Cancer Chemother Pharmacol (2002) 49: 211–216
DOI 10.1007/s00280-001-0401-7

ORIGINAL ARTICLE

Kaori Fujimoto-Ouchi · Fumiko Sekiguchi
Yutaka Tanaka

Antitumor activity of combinations of anti-HER-2 antibody trastuzumab and oral fluoropyrimidines capecitabine/5′-dFUrd in human breast cancer models

Received: 9 May 2001 / Accepted: 18 October 2001 / Published online: 22 December 2001

Abstract The anti-HER-2 antibody, trastuzumab (Herceptin), and the oral fluoropyrimidine, capecitabine (Xeloda), are both effective in breast cancer with different modes of action and toxicity profiles. Therefore, the efficacy in combination therapy of these agents for the treatment of HER-2-overexpressing breast cancer was of interest. An antagonistic interaction in vitro between trastuzumab and 5-fluorouracil (5-FUra) in combination has previously been reported. In the same study, the in vivo antitumor activity of this combination was investigated. However, the results were inconclusive since *5-FUra* at the dose used had sufficient antitumor efficacy as a single agent and therefore it was not possible to clarify the potential difference between 5-FUra alone and the combination. In the present study, we investigated the efficacy of trastuzumab in combination with capecitabine or its intermediate metabolite 5′-dFUrd in HER-2-overexpressing human breast cancer cell lines and xenograft models. In vivo antitumor activity of the combination was at least additive, in terms of tumor growth inhibition and tumor growth delay, in human breast cancer models KPL-4 and BT-474. The combination treatment in vivo was superior to the treatment with either single agent alone, even though in vitro treatment with trastuzumab and 5′-dFUrd/5-FUra showed antagonistic antiproliferative activity in these cell lines. The reason for the discrepancy between the in vivo and in vitro results was not clarified. However, observed additive in vivo antitumor activity clearly indicates that the clinical efficacy of the combination of trastuzumab and capecitabine/5′-dFUrd against HER-2-overexpressing breast cancer is worth investigating.

K. Fujimoto-Ouchi (✉) · F. Sekiguchi · Y. Tanaka
Oncology, Nippon Roche Research Center,
200 Kajiwara, Kamakura,
Kanagawa 247-8530, Japan
E-mail: kaori.ohuchi@roche.com
Tel.: +81-467-472220
Fax: +81-467-456782

Keywords Trastuzumab · Capecitabine · 5′-dFUrd · Breast cancer xenograft · Combination therapy

Abbreviations *5′-dFUrd:* 5′-deoxy-5-fluorouridine *5-FUra:* 5-fluorouracil · *CI:* combination index *dThdPase:* thymidine phosphorylase · *MTD:* maximum tolerated dose

Introduction

Trastuzumab (Herceptin) targets the oncogenic factor HER-2/neu, which is encoded by the c-erbB2 gene and is amplified in approximately 25% of breast cancer patients [1, 2, 3]. Trastuzumab is approved in the US, EU, Japan and other countries as a first-line therapy for use in combination with paclitaxel and as a second- and third-line monotherapy for breast cancer types that overexpress the HER-2/neu growth factor receptor [4, 5].

Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine, Xeloda) is a fluoropyrimidine carbamate that is approved in many countries worldwide and is used clinically in an oral form for the treatment of breast cancer patients who have failed paclitaxel- and anthracycline-containing regimens [6]. It has also recently been approved in the US in combination with docetaxel for advanced and metastatic breast cancer after failure of prior anthracycline-containing chemotherapy [7] and as first-line treatment of colorectal cancer in the US, EU and many other countries worldwide [8, 9]. In humans, it is sequentially converted first to 5′-deoxy-5-fluorocytidine by carboxylesterase located in the liver, then to 5′-deoxy-5-fluorouridine (5′-dFUrd, doxifluridine) by cytidine deaminase, also with high activity in the liver and in various solid tumors, and finally to 5-fluorouracil (5-FUra) by thymidine phosphorylase (dThdPase) with high activity in many types of tumors [10]. An oral form of intermediate metabolite 5′-dFUrd (doxifluridine, Furtulon) is prescribed in Japan, Korea and China for

the treatment of breast, colorectal, gastric, bladder, and cervical cancers [11].

The efficacy of trastuzumab and capecitabine/5′-dFUrd in combination for the treatment of breast cancer is worthy of investigation since both of these agents are effective in breast cancer with different modes of action and different toxicity profiles. Pegram et al. have reported that trastuzumab and 5-FUra in combination is antagonistic in in vitro antiproliferation assays of the human breast cancer cell line SK-BR-3 [12]. However, it was not possible to determine the in vivo antitumor activity of this combination since the 5-FU dose used had sufficient antitumor efficacy as a single agent and therefore the potential difference between the effects of 5-FU alone and of the combination could not be established [12]. In the present study, we investigated the efficacy of trastuzumab and the fluoropyrimidine prodrugs, capecitabine and 5′-dFUrd, in combination in HER-2-positive human mammary tumor xenograft models.

Materials and methods

Chemicals

Trastuzumab was synthesized at Genentech (South San Francisco, Calif.) and provided as an injectable formulation. Capecitabine was synthesized by a method described elsewhere [13]. 5′-dFUrd was synthesized at Hoffmann-La Roche (Basle, Switzerland). An injectable formulation of 5-FUra was purchased from Kyowa Hakko Company (Tokyo, Japan).

Animals

Female BALB/c nu/nu mice at 5 weeks of age were obtained from Charles River Japan (Yokohama, Japan). They were kept for 1 week in our animal facility prior to tumor inoculation. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals in Nippon Roche Research Center.

Tumors

The human inflammatory breast cancer cell line, KPL-4 [14, 15], was kindly provided by Dr. J. Kurebayashi (Kawasaki Medical School, Kurashiki, Japan). KPL-4, which naturally overexpresses HER-2 and is sensitive to trastuzumab [14] and is estrogen receptor-negative [15], was maintained in Dulbecco′s modified Eagle′s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The human breast cancer cell lines BT-474 and MDA-MB-231 were obtained from the American Type Culture Collection (Rockville, Md.). BT-474, which is known to naturally overexpress HER-2, is sensitive to trastuzumab and requires estrogen supplementation for in vivo growth [16, 17, 18, 19], was maintained in RPMI1640 supplemented with 10% FBS, 2 m*M* L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 m*M* HEPES, and 1 m*M* sodium pyruvate. MDA-MB-231 was maintained in L-15 supplemented with 0.7 g/l sodium bicarbonate and 10% FBS.

Cellular proliferation assay

Aliquots of KPL-4 (3×10^3 cells/well) and BT-474 (10^4 cells/well) cells were plated into 96-well microtiter plates. Following cell adhesion (24 h), experimental medium containing trastuzumab or control medium was added to the appropriate wells. 5′-dFUrd- and 5-FUra-containing medium or control medium was then added to duplicate wells and serial twofold dilutions were performed to give doses in a range suitable for the dose-effect analysis. Following incubation for 72 h, the numbers of viable cells were estimated using a WST-1 kit (Roche Diagnostics, Indianapolis, Ind.). Multiple drug effects were analyzed by calculating the combination indexes (CI) from parameters of the median effect plots [12, 20]. CI values <1.0 are defined as synergistic, values equal to 1.0 as additive, and values >1.0 as antagonistic in this model.

Human cancer xenograft models

A suspension of KPL-4 cells (0.8×10^7 cells/mouse) was orthotopically transplanted into the second mammary fat pad of the mice. BT-474 cells were transplanted at 1×10^7 cells/mouse after mixing with an equal volume of Matrigel (BD Biosciences, Bedford, Mass.) into the second mammary fat pad of mice which had received implants of slow-release estrogen pellets (0.25 mg 17β-estradiol; Innovative Research of America, Toledo, Ohio) the day before tumor inoculation. Several weeks after tumor inoculation, mice bearing a tumor of approximately 100–200 mm^3 in volume were selected and randomized into control and treatment groups, and the treatment was started. The day treatment started the numbers of mice per group were specified for each experiment. Tumor volumes (calculated as $ab^2/2$, where a is the longer diameter, and b the shorter diameter) were monitored two or three times a week. Capecitabine, 5′-dFUrd and their vehicle (40 m*M* citrate buffer containing 5% gum arabic, pH 6.0) were administered orally 5 days per week for 3 weeks. 5-FUra was dissolved in saline and administered intraperitoneally (i.p.) 5 days per week for 3 weeks. Control IgG (ICN/Cappel, Aurora, Ohio) was dissolved at 22 mg/ml in saline containing 90 μg/ml Tween 20 and 2 mg/ml of α-α-trehalose dihydrate which were the major components of the vehicle of the injectable trastuzumab formulation. IgG and trastuzumab were then diluted with saline to an appropriate concentration and administered by i.p. injection twice per week for 3 weeks. The initial dose of trastuzumab (loading dose) was twice the subsequent doses (maintenance dose).

HER-2 levels in tumor tissues and tumor cells

Tumor tissues were homogenized in phosphate-buffered saline (pH 7.4) with a glass homogenizer. Cultured tumor cells were collected and sonicated in the same buffer. The homogenate was then centrifuged at 10,000 *g* for 20 min at 4°C, and the supernatants were stored at –80°C until used. The protein concentration of the supernatant was determined using a DC protein assay kit (BioRad Laboratories, Hercules, Calif.). Levels of HER-2 expression in the tumor tissues and cultured cells were determined using a commercially available enzyme immunoassay (EIA) system (ErbB-2 EIA Nichirei; Nichirei, Tokyo, Japan). This system was originally developed for detecting circulating HER-2 fragments in blood. However, the same system has recently been found to be suitable for the determination of tumor levels of HER-2, since the EIA results correlate well with HER-2 overexpression as assessed by gene amplification and IHC [21, 22].

Results

HER-2 expression in KPL-4 and BT-474 cells in vitro and in vivo

The tumor cell lines used in the present study overexpressed HER-2. The levels were measured by an EIA. Both KPL-4 and BT-474 cell lines showed significantly higher levels of HER-2 expression in vitro and in vivo

(Table 1) than the control cell line MDA-MB-231 which is known to have a non-amplified HER-2 gene [16].

In vitro multiple drug effect analysis of trastuzumab in combination with 5′-dFUrd or 5-FUra against KPL-4 and BT-474 cells

Capecitabine is a prodrug that has to be metabolized initially by the liver enzyme carboxylesterase in vivo [10]. Therefore, for in vitro experiments, its metabolites 5′-dFUrd and 5-FUra were used instead of capecitabine itself. Tumor cells were treated with various concentrations of trastuzumab and 5′-dFUrd or 5-FUra either as single agents or in combination. Interactions between the agents were analyzed by calculating the CI as reported previously [12, 20]. As shown in Table 2, mean CI values at the IC_{50} in KPL-4 cells for the combinations of trastuzumab with 5′-dFUrd and 5-FUra were 2.4 and 2.5, respectively, indicating antagonistic interaction between trastuzumab and these fluoropyrimidines. Antagonistic interactions were also observed over the range of IC_{30} to IC_{70}. In BT-474 cells, the CI for the combination of trastuzumab and 5′-dFUrd was close to 1, indicating an additive interaction, while the CI for the combination of trastuzumab and 5-FUra was 2.3, indicating an antagonistic interaction.

Table 1 Levels of HER-2 expression in cells and tumor tissues measured as described in Materials and methods

Tumor line	HER-2 level (ng/mg protein)	
	In vitro	In vivo
KPL-4	173 ± 17	130 ± 19
BT474	689 ± 66	152 ± 19
MDA-MB-231	< 5	< 5

Antitumor activity of trastuzumab in combination with capecitabine/5′-dFUrd in in vivo models of KPL-4 and BT-474 human breast cancer

The antitumor activities of the combination of trastuzumab and capecitabine, 5′-dFUrd or 5-FUra were determined in the KPL-4 in vivo model. Doses of these agents were determined from a preliminary dose-finding experiment. In order to detect an interaction between the two agents distinctly, we chose suboptimal doses of each drug. Trastuzumab given twice a week at 20 mg/kg loading and 10 mg/kg maintenance doses showed similar antitumor activity to 40 mg/kg loading and 20 mg/kg maintenance doses, while 10 mg/kg loading and 5 mg/kg maintenance doses showed less activity. Thus 20 mg/kg loading and 10 mg/kg maintenance doses were the maximum effective regimen in this model. In the present combination therapy experiments the doses of trastuzumab used were three-fourths of the maximum effective doses, that is 15 mg/kg loading and 7.5 mg/kg maintenance. The MTD for capecitabine administered five times a week for 3 weeks in this model was determined as 189 mg/kg in a separate experiment. The MTDs for 5′-dFUrd and 5-FUra were calculated from capecitabine's MTD and the ratio of the MTDs for these fluoropyrimidines reported previously [23]. Doses of capecitabine (126 mg/kg), 5′-dFUrd (43 mg/kg) and 5-FUra (4.6 mg/kg) used in the present experiments corresponded to two-thirds of the MTD for each drug.

As shown in Fig. 1 and Table 3, trastuzumab, 5′-dFUrd and 5-FUra as single agents showed only marginal antitumor activity in this model at the doses

Table 2 Combination index (CI) values for trastuzumab in combination with 5′-dFUrd or 5-FUra in KPL-4 and BT-474 in vitro. The CI values are means ± SD at the IC_{50} derived from three plates. The dose ranges tested were as follows: in KPL-4, trastuzumab 20–10,000 μg/ml, 5′-dFUrd 3.1–1570 μg/ml, 5-FUra 0.25–130 μg/ml; in BT-474, trastuzumab 0.01–6.4 μg/ml, 5′-dFUrd 3.1–1570 μg/ml, 5-FUra 0.25–130 μg/ml

Cell line	Drug	CI (mean ± SD)	Effect
KPL-4	5′-dFUrd	2.4 ± 0.7	Antagonistic
	5-FUra	2.5 ± 1.1	Antagonistic
BT-474	5′-dFUrd	1.1 ± 0.2	Additive
	5-FUra	2.3 ± 1.1	Antagonistic

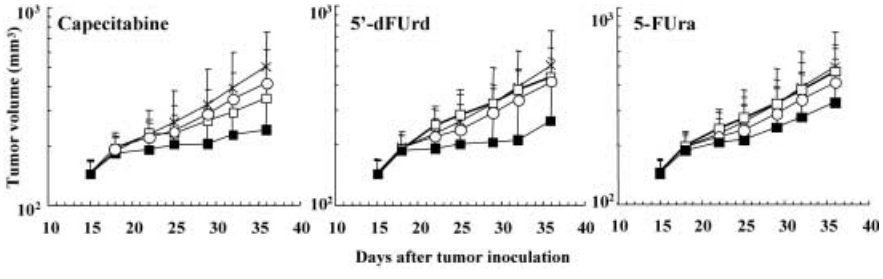

Fig. 1 Antitumor activity of three combinations of trastuzumab with fluoropyrimidines in KPL-4 human breast cancer model. Treatment was started 15 days after tumor inoculation. Mice were randomized into groups of 12 mice each. Capecitabine (126 mg/kg, orally), 5′-dFUrd (43 mg/kg, orally), and 5-FUra (4.6 mg/kg, i.p.) were administered five times a week for 3 weeks. Trastuzumab (15 mg/kg loading dose, 7.5 mg/kg maintenance dose) and the corresponding doses of IgG were administered i.p. twice a week for 3 weeks. The data points are mean ± SD tumor volumes (*crosses* control IgG and vehicle, *open circles* trastuzumab and vehicle, *open squares* control IgG and fluoropyrimidines, *filled squares* combination of trastuzumab and fluoropyrimidine). The significance of the differences between the groups is indicated in Table 3

Table 3 Effect of combinations of trastuzumab and fluorinated pyrimidines in the KPL-4 tumor model. The antitumor activities of the combinations of trastuzumab and three fluoropyrimidines shown in Fig. 1 are summarized. Percent tumor growth inhibition was evaluated on the day after the 3-week treatment period (day 36). The time taken for the tumors to grow to 400 mm^3 (days to TV_{400}) was determined. Body weight change from the day treatment started to the day after it ended was also determined

Group	Dose (mg/kg)	Tumor volume (mm^3, mean ± SD)	Tumor growth inhibition (%)	Days to TV_{400}		Body weight change (g, mean ± SD)
				Median	Range	
Control IgG		501 ± 112	–	33	29–47	–0.5 ± 0.8
Trastuzumab	15/7.5	415 ± 336	17	45	22–120	–0.3 ± 1.2
Capecitabine alone	126	345 ± 79*	31	47*	34–66	–0.2 ± 0.9
Capecitabine + trastuzumab		241 ± 152*	52	55*	34–120	0.1 ± 1.0
5′-dFUrd alone	43	441 ± 102	12	32	27–51	–0.4 ± 0.7
5′-dFUrd + trastuzumab		265 ± 165*,***	47	51*,***	30–120	0.0 ± 1.0
5-FUra alone	4.6	474 ± 174	5	35	25–54	–0.9 ± 0.8
5-FUra + trastuzumab		331 ± 213**	34	40**	27–120	0.4 ± 1.3

*$P < 0.01$ vs control, **$P < 0.05$ vs control, ***$P < 0.01$ vs fluoropyrimidine alone

and with the treatment schedule used. Capecitabine monotherapy showed moderate but significant antitumor activity with 31% inhibition of tumor growth. In contrast, all three combinations of trastuzumab with a fluoropyrimidine produced statistically significant inhibition of tumor growth compared with the control group. The antitumor activities of the combinations were superior to the activities of the single agents.

A similar tendency was also observed in the measurement of tumor growth delay induced by the treatment. The median time in days taken for the tumor to grow to 400 mm^3 (days to TV_{400}) was determined in every group (Table 3). The number of days in the groups treated with trastuzumab and capecitabine or 5′-dFUrd in combination was significantly greater than in the control group and also greater than in the groups treated with the single agents. There was no significant change in body weight in any group, indicating that the combination treatments did not enhance toxicity. Thus, the combination of trastuzumab and capecitabine or 5′-dFUrd showed additive antitumor activity in the in vivo KPL-4 model, even though the combination of trastuzumab and 5′-dFUrd against this tumor line in vitro showed an antagonistic interaction in the antiproliferation assays.

The activity of the combination of trastuzumab and capecitabine was also investigated in the BT-474 model. Since this tumor model is rather resistant to capecitabine treatment, capecitabine was given at its MTD of 503 mg/kg five times a week for 3 weeks. However, trastuzumab was given at a loading dose of 1.2 mg/kg and a maintenance dose of 0.6 mg/kg, which corresponds to only 1/25th of the maximum effective dose, because the model is highly susceptible to this drug [18]. Figure 2 shows the results of the combination treatment. Capecitabine alone did not show clear antitumor activity even at the MTD, while a low dose of trastuzumab as a single agent showed significant tumor growth inhibitory activity. In the group treated with the combination of these agents, very potent antitumor activity and even a reduction in tumor volume was observed. The volume of the tumors in the group treated with the combination was significantly less than that of the control group and also that of the groups treated with either agent alone. Thus, the combination of trastuzumab and capecitabine also showed at least additive antitumor activity in the BT-474 model.

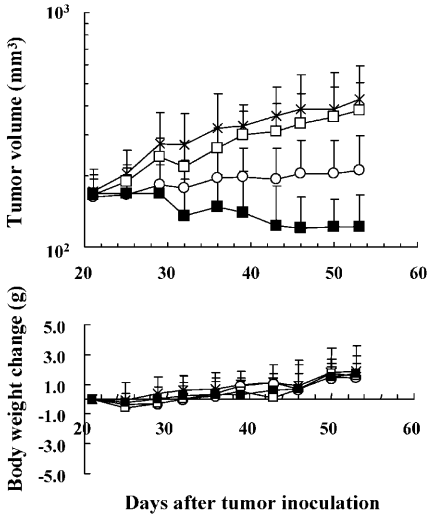

Fig. 2 Antitumor activity of the combination of trastuzumab and capecitabine in BT474 human breast cancer model. Treatment was started 21 days after tumor inoculation. Mice were randomized into groups of eight mice each. Capecitabine (503 mg/kg, orally) was administered five times a week for 3 weeks. Trastuzumab (1.2 mg/kg loading dose and 0.6 mg/kg maintenance dose) and the corresponding doses of IgG were administered i.p. twice a week for 3 weeks. The data points are mean ± SD tumor volumes (*crosses* control IgG and vehicle, *open circles* trastuzumab and vehicle, *open squares* control IgG and capecitabine, *filled squares* combination of trastuzumab and capecitabine). On the last day of measurement, the tumor volume of the combination group was significantly less than that of the control, capecitabine alone and trastuzumab alone groups ($P < 0.05$), and the tumor volume of the trastuzumab alone group was also significantly less than that of the control group ($P < 0.05$)

Discussion

In a previous study, Pegram et al. have demonstrated that the effect of the combination of 5-FUra and trastuzumab is antagonistic in the SK-BR-3 human breast

cancer model [12]. Although the mechanism of the antagonistic interaction between trastuzumab and 5-FUra in vitro has not yet been fully clarified, trastuzumab is able to reduce the fraction of SK-BR-3 cells in S phase and this might be responsible for the reduced sensitivity to 5-FUra of cells treated with trastuzumab [12]. In an in vitro antiproliferation assay that trastuzumab and 5-FUra showed an antagonistic interaction in KPL-4 and BT-474 human breast cancer cells, which is consistent with the reported results of the study with the SK-BR-3 human breast cancer cells. The combination of trastuzumab and 5′-dFUrd showed an antagonistic interaction in vitro in KPL-4 cells, whereas in BT-474 cells it showed an additive interaction. Since 5′-dFUrd exhibits antiproliferative activity after conversion into the active metabolite 5-FUra, it is likely that 5′-dFUrd and 5-FUra interact antagonistically with trastuzumab by the same mechanism. The reason for the discrepancies between 5′-dFUrd and 5-FUra found in combination in the BT-474 model has not been elucidated. Low levels of dThdPase expression, which makes the cells resistant to 5′-dFUrd (IC_{50} for 5′-dFUrd was 64-fold higher on a molar basis than that for 5-FUra), might make clarification of the antagonistic interaction difficult.

On the other hand, in in vivo experiments, the combination of trastuzumab and capecitabine/5′-dFUrd showed at least additive antitumor activity in the two models, KPL-4 and BT-474. The combination treatment was superior to treatment with either single agent alone in terms of tumor growth inhibition and tumor growth delay. Therefore, the antagonistic interaction found in vitro was simply not observed in vivo. It is likely that the mechanism of the antitumor activity of trastuzumab in vivo is different from that in vitro in the tumor models studied. Indeed, the antibody-dependent cell-mediated cytotoxicity (ADCC) reaction has recently been shown to be important in the in vivo activity of trastuzumab using the Fc receptor γ knockout mouse model [24]. ADCC is not associated with in vitro antiproliferative activity, and the in vivo activity of trastuzumab may not be antagonistic to the activity of 5-FUra in vivo.

In separate experiments, we also investigated the possibility that trastuzumab enhances the efficacy of capecitabine in vivo by upregulating the activity of the key metabolic enzyme dThdPase, as do taxanes [25], CPA [26] and X-rays [27], or by downregulating the activity of the other key metabolic enzyme dihydropyrimidine dehydrogenase. However, administration of trastuzumab did not affect the tumor levels of these enzymes in the two models (data not shown). Therefore, it seems rather unlikely that the interaction of two agents is mediated by modulation of enzymes involving fluoropyrimidine metabolism.

A rather high dose of trastuzumab was required to produce antitumor activity in the KPL-4 model, although the level of HER-2 expression in the tumors was comparable to that in BT-474 tumors. In KPL-4 tumor-bearing mice, a high level of circulating HER-2 (1.23 μg/ml) was detected by EIA while in BT-474 tumor-bearing mice the level was 0.03 μg/ml. This might be one reason why KPL-4 tumors were rather refractory to trastuzumab treatment compared with BT-474 tumors.

Trastuzumab has been investigated in combination with various chemotherapeutic agents including paclitaxel and CDDP in preclinical and clinical studies [18, 28]. In addition to these agents, the observed additive in vivo antitumor activity in the present study prompted us to examine the clinical efficacy of the combination of trastuzumab and capecitabine/5′-dFUrd against HER-2-overexpressing breast cancer. Indeed, Bangemann et al. have recently reported a 50% objective tumor response with capecitabine/trastuzumab combination therapy in HER-2-overexpressing metastatic breast cancer [29]. To fully understand the reason for the discrepancy between the in vivo and in vitro effects of the combination, further studies are needed. The in vivo antitumor mechanism of trastuzumab may be different from that in vitro, particularly in combination with fluoropyrimidines, and a clinical assessment of these drugs in HER-2-overexpressing breast cancer is warranted.

Acknowledgements We would like to thank Dr. J. Kurebayashi (Kawasaki Medical School, Kurashiki, Japan) for generously providing the KPL-4 cell line used in this study.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235:177
2. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF (1989) Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707
3. Pauletti G, Godolphin W, Press MF, Slamon DJ (1996) Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. Oncogene 13:63
4. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpress HER2. N Engl J Med 344:783
5. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ (1999) Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639
6. Blum JL, Jones SE, Buzdar AU, Lo Russo PM, Kuter I, Vogel C, Osterwalder B, Burger HU, Brown CS, Griffin T (1999) Multicenter phase II study of capecitabine in paclitaxel-refractory metastatic breast cancer. J Clin Oncol 17:485
7. O'Shaughnessy J, Vukelja S, Moiseyanko V, Miles D, Cervantes G, Navarro C, Mauriac L, Van Hazel G, Lui W-Y, Ayaoub J-P, Rizel S, Osterwalder B, Rutman O, Leonald R (2000) Results of a large phase III trial of Xeloda/Taxotere combination therapy vs Taxotere monotherapy in metastatic breast cancer (MBC) patients. Breast Cancer Res Treat 64:381

8. Hoff PM, Ansari R, Batist G, Cox J, Kocha W, Kuperminc M, Maroun J, Walde D, Weaver C, Harrison E, Burger HU, Osterwalder B, Wong AO, Wong R (2001) Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. J Clin Oncol 19:2282
9. Twelves C, Boyer M, Findlay M, Cassidy J, Weitzel C, Barker C, Osterwalder B, Jamieson C, Hieke K, The Xeloda Colorectal Cancer Study Group (2001) Capecitabine (Xeloda) improves medical resource use compared with 5-fluorouracil plus leucovorin in a phase III trial conducted in patients with advanced colorectal carcinoma. Eur J Cancer 37:597
10. Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, Shimma N, Umeda I, Ishitsuka H (1998) Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumours by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 34:1274
11. Niitani H, Kimura K, Saito T, Nakao I, Abe O, Urushizaki I, Ohta K, Yoshida Y, Kimura T, Kurihara M, Takeda C, Taguchi T, Terasawa T, Tominaga K, Furue H, Wakui A, Ogawa N (1985) Phase II study of 5′-deoxy-5-fluorouridine (5′-DFUR) in patients with malignant cancer. Multi-institutional cooperative study. Jpn J Cancer Chemother 12:2044
12. Pegram M, Hsu S, Lewis G. Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinavar F, Slamon D (1999) Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene 18:2241
13. Arasaki M, Ishitsuka H, Kuruma I, Miwa M, Murasaki C, Shimma N, Umeda I (1992) *N*-Oxycarbonyl substituted 5′-deoxy-5-fluorocytidines. European Patent Application no. 92121538.0
14. Kurebayashi J, Otsuki T, Tang CK, Kurosumi M, Yamamoto S, Tanaka K, Mochizuki M, Nakamura H, Sonoo H (1999) Isolation and characterization of a new human breast cancer cell line, KPL-4, expressing the Erb B family receptors and interleukin-6. Br J Cancer 79:707
15. Kunisue H, Kurebayashi J, Otsuki T, Tang CK, Kurosumi M, Yamamoto S, Tanaka K, Doihara H, Shimizu N, Sonoo H (2000) Anti-HER2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER2. Br J Cancer 82:46
16. Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shepard HM (1993) Differential responses of human tumor cell lines to anti-p185 HER2 monoclonal antibodies. Cancer Immunol Immunother 37:255
17. Kumar R, Shepard HM, Mendelsohn J (1991) Regulation of phosphorylation of the c-erbB-2/HER2 gene product by a monoclonal antibody and serum growth factor(s) in human mammary carcinoma cells. Mol Cell Biol 11:979
18. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J (1998) Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res 58:2825
19. Slooten H-J van, Bonsing BA, Hiller AJ, Colbern GT, van Dierendonck JH, Cornelisse CJ, Smith HS (1995) Outgrowth of BT-474 human breast cancer cells in immune-deficient mice: a new in vivo model for hormone-dependent breast cancer. Br J Cancer 72:22
20. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27
21. Imoto S, Ohkura H, Sugano K, Sasaki Y, Ito K, Igarashi T, Ohtsu T, Fujii H, Minami H, Hasebe T, Mukai K (1998) Determination of cytosol c-erbB-2 protein in breast cancer by sandwich enzyme immunoassay. Jpn J Clin Oncol 28:92
22. Sugano K, Kitajima M, Ishii M, Koyama H, Kasumi F, Demura R, Mori S, Ohkura H (1994) Clinical evaluation of ErbB-2 protein in tissue extract using an enzyme immunoassay (ErbB-2 EIA ''Nichirei''). Gan To Kagaku Ryoho 21:1957
23. Ishikawa T, Utoh M, Sawada N, Nishida M, Fukase Y, Sekiguchi F, Ishitsuka H (1998) Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. Biochem Pharmacol 55:1091
24. Clynes RA, Towers TL, Presta LG, Ravetch JV (2000) Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. Nat Med 6:443
25. Sawada N, Ishikawa T, Fukase Y, Nishida M, Yoshikubo T, Ishitsuka H (1998) Induction of thymidine phosphorylase activity and enhancement of capecitabine efficacy by Taxol/Taxotere in human cancer xenografts. Clin Cancer Res 4:1013
26. Endo M, Shinbori N, Fukase Y, Sawada N, Ishikawa T, Ishitsuka H, Tanaka Y (1999) Induction of thymidine phosphorylase expression and enhancement of efficacy of capecitabine or 5′-deoxy-5-fluorouridine by cyclophosphamide in mammary tumor models. Int J Cancer 83:127
27. Sawada N, Ishikawa T, Sekiguchi F, Tanaka Y, Ishitsuka H (1999) X-ray irradiation induces thymidine phosphorylase and enhances the efficacy of capecitabine (Xeloda) in human cancer xenografts. Clin Cancer Res 5:2948
28. Pietras RJ, Pegram MD, Finn RS, Maneval DA, Slamon DJ (1998) Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. Oncogene 17:2235
29. Bangemann N, Kuhle A, Willrodt RG, Buehler H, Schaller G (2000) Treatment of HER2 overexpressing metastatic breast cancer (MBC) with trastuzumab (Herceptin) and chemotherapy. Breast Cancer Res Treat 64:123