A Novel Anti-HER2 Antibody–Drug Conjugate XMT-1522 for HER2-Positive Breast and Gastric Cancers Resistant to Trastuzumab Emtansine

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Abstract

Most patients with HER2-positive breast or gastric cancer exhibit primary or acquired resistance to trastuzumab emtansine (T-DM1), and such patients may have limited therapeutic options. XMT-1522 is a novel anti-HER2 antibody–drug conjugate. We compared XMT-1522 to T-DM1 in preclinical models. The effects of XMT-1522 and T-DM1 on cell survival and apoptosis were compared in six HER2-positive breast cancer or gastric cancer cell lines, of which three lines were T-DM1–sensitive (N-87, OE-19, JIMT-1) and three T-DM1–resistant (RN-87, ROE-19, SNU-216). We compared these agents also in the HER2-negative breast cancer cell line MCF-7, and in mouse RN-87 and JIMT-1 xenograft models. Cell survival was assessed using the AlamarBlue method and apoptosis with the Caspase-Glo 3/7 method. XMT-1522 inhibited the growth of all six HER2-positive cell lines. The proportions of cells that survived XMT-1522 treatment were smaller as compared with T-DM1, particularly in the T-DM1–resistant cell lines. XMT-1522 induced more cell apoptosis compared with T-DM1. While RN-87 and JIMT-1 xenograft tumors progressed on T-DM1 treatment, all tumors responded to XMT-1522, and all but one tumor disappeared during the XMT-1522 treatment. XMT-1522 had a strong antitumor effect on RN-87 and JIMT-1 xenografts that progressed on T-DM1. We conclude that XMT-1522 was effective in HER2-positive breast cancer and gastric cancer cell lines resistant to T-DM1, and in xenograft models resistant to T-DM1. The results support the testing of XMT-1522 in clinical trials in patients with HER2-positive cancer.

Introduction

Overexpression and amplification of the human epidermal growth factor receptor-2 (HER2) is present in about 15%–20% of human breast cancers and gastric cancers (1–3). Trastuzumab, a humanized mAb that binds to the extracellular domain IV of HER2, has substantial antitumor activity, and is approved for the treatment of HER2-positive breast cancer and for HER2-positive metastatic gastric or gastroesophageal junction adenocarcinoma (4, 5). Although responses to trastuzumab are often durable, most advanced cancers eventually become resistant to trastuzumab (6–8).

Antibody–drug conjugates (ADC) can deliver cytotoxic payloads to cancer cells. Trastuzumab emtansine (T-DM1, Kadcyla) is an anti-HER2 ADC where trastuzumab is linked with DM1 (a derivative of maytansine) via a nonreducible thioether linker (9). One T-DM1 carries an average of 3.5 DM1 moieties. DM1 and other T-DM1 catalobites such as MCC-DM1 (4-[N-maleimidomethyl]cyclohexane-1-carbonyl-DM1) and lysine-MCC-DM1 are released following receptor-mediated internalization and lysosomal degradation of the conjugate (10). Intracellular DM1 is a potent inhibitor of the microtubule assembly thereby causing cell death (9, 11). The FDA approved T-DM1 as monotherapy for the treatment of patients with HER2-positive advanced breast cancer who had previously received trastuzumab and a taxane in 2013 (12, 13). As with trastuzumab, the majority of the initially responding advanced breast cancers eventually became resistant to T-DM1 (12, 13). Adjuvant T-DM1 was superior to trastuzumab in a randomized trial in patients with HER2-positive breast cancer who had residual cancer in the breast or axilla at surgery after neoadjuvant chemotherapy and trastuzumab (14), but it was not superior to taxane chemotherapy in a patient population with previously treated HER2-positive advanced gastric cancer in a randomized trial (15).

XMT-1522 is a novel anti-HER2 ADC containing a human IgG1 anti-HER2 mAb (HT-19) that binds to domain IV of HER2 to an epitope that is distinct from the trastuzumab-binding site, and it does not compete with trastuzumab for HER2 binding. In XMT-1522, each antibody is armed with an average of 12 auristatin F-hydroxypropylamide (AF-HPA) moieties, linked to HT-19 via a cysteine linkage using a biodegradable hydrophilic linker (9). One T-DM1 carries an average of 3.5 DM1 moieties. DM1 and other T-DM1 catalobites such as MCC-DM1 (4-[N-maleimidomethyl]cyclohexane-1-carbonyl-DM1) and lysine-MCC-DM1 are released following receptor-mediated internalization and lysosomal degradation of the conjugate (10). Intracellular DM1 is a potent inhibitor of the microtubule assembly thereby causing cell death (9, 11). The FDA approved T-DM1 as monotherapy for the treatment of patients with HER2-positive advanced breast cancer who had previously received trastuzumab and a taxane in 2013 (12, 13). As with trastuzumab, the majority of the initially responding advanced breast cancers eventually became resistant to T-DM1 (12, 13). Adjuvant T-DM1 was superior to trastuzumab in a randomized trial in patients with HER2-positive breast cancer who had residual cancer in the breast or axilla at surgery after neoadjuvant chemotherapy and trastuzumab (14), but it was not superior to taxane chemotherapy in a patient population with previously treated HER2-positive advanced gastric cancer in a randomized trial (15).

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and polymer that enables high AF-HPA loading (ref. 16; the structure of XMT-1522 is provided in the Supplement). Both AF-HPA and its intracellular catabolite auristatin F (AF) are potent inhibitors of tubulin polymerization (17).

We compared XMT-1522 to T-DM1 in both T-DM1–sensitive and T-DM1–resistant in vitro and in vivo models of breast cancer and gastric cancer, and found XMT-1522 to be generally more effective than T-DM1. To our knowledge, this is the first study to demonstrate that XMT-1522 has pronounced anticancer effect on HER2-positive T-DM1–resistant cancers.

Materials and Methods

Cell lines

The cell lines used are summarized in Supplementary Table S1. OE-19 was obtained from the European Collection of Cell Culture (CARM Centre for Applied Microbiology and Research, Wilshire, United Kingdom), NCI-N87 (N-87) and MCF-7 from the ATCC, SNU-216 from the Korean Cell Line Bank, and JIMT-1 (Tampere, Finland; ref. 18). The cell lines were cultured according to the recommended specifications.

We generated the T-DM1–resistant HER2-positive gastric cancer cell lines ROE-19 and RN-87 by treating OE-19 and N-87 cells, respectively, with increasing concentrations of T-DM1 (Roche Ltd.). N-87 and OE-19 cells sensitive to T-DM1 were initially exposed to 0.12 μg/mL or 0.08 μg/mL of T-DM1, respectively, and then to gradually increasing concentrations of T-DM1 up to a maximum concentration of 2 μg/mL over 7 months (N-87) or 9 months (OE-19).

Authentication of cell lines was performed using short tandem repeat analysis. All lines were tested for Mycoplasma routinely and only Mycoplasma-free cells were used.

In vitro assays of cell viability and caspase activation

The effects of T-DM1 and XMT-1522 (Mesana Therapeutics Inc.) on the cell growth were studied using the AlamarBlue method (Thermo Fisher Scientific). The cells were trypsinized and plated in 96-well, flat-bottomed tissue culture plates. The effects of T-DM1 and XMT-1522 were tested at concentrations of 0.0001, 0.0006, 0.003, 0.016, 0.08, 0.4, 1, 2, and 10 μg/mL. The MCF-7 breast cancer cell line with low HER2 expression was used as the negative control. The numbers of viable cells were assessed after 5 days of incubation by addition of the AlamarBlue reagent (Thermo Fisher Scientific). Fluorescence was measured with excitation at 540 nm and emission at 590 nm using a PHERAstar FS plate reader (BMG Labtech). The fluorescence of the samples was normalized to the fluorescence of the cell-free culture media. The results are presented as the proportion of viable cells, obtained by dividing the fluorescence of the test samples by the fluorescence of the PBS-treated control samples. The dose achieving half-maximal (50%) inhibitory concentration (IC50) with the drugs was calculated using the GraphPad Prism software (GraphPad Software).

To assess the rate of apoptosis, caspase activation was measured using the Caspase-Glo 3/7 method (Promega; ref. 19). The cells were trypsinized and plated in 96-well flat-bottomed tissue culture plates. After overnight culture, the medium was exchanged to a medium containing 0.0006, 0.003, 0.016, 0.08, 0.4, 1, or 2 μg/mL concentration of either T-DM1 or XMT-1522. After 48 hours of incubation, 100 μL of the medium was transferred into white-walled 96-well plates, mixed with 100 μL Caspase-Glo 3/7 reagent, incubated for 30 minutes at room temperature, and the luminescence was recorded using a PHERAstar FS plate reader (BMG Labtech). The results are presented as luminescence units obtained after subtracting the luminescence value from a blank reaction (without T-DM1 or XMT-1522 treatment).

Mouse xenografts

The National Animal Experiment Board of Finland approved the mouse experiments. Five- to 8-week-old female SCID mice (Envigo RMS B.V., Horst, The Netherlands) were injected subcutaneously with 1.5 × 106 or 1.6 × 107 of human breast cancer cells (BMI-1) in 150 μL of the culture medium (DMEM supplemented with 10% FBS), 1.4 × 107 of human gastric cancer cells (N-87), or with 2.5 × 107 of T-DM1–resistant human gastric cancer cells (RN-87) in 150 μL of the cell culture medium (RPMI supplemented with 10% FBS) to establish xenograft tumors. Following this, T-DM1 (5 mg/kg) or XMT-1522 (1 mg/kg or 3 mg/kg) were administered intravenously (i.v.) at 7-day intervals. As a control, PBS was administered intraperitoneally (i.p.) at 7-day intervals. Tumor size was measured using a caliper, and tumor volume was calculated using the formula \( \text{Volume} = \frac{\pi r^2 h}{6} \) (smaller diameter) \( \times \) (larger diameter) \( \times \) (smaller diameter) \( \times \) (larger diameter). Mice with tumor > 20 mm in any one dimension or tumor ulceration were sacrificed using CO2 inhalations and cervical dislocations.

Electron microscopy

Extracellular vesicle (EV) samples were prepared for electron microscopy and imaged as described elsewhere (19, 20) using an immunostaining procedure. In brief, after paraformaldehyde fixation, the samples were blocked with 0.5% BSA in 0.1 M sodium phosphate buffer (pH 7.0) for 10 minutes at room temperature, incubated with a 12 nm colloidal gold-conjugated goat-anti-human-IgG secondary antibody (gold-GAHIG; Jackson ImmunoResearch) in the same sodium phosphate buffer for 30 minutes at room temperature, washed with the sodium phosphate buffer and deionized water, stained with uranyl acetate, and embedded in a methyl cellulose uranyl acetate mixture. The samples were viewed using either a Tecnai 12 transmission electron microscope (FEI Company) or a Jeol JEM-1400 transmission electron microscope (Jeol Ltd.), each operating at 80 kV.

Other methods

The methods used for RNA isolation, RNA expression analysis, IHC, FISH, flow cytometry, EV sample preparation and analysis, and the nanoparticle tracking analysis are provided in the Supplementary Data.

Statistical analysis

Data are expressed as the mean ± SE. Groups were compared using Student t test when the data passed the normality test. Gene expression data were compared using two-way ANOVA. Unpaired groups were compared with the Mann–Whitney test. Survival was analyzed using the Kaplan–Meier method, and survival between groups was compared with the log-rank test. Statistical calculations were carried out using the IBM SPSS version 24 (IBM). All P values are two-sided.
Results

XMT-1522 inhibits the growth of T-DM1–resistant cells in vitro

The growth-inhibitory effects of T-DM1 and XMT-1522 were compared in six HER2-positive cancer cell lines and in the control cell line (MCF-7) that does not harbor HER2 amplification and has a low level of HER2 protein expression (21). XMT-1522 inhibited the growth of all HER2-positive cell lines in a dose-dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner. The proportion of cells surviving the treatments was smaller with XMT-1522 than with T-DM1 particularly in a dependent manner.

As expected, neither T-DM1 nor XMT-1522 was effective in the HER2-negative MCF-7 cell line (Fig. 1A–G).

To estimate the rate of apoptosis, we studied the influence of T-DM1 and XMT-1522 on caspase-3 and/or caspase-7 in the HER2-positive cell lines JIMT-1 and RN-87. In both cell lines, XMT-1522 increased caspase-3 and/or caspase-7 activity in a dose-dependent manner more than T-DM1 (Fig. 1H and I).

XMT-1522 eradicates T-DM1-resistant RN-87 xenografts in vivo

The effects of XMT-1522 and T-DM1 on the growth of RN-87 xenografts were compared in mice. The RN-87 gastric cancer xenografts expressed HER2 at the same level as the T-DM1-sensitive parental N-87 xenografts (Supplementary Fig. S1A and S1B). Tumors formed in all 44 mice inoculated with RN-87 cell suspension within 7 days from the date of inoculation reaching a mean tumor volume of 39.5 ± 11.4 mm³ on day 7. On day 18 since inoculation, the mice were split into four groups, and were treated weekly with either PBS (n = 12), T-DM1 5 mg/kg (n = 20), XMT-1522 1 mg/kg (n = 6), or XMT-1522 3 mg/kg (n = 6). T-DM1 did not inhibit tumor growth, whereas both doses of XMT-1522 inhibited tumor growth in a dose-dependent manner (Fig. 1H and I).

Figure 1.

Effect of T-DM1 and XMT-1522 on the growth (A–G) and caspase activity (H and I) of breast cancer and gastric cancer cells lines. Cell growth rate was assessed with the AlamarBlue method and apoptosis with the Caspase-Glo 3/7 method.
induced rapid and complete tumor shrinkage (Fig. 2A and B). Six out of the 20 T-DM1-treated mice were euthanized due to the presence of a large ulcerated tumor by day 35 since the inoculation, the T-DM1 treatment of nine mice was discontinued on day 35, and five mice continued to receive the T-DM1 treatment. From day 35 onwards, 4 of the 9 mice whose T-DM1 treatment was discontinued were treated with 1 mg/kg of XMT-1522 and 5 mice with 3 mg/kg of XMT-1522. All 9 mice treated with XMT-1522 following the T-DM1 treatment had tumor shrinkage, whereas the five mice that continued on T-DM1 showed persistent tumor growth. Two of the 5 mice treated with 3 mg/kg of XMT-1522 were euthanized due to ulceration of the tumor on day 43 since the inoculation, and the three remaining mice had unmeasurable tumor by day 67. All four tumors treated with 1 mg/kg of XMT-1522 became unmeasurable by day 71. XMT-1522 treatment was discontinued for all the remaining animals on day 63 with follow-up until day 85. No tumor relapse was observed during the follow-up time. No significant difference in the tumor growth rate was observed between the two XMT-1522 dosing groups.

On day 35 since the date of tumor inoculation, 3 of the 12 mice allocated to PBS were switched to 1 mg/kg or 3 mg/kg in a few mice, and PBS treatment to either XMT-1522 1 mg/kg, XMT-1522 3 mg/kg, or T-DM1. Figure 2B, The drug administration schedule.

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**Figure 2.**
Effect of T-DM1 and XMT-1522 on the growth of RN-87 xenografts in SCID mice (A). On day 35 after tumor inoculation, the T-DM1 treatment was switched to either XMT-1522 1 mg/kg or XMT-1522 3 mg/kg in a few mice, and PBS treatment to either XMT-1522 1 mg/kg, XMT-1522 3 mg/kg, or T-DM1. B, The drug administration schedule.
group were euthanized due to ulceration of the tumor on day 43 and on day 46 since the inoculation, respectively, but the four remaining tumors became unmeasurable by day 71 in the 1 mg/kg group and by day 67 in the 3 mg/kg group similar to the mice that were switched from T-DM1 to XMT-1522.

XMT-1522 increased mice survival compared with T-DM1 ($P < 0.001$; Supplementary Fig. S2A). The mice whose T-DM1 treatment was switched to XMT-1522 survived longer than the mice that had been treated continuously with T-DM1 ($P = 0.010$; Supplementary Fig. S2B).

**XMT-1522 eliminates breast cancer xenografts**

The effects of XMT-1522 and T-DM1 were next compared using a JIMT-1 HER2-positive breast cancer xenograft model. Tumors formed in all 32 mice inoculated with the JIMT-1 cell suspension by day 7 with a mean tumor volume of $80.6 \pm 26.9 \text{ mm}^3$ on day 7. On day 15 after the inoculation, the mice were split into four treatment groups, and were treated weekly for 3 weeks with either PBS ($n = 10$), T-DM1 (5 mg/kg, $n = 10$), XMT-1522 1 mg/kg ($n = 6$), or XMT-1522 3 mg/kg ($n = 6$). While the T-DM1-treated tumors grew continuously, tumor shrinkage was rapid in both XMT-1522 dosing groups. All six mice treated with 3 mg/kg of XMT-1522 and 5 of the 6 mice treated with 1 mg/kg of XMT-1522 had no palpable tumor from day 70 onwards. The one remaining tumor shrunk from the size of $301.4 \text{ mm}^3$ on day 15 when XMT-1522 treatment was started to $4.2 \text{ mm}^3$ on day 63, and then started to grow reaching $14.1 \text{ mm}^3$ on day 98. From day 99 onwards this mouse was treated with XMT-1522 3 mg/kg for three weeks, which resulted in tumor shrinkage to $1.5 \text{ mm}^3$ on day 131. Mice allocated to XMT-1522 survived longer than those allocated to T-DM1 ($P < 0.001$; Fig. 3).

Next, the XMT-1522 and T-DM1 treatments were started at the time of JIMT-1 cell inoculation to compare the drug effects on tumor formation. Six mice treated with PBS were used as a control. The tumors in the T-DM1-treated mice ($n = 18$) remained small until day 27, but then started to grow. In the two XMT-1522 dosing groups (1 mg/kg and 3 mg/kg, $n = 6$ each), no tumors were detectable from day 20 onwards (Fig. 4A and B). The mice treated with T-DM1 survived longer than the control group mice ($P < 0.001$) while the mice allocated to XMT-1522 survived longer than those treated with T-DM1 ($P < 0.001$; Fig. 4C).

XMT-1522 was also tested in mice that had cancer progression while on T-DM1 5 mg/kg weekly. Two of these 18 mice were euthanized due to a large ulcerated tumor by day 43 since the date of tumor inoculation.

**Figure 3.**

Effect of T-DM1 and XMT-1522 on the growth of JIMT-1 xenografts in SCID mice (A). The drug administration times are indicated with arrows below the x-axis. One tumor treated with XMT-1522 1 mg/kg started to grow after initial shrinkage and was re-treated with the higher dose of XMT-1522 from day 99 onwards (pink arrows). B, Survival of mice in the four treatment groups since the date of tumor inoculation.
of tumor inoculation. On day 43, 10 of the remaining 16 mice had the treatment switched to either 1 mg/kg of XMT-1522 (n = 5) or 3 mg/kg of XMT-1522 (n = 5), and 6 mice continued with T-DM1 treatment. The tumors of the 6 mice that continued with T-DM1 treatment progressed, while the mice treated with XMT-1522 had rapid tumor shrinkage irrespective of the XMT-1522 dosage (Fig. 4A and B). Four of the 5 mice treated with XMT-1522 3 mg/kg had no palpable tumor from day 76 onwards, but one tumor became detectable on day 125 and then progressed. Two out of the 5 mice treated with XMT-1522 1 mg/kg had no palpable tumor from day 83 onwards, the 3 remaining tumors first shrank (to unmeasurable, 14.1 mm³, and 6.3 mm³), but then started to
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Figure 5.
XMT-1522 evokes apoptosis. More apoptotic cancer cells (arrows) were present in RN-87 tumors (B) and JIMT-1 tumors (D) treated with XMT-1522 than in those treated with T-DM1 (A and C). Many apoptotic cells were present in a JIMT-1 tumor that relapsed after being treated with the 1 mg/kg dose of XMT-1522 but was sensitive (s.) to the 3 mg/kg dose of XMT-1522 (E). Few apoptotic cells were present in the tumors that progressed first on T-DM1 then on XMT-1522 (F). Scale bar, 30 μm.

Expression of HER2 and EGFR on T-DM1–treated and XMT-1522–treated JIMT-1 xenografts
JIMT-1 tumors treated continuously with T-DM1 retained their HER2 expression, but gained some EGFR expression (Supplementary Fig. S4A and S4B, S4E, and S4G). The JIMT-1 tumor that ceased to respond to 1 mg/kg of XMT-1522 but responded to 3 mg/kg of XMT-1522 remained HER2-positive and EGFR-negative (Supplementary Fig. S4D and S4I). All 4 tumors that progressed on T-DM1 and were subsequently treated with XMT-1522 had lost the HER2 expression, and two of the four progressing tumors expressed slightly also EGFR (Supplementary Fig. S4E and S4I). Giant multinucleated cells and cells with aberrant mitotic morphology, the hallmarks of mitotic catastrophe (22), were observed in both T-DM1–treated and XMT-1522–treated xenografts (Supplementary Fig. S4B–S4D and S4G–S4I).

Loss of HER2 amplification in T-DM1–pretreated JIMT-1 xenografts that progressed on XMT-1522 treatment
Interestingly, JIMT-1 tumors that progressed on T-DM1 and were subsequently treated with XMT-1522 and that after an initial response progressed also on XMT-1522 showed loss of HER2 gene amplification unlike JIMT-1 tumors that were continuously treated with T-DM1 (P < 0.0001; Supplementary Fig. S5A–S5D).

HER2 and ATP-binding cassette (ABC) transporter mRNA and protein expression in the T-DM1–resistant cell lines
To elucidate the mechanisms associated with the T-DM1 resistance, gene expression of the T-DM1–sensitive parental gastric cancer cell lines (N-87, OE-19) was compared to their resistant counterparts (RN-87, ROE-19). Slight downregulation of HER2 mRNA was detected in the resistant cells as compared with the corresponding sensitive cell lines (RN-87 vs. N-87, 1.35-fold decrease; ROE-19 vs. OE-19, 1.72-fold decrease). ATP-binding cassette (ABC) transporters may cause resistance to anticancer therapy by increasing the efflux of chemotherapy agents from cancer cells (23). Substantial upregulation of ABCC2 and ABCG2 mRNA was found in the resistant RN-87 cells as compared with the sensitive N-87 cells (1385-fold and 116-fold increase, respectively), and significant upregulation of ABCB1 and ABCG2 was detected in the resistant ROE-19 cells as compared with the sensitive OE-19 cells (98-fold and 37-fold increase, respectively; Fig. 6A).

Flow cytometric analyses of the corresponding protein levels supported these findings. Resistant RN-87 and ROE-19 cells had lower cell surface HER2 expression than the sensitive N-87 cells and OE-19 cells (P = 0.005 and P = 0.007, respectively; Fig. 6B). There were no significant associations between the drug responses (IC50 values) to either T-DM1 or XMT-1522 and the numbers of cell surface HER2 receptors (r = −0.94, P = 0.23; r = −0.49, P = 0.32, respectively; Fig. 6C). The expression of ABCB1, ABCG2, and ABCG2 proteins in the cell lines were in agreement with the corresponding mRNA expression levels. SNU-216 gastric cancer cells had very low levels of ABCB1 and ABCG2 (Fig. 6D–F).

Inhibition of ABC transporters sensitizes the resistant cells to T-DM1
To investigate the role of ABC transporters in resistance to T-DM1, three ABC transporter inhibitors tetrandrine, MK571, and...
elacridar were studied either as single agents or in a combination with T-DM1 in HER2-positive T-DM1-resistant gastric cancer cell lines RN-87 and ROE-19. MK571 (Sigma-Aldrich) is an inhibitor of ABCB1, ABCB2, and ABCG2 (24, 25), elacridar (Sigma-Aldrich) inhibits ABCB1 and ABCB2 (26), and tetrateridine (Abcam) inhibits ABCB1 and ABCG2 (27). Tetraderidene (5 μM/L), MK571 (50 μM/L), and elacridar (1 μM/L) were used either alone or combined with T-DM1 at concentrations of 10 μg/mL, 10 μg/mL, and 2 μg/mL, respectively. In each experiment, the combination of an ABC transporter inhibitor and T-DM1 resulted in the lowest cell survival (Fig. 6G-I). These data suggest that overexpression of ABCB1, ABCB2, and ABCG2 transporters decreases biological activity of T-DM1 in RN-87 and ROE-19 cells.

T-DM1-resistant cells dispose T-DM1 by extracellular vesicle (EV) secretion

Cancer cells can dispose T-DM1 by EV secretion (19), and we, therefore, compared this mechanism between the T-DM1-sensitive N-87 and T-DM1-resistant RN-87 cells. EVs of 30 to 300 nm in diameter were detected by using transmission electron microscopy and no difference in the EV particle size distributions were detected between the two cell lines by nanoparticle tracking analysis (Supplementary Fig. S6). At immuno-electron microscopy, T-DM1 was present on the surface of EVs derived from both N-87 and RN-87 cells treated with T-DM1 (Supplementary Fig. S7A and S7B). Higher amounts of T-DM1 were found on the EVs prepared from RN-87 cells than on those derived from N-87. Except for T-DM1, no difference in the EV particle size distributions were detected between the two cell lines by nanoparticle tracking analysis (Supplementary Fig. S6). Higher amounts of T-DM1 were found on the EVs prepared from RN-87 cells than on those derived from N-87. These data suggest that the resistant cells dispose more T-DM1 by secreting EVs than the T-DM1-sensitive cells. The T-DM1-binding capacity (TBC) of the EVs harvested from the RN-87 cells was also higher than that of the EVs harvested from N-87 cells (P = 0.037; Supplementary Fig. S7C and S7D).

Discussion

Most cancers treated with T-DM1 eventually progress (12, 13), and, therefore, novel agents for treating T-DM1-resistant HER2-positive cancers are needed. We report here that both breast and
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gastric cancer cells resistant to T-DM1 are sensitive to XMT-1522, a next-generation anti-HER2 ADC. XMT-1522 had a more pronounced growth-inhibitory effect on breast and gastric cancer cells sensitive to T-DM1 than T-DM1 in vitro. XMT-1522 had marked antitumor efficacy in vitro and in mouse xenograft models of breast cancer and gastric cancer with resistance to T-DM1.

The biological activity of T-DM1 likely depends on the cytosolic concentration of its cytotoxic moiety, DM1. Therefore, the factors that decrease the intracellular DM1 concentration may contribute to resistance (11), such as decreased expression of HER2 leading to delivery of less T-DM1 into the cells (28), and overexpression of drug efflux proteins that can discard DM1 from the cells (24, 28). In line with these observations, we detected decreased expression of HER2 on the T-DM1–resistant RN-87 and ROE-19 gastric cancer cells as compared with their sensitive parental cells. In addition, the resistant cells expressed higher levels of ABCB1 (multi-drug resistance protein 1), ABCB2 (multi-drug resistance protein 2), and ABCG2 (breast cancer resistance protein). Inhibitors of these transporters (MK571, elacridar, and tetratidine) restored the sensitivity of the resistant cells to T-DM1, suggesting that these transporters play a role in the T-DM1 resistance in RN-87 and ROE-19 cells. We also propose a novel mechanism that can further decrease intracellular DM1 concentrations. When we compared T-DM1–sensitive and T-DM1–resistant cancer cells (N-87 and RN-87, respectively), the secreted extracellular vesicles (EV) derived from the resistant cells contained more HER2 compared with the EVs derived from the sensitive cells (although the resistant cells had less cell surface HER2), and more T-DM1 was present on EVs harvested from the T-DM1–resistant RN-87 cells. Therefore, disposal of T-DM1 on the secreted EVs by the resistant cells may contribute to T-DM1 resistance.

Loss of HER2 expression and increased EGFR expression were linked to the acquired T-DM1 resistance based on an in vitro JIMT-1 breast cancer cell model (29). We found decreased, but still substantial expression of HER2 protein in the T-DM1–resistant JIMT-1 xenografts, and increased expression of EGFR. While higher EGFR expression could serve as a growth signal bypassing HER2, the mechanisms causing T-DM1 resistance in JIMT-1 cells remain largely unknown.

We tested three strategies to administer XMT-1522 in mouse xenograft models, and found XMT-1522 effective in each setting. When XMT-1522 was administered to mice, mice carrying small subcutaneous tumors that had not been treated previously, all RN-87 gastric cancer xenografts and all but one JIMT-1 breast xenograft shrank rapidly and disappeared. XMT-1522 was also effective on larger xenograft tumors that were resistant to T-DM1. In this setting, most RN-87 gastric cancer xenografts and most JIMT-1 breast cancer xenografts disappeared during the XMT-1522 treatment. Interestingly, several of the few tumors that did not regress completely on XMT-1522 treatment or recurred following XMT-1522 treatment were HER2-negative in both IHC and FISH, suggesting that loss of HER2 expression, which might be caused by loss of HER2 amplification, is a resistance mechanism to XMT-1522. In the third setting where drug administration was started already at the time of tumor cell inoculation mimicking micrometastatic disease and the adjuvant setting, XMT-1522 inhibited tumor formation, whereas in the T-DM1–treated mice the tumors progressed.

Retrospective analyses of two phase II trials evaluating T-DM1 showed lower response rates in patients with lower HER2 levels (30, 31), suggesting that response to T-DM1 depends on the amount of tumor HER2 expression even in the subset of cancers that were judged as HER2-positive. Hypothetically, high response rates may be achieved with ADCs that have a high drug antibody ratio (DAR). In XMT-1522 the cytotoxic anti-HER2 moiety is conjugated to an anti-HER2 antibody via a polymer linker that allows high drug loading (16). The higher DAR of XMT-1522 compared with T-DM1 (12 vs. 3.5, respectively) may in part explain the superior antitumor efficacy of XMT-1522 in our breast and gastric cancer models as compared with T-DM1. Further study is needed to find out whether AF-HPA and its catabolite auristatin F (AF) are less avid substrates for the ABCB1, ABCB2, and ABCG2 transporters than lysine-MCC-DM1, the active catabolite of DM1.

The greater antitumor effect of XMT-1522 might also be related to the bystander effect. AF-HPA is membrane-permeable and can, therefore, enter the neighboring cells where AF-HPA is converted to highly cytotoxic but non-cell membrane-permeable AF, which is trapped in the cells (17). In contrast, lysine-MCC-DM1 that is formed at proteolytic degradation of T-DM1 in the lysosomes cannot cross the cell membranes, and, therefore, T-DM1 lacks the bystander effect (9). Both DM1 and auristatins are highly cytotoxic payloads causing cell death by inhibiting tubulin polymerization (31). Antibody-dependent cellular cytotoxicity (ADCC) likely contributes substantially to the efficacy of trastuzumab and T-DM1 in vivo (22, 32). HT-19, the antibody part of XMT-1522, may also evoke ADCC (33).

In summary, XMT-1522, an anti-HER2 ADC showed a stronger inhibitory effect on breast and gastric cancer cells sensitive to T-DM1 than T-DM1. In addition, it was efficacious on gastric cancer cells with primary or acquired resistance to T-DM1 in vivo, and XMT-1522 had antitumor efficacy also in T-DM1–resistant breast cancer and gastric cancer xenografts in vivo. The results support clinical evaluation of XMT-1522 in patients with HER2-positive breast cancer or gastric cancer.

Disclosure of Potential Conflicts of Interest

H. Joensuu is a vice president (employment or paid consulting) at Orion Pharma, has ownership interest (including patents) at Sartar Therapeutics, has an unpaid consultant/advisory board relationship at Neutron Therapeutics. M. Barok reports receiving a commercial research grant from Mersana Therapeutics Inc. No potential conflicts of interest were disclosed from the other authors.

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