------------------|
| Breast cancer| BC#046       | 3+/Yes                          | 7              | ++++               | 45             | 0                  | ++++               |
|              | BC#197       | 2+/Yes                          | /              | /                  | /              | 14                 | 2                  | ++++               |
|              | BC#239       | 2+/Yes                          | /              | /                  | /              | 10                 | 3                  | ++++               |
| Gastric cancer| STO#041       | 3+/Yes                          | 84             | /                  | /              | 1                  | /                  | ++++               |
|              | STO#053       | 1+/No                           | /              | /                  | /              | 19                 | /                  | ++++               |
|              | STO#069       | 3+/No                           | /              | /                  | /              | 71                 | 6                  | ++++               |
|              | STO#151       | 2+/Yes                          | 90             | /                  | /              | 1                  | /                  | ++++               |
|              | STO#179       | 3+/Yes                          | /              | /                  | /              | 1                  | /                  | ++++               |
|              | STO#240       | 1+/No                           | /              | /                  | /              | 41                 | /                  | ++++               |
|              | STO#395       | 2+/No                           | /              | /                  | /              | 99                 | 9                  | ++++               |
|              | STO#410       | 2+/Yes                          | 109            | /                  | /              | 40                 | 0                  | ++++               |

1. HER2 expression in breast cancer models was graded via IHC according to the “Guidelines for HER2 Detection in Breast Cancer (2014)”, and HER2 expression in gastric cancer models was graded via IHC according to the “Guidelines for HER2 Detection in Gastric Cancer (2011)”.  
2. "/" Indicates that the dose was not administered.  
3. Relative tumor growth rate (T/C(%) = (relative TV [RTV] of test article group/RTV of vehicle group) × 100%; RTV = Vf/V0, where V0 is the TV measured before grouping (i.e., Day 0) and Vf is the TV measured on the last day of experiment; TV = l x w^2/2, where “l” and “w” represents the length and width of a tumor. T/C(%) value is rounded to whole number.

resistance- causing mechanisms, such as defects in the PTEN-PI3K/AKT pathway, autocrine production of EGF-related ligands, and impaired ADCC activity [20]. In gastric cancer, it has been reported that the ADCC activity of trastuzumab is impaired due to NK cell dysfunction [31]. In contrast to trastuzumab, which relies largely on ADCC activity and other immune responses to kill targeted cancer cells, MRG002 kills cancer cells via potent cytotoxins it delivers to the cells regardless of immune status. In addition, the reduced ADCC activity due to hyperfucosylation will potentially reduce unwanted side effects in the clinic. The superior efficacy of MRG002 observed in comparison with Kadcyla® in gastric cancer models would at least partially ascribe to the high heterogeneity of gastric cancer [32]. MMAE is also capable of diffusing out of the target cells and exerts bystander killing [33]. This trait is especially advantageous for treating those tumors with heterogenous target expression.

Admittedly, the tumor-inhibition effect of MRG002 does not always correlate well with HER2 expression level. This may be perhaps due to those unknown mutations that may jeopardize the endocytosis or trafficking of MRG002, or those that promote tumor cell survival. In fact, such resistance mechanisms have been studied for Kadcyla® [20]. However, it should be noted that even though the anti-tumor effect may be to certain extent affected by these mechanisms, MRG002 is still more potent in all the breast cancer and gastric cancer models that have been tested. The combination therapy with PD-1 inhibitor explored further therapeutic potential of MRG002 in patients with PD-1-resistant, HER2-overexpressing cancers.

In summary, the encouraging preclinical results support clinical evaluation of MRG002 for treating HER2-positive breast cancer and gastric cancer. In fact, a phase I clinical study for patients with HER2-expressing solid tumors has been ongoing (CTR20181778).

Supplementary Data

Supplementary Data are available at ABT Online.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

Acknowledgment

We wish to thank Ms Xiaojing Shi for her assistance in reformating the figures and references of this manuscript.

Conflict of interest statement

None declared.

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