Original Articles

ARX788, a novel anti-HER2 antibody-drug conjugate, shows anti-tumor effects in preclinical models of trastuzumab emtansine-resistant HER2-positive breast cancer and gastric cancer

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A B S T R A C T

The majority of HER2-positive breast or gastric cancers treated with T-DM1 eventually show resistance to this agent. We compared the effects of T-DM1 and ARX788, a novel anti-HER2 antibody-drug conjugate, on cell growth and apoptosis in HER2-positive breast cancer and gastric cancer cell lines sensitive to T-DM1, gastric cancer cell lines resistant to T-DM1, HER2-negative breast cancer cell lines, and T-DM1-resistant xenograft models. ARX788 was effective in T-DM1-resistant in vitro and in vivo models of HER2-positive breast cancer and gastric cancer. ARX788 showed a pronounced growth inhibitory effect on all five HER2-positive cell lines tested, of which two gastric cancer cell lines had acquired resistance to T-DM1. ARX788 evoked more apoptotic events compared to T-DM1. While JIMT-1 and RN-87 xenograft tumors progressed on T-DM1 treatment, all such tumors responded to ARX788, and four out of the six JIMT-1 tumors and nine out of the twelve RN-87 tumors disappeared during the ARX788 treatment. Mice treated with ARX788 survived longer than those treated with T-DM1. The data support evaluation of ARX788 in patients with HER2-positive breast cancer or gastric cancer including cancers that progress during T-DM1 therapy.

1. Introduction

The human epidermal growth factor receptor-2 (HER2) is overexpressed in 15%–20% of human breast and gastric cancers [1–3]. Trastuzumab, a humanized monoclonal antibody targeting HER2, has shown substantial anti-cancer efficacy in clinical trials of patients with HER2-positive breast or gastric cancer, and it is approved for the treatment of HER2-positive early breast cancer, advanced breast cancer, and advanced gastric cancer [4,5]. However, resistance to trastuzumab eventually emerges in the great majority of treated patients [6–8], and, therefore, further improvements are required in the systemic treatment of such patients.

Antibody-drug conjugates (ADC) are designed to deliver cytotoxic payloads specifically to cancer cells. Trastuzumab emtansine (T-DM1, Kadcyla) is an anti-HER2 ADC, in which the monoclonal antibody, trastuzumab, has been armed with DM1 (a derivative of maytansine) payloads via a non-reducible thioether linker [9]. One T-DM1 molecule delivers an average of 3.5 DM1 payloads to the target cells. Binding of T-DM1 to cell surface HER2 receptors induces receptor-mediated internalization, which is followed by lysosomal degradation of the conjugate leading to intracellular release of lysine-MCC-DM1 (4-[N-maleimidomethyl] cyclohexane-1-carbonyl-DM1). Intracellular DM1 is a powerful inhibitor of the microtubule assembly causing cancer cell death [9,10]. The U.S. Food and Drug Administration (FDA) approved T-DM1 as monotherapy for the treatment of patients with HER2-positive advanced breast cancer in 2013 [11,12], and for the adjuvant treatment of patients with HER2-positive early breast cancer who have residual invasive disease after neoadjuvant taxane and trastuzumab-based treatment in 2019 [13]. Similarly to trastuzumab, the majority of breast cancer patients treated with T-DM1 acquire resistance to T-DM1...
In addition, in one randomized trial, T-DM1 was not superior to chemotherapy in patients with HER2-positive advanced gastric cancer [14]. Such data suggest that both primary and acquired resistance to T-DM1 are common.

ARX878 is a next generation anti-HER2 ADC containing an anti-HER2 monoclonal antibody site-specifically conjugated to Amberstatin 269 (AS269), a proprietary version of monomethyl auristatin F (MMAF) payload, via a non-cleavable linker [15,16]. Site-specific conjugation was performed through para-acetylphenylalanine (pAp), a non-natural amino acid, which was incorporated into the primary sequence of the antibody at a defined site and serves as a linkable platform for conjugation of the payload [15]. MMAF is a potent inhibitor of tubulin polymerization, thereby causing cell death [17].

Here we report that ARX878 shows a stronger inhibitory effect than T-DM1 on breast cancer and gastric cancer cells sensitive to T-DM1 in vitro. In addition, ARX878 had pronounced anti-cancer effects on in vivo and in vitro models of HER2-positive breast cancer and gastric cancer with resistance to T-DM1.

2. Materials and methods

2.1. Cell lines

The cell lines used and their origins are summarized in Table 1. The HER2-positive gastric cancer cell line NCi-N87 (N-87), and the HER2-negative breast cancer cell lines MCF-7 and Hs-578T were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), the HER2-positive gastric cancer cell line OE-19 from the European Collection of Cell Culture (CAMR Centre for Applied Microbiology and Research, Wiltshire, UK), and the HER2-positive breast cancer cell line JIMT-1 from the laboratory of Cancer Biology, University of Tampere, Finland [18]. The cell lines were cultured according to the recommended specifications. The T-DM1-resistant HER2-positive gastric cancer cell lines RN-87 and ROE-19 were generated in our laboratory by treating the N-87 and OE-19 cells, respectively, with increasing concentrations of T-DM1 (Roche Ltd., Basel, Switzerland) as described previously [19]. Authentication of the cell lines was performed using a short tandem repeat analysis.

2.2. In vitro drug sensitivity assays and caspase activation

The effects of T-DM1 and ARX878 (Ambrx Inc., La Jolla, CA, USA) on the cell growth were studied using the AlamarBlue method (Thermo Fisher Scientific, Waltham, USA). The cells were trypsinized and plated in flat-bottomed 96-well tissue culture plates. The effects of T-DM1 and ARX878 were tested at concentrations of 0.0001, 0.003, 0.016, 0.08, 0.4, 1, 2, and 10 μg/mL. The MCF-7 and Hs-578T breast cancer cell lines with low HER2 expression were used as negative controls. The numbers of viable cells were assessed after a five-day incubation by addition of the AlamarBlue reagent (Thermo Fischer Scientific). Fluorescence was measured with excitation at 540 nm and emission at 590 nm 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 ARX878 treatment).

2.3. Immunohistochemistry

Tissue samples from xenograft tumors were fixed in 4% buffered formaldehyde for 24 h, processed into paraffin, and sectioned. For immunohistochemistry, 4 μm sections were deparaffinized followed by antigen-retrieval in a sodium citrate buffer (10 mM Sodium Citrate, pH 6.0) using a 2100 Antigen Retriever (Aptum Biologies Ltd., Southampton, UK) following the manufacturer’s recommendations. After blocking the non-specific binding, the primary antibodies anti-HER2 (CB11, Leica Biosystems), M30 CytoDeath (Sigma-Aldrich, St. Louis, USA), anti-ABCC1 (HPA002380, Sigma-Aldrich), anti-ABCC2 (ab3373, Abcam, Cambridge, UK), and anti-ABCG2 (BXP-21, ab3380, Abcam) were applied at optimized concentrations, and incubated overnight at 4 °C. The primary antibody binding was detected using a BrightVision Poly-HRP anti mouse kit (VWR, Radnor, USA) and 3,3′-diaminobenzidine (ImmPACT DAB, Vector Laboratories, Burlingame, CA, USA) following the manufacturer’s recommendations. The tissue sections were counterstained with hematoxilyn. HER2 expression visualized by immunohistochemistry was quantified semi-quantiatively using a scale negative (0), weakly positive (+), moderately positive (++), or strongly positive (+++). M30 CytoDeath-positive apoptotic cells were counted from a minimum of 10 randomly selected representative tumor sections using 40x magnification of the Olympus B×50 microscope (Olympus Corporation, Tokyo, Japan). The data are presented as the average ± standard deviation of positive cells per one microscope field.

2.4. Fluorescence in situ hybridization

HER2 fluorescence in situ hybridization (FISH) was performed with a fully automated staining robot (PathCom Systems, Dublin, USA) using a fluorescein labeled genomic probe and a reagent kit from the manufacturer (Pathcom Systems). To visualize nuclei, 0.3 μg/mL DAPI fluorescence of the test samples by the fluorescence of the phosphate-buffered saline (PBS)-treated control samples. The dose achieving the half-maximal (50%) inhibitory concentration (IC₅₀) with the drugs was calculated using the Graphpad Prism software (GraphPad Software, San Diego, CA, USA).

To assess the rate of apoptosis, caspase activation was measured using the Caspase-Glo 3/7 method (Promega, Madison, USA) [20]. The cells were trypsinized and plated in flat-bottomed 96-well tissue culture plates. After overnight culture, 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 ARX878. After 48 h 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 min at RT, 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 ARX878 treatment).

Table 1

The cell lines investigated.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>HER2 status</th>
<th>Reported sensitivity to T-DM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE-19</td>
<td>Human gastric cancer</td>
<td>Positive [22]</td>
<td>Sensitive [22]</td>
</tr>
<tr>
<td>N-87</td>
<td>Human gastric cancer</td>
<td>Positive [22]</td>
<td>Sensitive [22]</td>
</tr>
<tr>
<td>RN-87</td>
<td>Human gastric cancer, derived from N-87</td>
<td>Positive [19]</td>
<td>Resistant [19]</td>
</tr>
<tr>
<td>JIMT-1</td>
<td>Human breast cancer</td>
<td>Positive [29]</td>
<td>Sensitive [29]</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Human breast cancer</td>
<td>Negative [22,29]</td>
<td>Resistant [22,29]</td>
</tr>
<tr>
<td>Hs-578T</td>
<td>Human breast cancer</td>
<td>Negative [23]</td>
<td>Resistant, reported here</td>
</tr>
</tbody>
</table>

Abbreviation: HER2, human epidermal growth factor receptor-2.
(Sigma-Aldrich) was used. A Zeiss Axio Imager Z2 epifluorescence microscope (Carl Zeiss, Götttingen, Germany) equipped with selective filters for the detection of fluorescein and DAPI was used to image samples. Fluorescence images were taken using a 100X (NA = 1.3) oil immersion objective. A total of 100 nonoverlapping tumor cells from each sample were scored for the green signal (the copies of HER2).

2.5. Flow cytometry

Flow cytometry was performed by using an Accuri C6 Flow Cytometer (Accuri Cytometers, Inc., Ann Arbor, USA). To assess cell surface HER2, the cells were trypsinized and washed with 1% BSA in PBS. HER2 receptors were labeled using an anti-HER2 primary antibody (9G6.10, Thermo Fisher Scientific) that does not compete with trastuzumab for binding [21] for 30 min at 4 °C. Then the cells were washed twice with PBS, and labeled with AlexaFluor488-goat anti-rabbit-IgG (A488-GAMiG; Jackson ImmunoResearch, West Grove, USA) for 30 min at 4 °C. The cells were then washed twice with PBS and fixed in 1% formaldehyde.

2.6. In vivo models of drug efficacy

The National Animal Experiment Board of Finland approved the mouse experiments. Five to 8-week-old female SCID mice (C.B-17/ IcrHan Hsd-Prkdcsdx), Envigo RMS B.V., Horst, The Netherlands) were injected subcutaneously with 15 × 10⁶ of human breast cancer cells (JIMT-1) in 150 μL of the cell culture medium, 14 × 10⁶ of human gastric cancer cells (N-87), or with 20 × 10⁶ or 26 × 10⁶ of T-DM1-resistant human gastric cancer cells (RN-87) in 150 μL of the cell culture medium to establish xenograft tumors. Following this, T-DM1 (5 mg/kg) or ARX788 (5 mg/kg) were administered intravenously (i.v.) at 7-day intervals. Tumor size was measured using a caliper, and at 7-day intervals. As a control, PBS was administered intraperitoneally (i.p.) at 7-day intervals. Tumor size was analyzed using the Kaplan-Meier method, and survival between unpaired groups was compared with the Mann-Whitney test. Survival unpaired groups was compared with the Student’s t-test when the data passed the normality test. All P-values are 2-sided.

2.7. Statistical analysis

The data are expressed as the mean ± SE. Groups were compared using the Student’s t-test when the data passed the normality test. 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, Armonk, USA). All P-values are 2-sided.

3. Results

3.1. ARX788 treatment inhibits the growth of T-DM1-resistant cancer cells in vitro

The growth inhibitory effects of ARX788 and T-DM1 were compared in five HER2-positive cancer cell lines and in two control cell lines (MCF-7, Hs-578T) without HER2 amplification and with low levels of HER2 expression [22, 23]. ARX788 inhibited the growth of all HER2-positive cell lines in a dose-dependent manner (Fig. 1). Compared to T-DM1, the ARX788 treatment resulted in a smaller percentage of surviving cells particularly in the T-DM1-resistant ROE-19 and RN-87 gastric cancer cell lines (Fig. 1, D&E). The 50% inhibitory concentration (IC₅₀) was achieved with ARX788 in all five HER2-positive cell lines, whereas T-DM1 reached the IC₅₀ only in the OE-19, N-87, and JIMT-1 cell lines. In these cell lines ARX788 yielded 1.35-fold (OE-19), 3.6-fold (N-87), and 29.35-fold (JIMT-1) greater potency compared to T-DM1 (Fig. 1A-E). The ARX788 IC₅₀ values on OE-19 and ROE-19 cells (0.033 μg/mL and 0.009 μg/mL, respectively) and on N-87 and RN-87 cells (0.01 μg/mL and 0.041 μg/mL, respectively) were roughly similar, suggesting similar activity of ARX788 in the parental, T-DM1-sensitive cells and the corresponding T-DM1-resistant cells (Fig. 1, A&D and B&E). Neither T-DM1 nor ARX788 was effective in the HER2-negative MCF-7 and Hs-578T cell lines (Fig. 1, F&G).

Next, we measured the caspase-3 and/or caspase-7 activity in the HER2-positive cell lines JIMT-1 and RN-87 treated with T-DM1 or ARX788 to assess apoptosis induction rate. ARX788 increased caspase-3 and/or caspase-7 activity in a dose-dependent manner and exceeded the effect of T-DM1 in both cell lines (Fig. 1, H&I).

3.2. ARX788 eliminates T-DM1-resistant RN-87 gastric cancer xenografts in vivo and prolongs survival

The effects of ARX788 and T-DM1 on the growth of RN-87 xenografts was compared in SCID mice. The RN-87 gastric cancer xenografts expressed HER2 at similar levels as the T-DM1-sensitive parental N-87 xenografts (Supplementary Fig. 1 A&B). Tumors formed in all 14 mice inoculated with RN-87 cell suspension within 5 days from the date of inoculation reaching a mean tumor volume of 52.5 ± 25.0 mm³ on day 5. On day 13 post-inoculation the mice were split into two groups, and treated once a week with either PBS (n = 7) or T-DM1 5 mg/kg (n = 7). No difference in the tumor growth was observed between the PBS or T-DM1 treatments (Fig. 2). Therefore, T-DM1 treatment was discontinued on day 22 post-inoculation in three out of the seven mice and switched to 5 mg/kg of ARX788, while the remaining four mice continued to receive T-DM1. All three mice treated with ARX788 following the T-DM1 treatment showed rapid tumor shrinkage, whereas the four mice that continued on T-DM1 showed persistent tumor growth. All four mice treated continuously with T-DM1 were euthanized due to tumor ulceration on day 34 (2 mice) or day 40 (2 mice). One of the three ARX788-treated mice was euthanized on day 34 and one on day 40 in order to harvest tumor samples for histological examination. The tumor of the third ARX788-treated mouse became unmeasurable by day 40, and no tumor relapse was observed during the follow-up time of 13 days (Fig. 2).

In the PBS control treatment group, all seven mice were switched to either T-DM1 (5 mg/kg, n = 4) or ARX788 (5 mg/kg; n = 3) from day 22 onwards. Mice treated with ARX788 following the PBS treatment showed a rapid tumor shrinkage, whereas mice that received T-DM1 after PBS showed persistent tumor growth (Fig. 2). Two out of the four T-DM1-treated mice were euthanized due to tumor ulceration on day 34 and the last two on day 40. Two out of the three ARX788-treated tumors became unmeasurable by day 43 and day 53, and the third tumor treated with ARX788 shrank to 1.0 mm³ by day 53 (Fig. 2).

To further examine the efficacy of ARX788 and to generate survival data, we next inoculated T-DM1 resistant RN-87 cells into 12 SCID mice. On day 13 post-inoculation the mice were split into two groups and treated once a week with either T-DM1 (5 mg/kg, n = 6) or ARX788 (5 mg/kg, n = 6). The tumors formed continued to grow in the T-DM1-treated mice, and all these mice were euthanized due to tumor ulceration between day 24 and day 52. ARX788 treatment led to complete tumor regression: no measurable tumors were observed by day 59 in these mice (Fig. 3, A). The mice treated with ARX788 survived longer as compared with the mice treated with T-DM1 (P = 0.0007; Fig. 3, B).

3.3. ARX788 treatment eliminates JIMT-1 breast cancer xenografts in vivo and prolongs survival

Next, we compared the efficacy of ARX788 and T-DM1 in a HER2-positive breast cancer xenograft model (JIMT-1). Tumors formed in all 20 SCID mice inoculated with a JIMT-1 cell suspension by day 7 and reached a mean tumor volume of 229.0 ± 87.5 mm³ on day 14. On day 15 after the inoculation, the mice were split into three treatment
groups, which were treated once a week for three weeks with either PBS (n = 6), T-DM1 (5 mg/kg, n = 8), or ARX788 (5 mg/kg, n = 6). While tumors treated with either PBS or T-DM1 continued to grow, rapid tumor shrinkage was observed in the ARX788-treated group (Fig. 4, A). Four of the six mice treated with ARX788 had no palpable tumor from day 77 onwards. The remaining two tumors shrank from the initial size of 381.5 mm$^3$ and 256.4 mm$^3$ on day 14 to 6.3 mm$^3$ and 1.0 mm$^3$ on day 63, respectively, and then started to regrow reaching the size of 78.5 mm$^3$ and 33.5 mm$^3$ on day 91, and progressed further despite administration of five additional doses of ARX788 (Fig. 4, A). Also in this setting ARX788 significantly prolonged the survival of the tumor-bearing mice compared to T-DM1 (P = 0.0006; Fig. 4, B).

3.4. ARX788 induces apoptotic cell death in T-DM1-resistant breast cancer and gastric cancer xenografts

Formalin-fixed paraffin-embedded xenograft tumor sections were stained with the M30 CytoDeath antibody to detect apoptotic cells using immunohistochemistry. More apoptotic cells were detected in the RN-87 gastric cancer xenografts when the treatment was switched from T-DM1 to ARX788 than in RN-87 xenograft tumors that were continuously treated with T-DM1 (P < 0.001, Fig. 5, A&B, Supplementary Fig. 2). Moreover, more apoptotic cells were detected in the JIMT-1 breast cancer xenografts treated with ARX788 than in tumors treated with T-DM1 (P < 0.001; Fig. 5 D-E, Supplementary Fig. 2). Only a few apoptotic cells were observed in the JIMT-1 tumors that progressed during ARX788 treatment (Fig. 5 F, Supplementary Fig. 2).
3.5. Loss of HER2 protein expression and HER2 gene amplification in JIMT-1 xenograft tumors that progressed on ARX788 treatment

JIMT-1 tumors treated continuously with T-DM1 retained their HER2 protein expression and HER2 gene amplification, but the two JIMT-1 tumors that initially responded to ARX788 but later progressed despite continued ARX788 treatment lost both HER2 protein expression and HER2 gene amplification (Supplementary Fig. 1 C-F and Supplementary Fig. 3, respectively).

3.6. HER2 expression may decrease as gastric cancer cells become resistant to T-DM1

Cell surface HER2 protein levels were quantitated using flow cytometry. The T-DM1-resistant RN-87 and ROE-19 cells had slightly lower HER2 expression than their T-DM1-sensitive counterparts (N-87 and OE-19; P = 0.006 and P = 0.008, respectively), suggesting that other mechanisms may contribute to resistance. As expected, MCF-7 and Hs-578T cells expressed low levels of HER2 (Fig. 6).

4. Discussion

The majority of cancer patients who respond to T-DM1 eventually cease to respond to it [11,12]. Since patients with T-DM1-resistant cancer have limited therapeutic options, there is an unmet medical need to develop novel agents for HER2-positive cancers. We compared the in vitro and in vivo efficacy of ARX788, a next generation anti-HER2 ADC, to T-DM1 in a panel of breast and gastric cancer cell lines including T-DM1 resistant cell lines. ARX788 had a more pronounced inhibitory effect on breast cancer and gastric cancer cells sensitive to T-DM1 (N-87, and OE-19) or sensitive to higher concentrations of T-DM1 (JIMT-1) than T-DM1. Moreover, ARX788 showed a substantial anticancer effect in vitro models of gastric cancer with acquired resistance to T-DM1 (RN-87, ROE-19). ARX788 induced caspase-3 and/or caspase-7 activity more efficiently than T-DM1 indicating that ARX788 evoked a higher rate of apoptotic cell death. Importantly, ARX788 showed a...
strong anti-cancer effect in the T-DM1-resistant breast cancer and gastric cancer xenografts.

The cytotoxic effect of T-DM1 likely depends on achieving a sufficient intracellular concentration of its cytotoxic payload, DM1 [10]. Hence, the mechanisms that can lower the intracellular DM1 concentration could play a role in T-DM1 resistance. For example, downregulation of HER2 receptors on the cell surface might result in internalization of fewer T-DM1 molecules into the cancer cells [24], or increased expression of ABC drug transporters may lead to efflux of more DM1 from cancer cells [24,25]. In accordance with these findings, we observed lower expression of HER2 on the T-DM1-resistant RN-87 and ROE-19 gastric cancer cells as compared to their sensitive counterparts. In addition, the T-DM1-resistant RN-87 and ROE-19 cells have higher ABC transporter expression than the sensitive N-87 and OE-19 cells [19]. A complete loss of HER2 expression caused acquired T-DM1-resistance in an in vitro model using JIMT-1 breast cancer cells [26]. Here we report retained expression of HER2 in T-DM1-resistant JIMT-1 xenografts. The mechanisms responsible for T-DM1 resistance are still not completely understood and require further study.

We applied two treatment strategies to test ARX788 efficacy on
HER2-positive breast cancer and gastric cancer xenografts. First, ARX788 was highly effective on relatively large RN-87 xenograft tumors that were previously nonresponsive to T-DM1. In this setting, a rapid tumor shrinkage was observed when the treatment was switched from T-DM1 to ARX788. Second, a rapid and complete tumor shrinkage was observed in all RN-87 xenografts and in four out of six JIMT-1 xenografts when ARX788 was given to mice carrying small to moderate size subcutaneous tumors that had not been previously treated with T-DM1. The remaining two JIMT-1 xenograft tumors initially responded to ARX788 treatment and showed pronounced shrinkage, but 48 days after the treatment was discontinued both tumors progressed, and despite additional ARX788 treatments these tumors continued to grow. Notably, the ARX788 nonresponsive tumors had lost detectable HER2 protein expression in immunohistochemical analyses and showed loss of HER2 gene amplification in FISH, which explains the lack of response to ARX788. Importantly, ARX788 significantly increased survival of mice bearing RN-87 or JIMT-1 tumors as compared to T-DM1. The anti-tumor effect of ARX788 seemed not to be dependent on the tumor size, since ARX788 caused complete tumor shrinkage of both ARX788 and T-DM1.

In summary, we compared the anti-tumor effects of T-DM1 and ARX788, a next generation anti-HER2 ADC. ARX788 showed a stronger inhibitory effect than T-DM1 on breast and gastric cancer cells sensitive to T-DM1 and on gastric cancer cells with acquired resistance to T-DM1 in vitro. ARX788 caused complete tumor regression of T-DM1-resistant breast cancer and gastric cancer xenograft tumors in vivo. The results support clinical investigation of ARX788 in patients with HER2-positive breast cancer or gastric cancer including patients with T-DM1-resistant HER2-positive cancer. ARX788 is currently being evaluated in a phase I multicenter study in patients with HER2-positive breast cancer or gastric cancer (NCT03255070).

Authors’ contributions

MB conceived the hypothesis, led the project, designed the experimental approach, performed the experimental work, analyzed data, coordinated the project, and drafted the manuscript together with HJ. VLJ discussed the hypothesis, designed the experimental approach, performed the experimental work, and supervised AM.

AM discussed the hypothesis, designed the experimental approach, and performed the experimental work.

JI discussed the hypothesis, designed the experimental approach, performed the experimental work, and interpreted data.

MS discussed the hypothesis, designed the experimental approach, and performed the experimental work.

PL discussed the hypothesis, designed the experimental approach, interpreted data, supervised VLJ and AM, and edited manuscript.

HJ led the project, discussed the hypothesis, designed the experimental approach, interpreted data, and drafted the manuscript together with MB.

All authors approved the final manuscript.

Declaration of competing interest

Heikki Joensuu is a board member of Sartner Therapeutics, has a co-appointment at Orion Pharma, and has received fees from Orion Pharma and Neutron Therapeutics Ltd.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.12.037.

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