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## Antitumor activity of erlotinib in combination with capecitabine in human tumor xenograft models

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**Abstract Purpose:** To examine the antitumor activity and tolerability of a combination comprising erlotinib and capecitabine in human colorectal, breast and epidermal cancer xenograft models. Further aims of the study were to examine the effects of single-agent erlotinib therapy on tumor growth, and on thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) levels, (enzymes which activate and deactivate capecitabine, respectively) in tumor tissue. **Methods:** BALB/c nu/nu mice bearing LoVo and HT-29 (colon cancer), A-431 (vulval cancer), and KPL-4 and MAXF401 (breast cancer) human tumors were treated with erlotinib 100 mg/kg/day and/or capecitabine 359 or 90 mg/kg/day, by oral administration once daily for 14 days. **Results:** The maximum tolerated dose (MTD) of erlotinib, formulated in carboxymethylcellulose/Tween 80, was identified as 125 mg/kg/day. Erlotinib at a dose of 100 mg/kg/day achieved significant tumor-growth inhibition in the, LoVo, KPL-4, and A-431 models. Some inhibition of MAXF401 tumor growth was observed, but was not significant. In the HT-29 model, erlotinib showed less marked but statistically significant antitumor activity. On day 15, mean tumor-growth inhibition in HT-29, LoVo, KPL-4, MAXF401, and A-431 models was 46, 74, 71, 20, and 85%, respectively. Evaluation of erlotinib/capecitabine combination therapy, at sub-optimal doses, in the three erlotinib-sensitive tumor models LoVo, KPL-4 and A-431, demonstrated at least additive activity with the combination compared with the single agents. In the A-431 and LoVo models, the combination of agents had greater antitumor activity than the single agent capecitabine alone at the MTD. Erlotinib in combination with capecitabine was not associated with significantly increased toxicity compared with single-agent therapy.

Erlotinib 100 mg/kg/day induced significant upregulation of TP and DPD in the LoVo model, a significant upregulation of TP in the HT-29, MAXF401 and A-431 models, but had no obvious effect on TP and DPD levels in the KPL-4 model. In the A-431 model, selective upregulation of TP by erlotinib 100 mg/kg resulted in an increased TP:DPD ratio. In the LoVo model, immunohistochemistry revealed marked upregulation of TP (but not DPD by erlotinib). **Conclusions:** Erlotinib inhibits tumor growth in a range of human tumor xenograft models, including breast and colorectal cancer (CRC). Erlotinib and capecitabine demonstrated at least additive activity in LoVo, KPL-4 and A-431 tumor models. The antitumor activity of the combination was greater than that of capecitabine alone at the MTD. Erlotinib treatment did affect the TP in the CRC tumor models as confirmed immunohistochemically. The findings of this study support clinical evaluation of erlotinib, both as a single agent and in combination with capecitabine, for the treatment of CRC and breast cancer.

**Keywords** Erlotinib · Capecitabine · Antitumor activity · Human xenograft models · Epidermal growth factor receptor (HER1/EGFR) · 5-Fluorinated pyrimidine

### Introduction

The epidermal growth factor receptor (EGFR) family comprises four closely related receptors (HER1/EGFR, HER2, HER3 and HER4) involved in cellular responses such as differentiation and proliferation (reviewed in [45]). HER1/EGFR is overexpressed in many human cancers, including breast, colorectal, head and neck, ovarian, renal cell and non-small-cell lung cancers (NSCLC) [4, 37] and this is frequently linked to advanced disease, metastases and poor prognosis [32, 48]. The role of HER1/EGFR in tumorigenesis has stimulated interest in it as a potential therapeutic target for novel anticancer therapies [4].

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Erlotinib (Tarceva, OSI-774) is an orally available inhibitor of HER1/EGFR tyrosine kinase. In vitro, erlotinib has demonstrated substantial inhibitory activity against HER1/EGFR tyrosine kinase in a number of human tumor cell lines, including CRC and breast cancer [27], and preclinical evaluation has demonstrated activity against a number of HER1/EGFR-expressing human tumor xenografts [34]. More recently, erlotinib has demonstrated promising activity in phase I and II trials in a number of indications, including head and neck cancer [43], NSCLC [33], CRC [31] and MBC [46]. In a phase III trial, erlotinib monotherapy significantly prolonged survival, delayed disease progression and delayed worsening of lung cancer-related symptoms in patients with advanced, treatment-refractory NSCLC [42].

Oral capecitabine is a highly effective fluoropyrimidine, which generates 5-fluorouracil (5-FU) preferentially in tumor tissue by exploitation of the increased activity of thymidine phosphorylase (TP) in tumors compared with normal tissue [24, 41]. 5-FU is deactivated by the enzyme dihydropyrimidine dehydrogenase (DPD) [29], and the TP:DPD ratio has been shown to correlate with susceptibility to capecitabine in human tumor xenograft models [14]. Capecitabine has demonstrated consistent and impressive activity in patients with chemo-naïve and pretreated advanced breast cancer [1, 2, 35], and advanced CRC [3, 44].

Target-specific therapeutic approaches, such as erlotinib, are generally associated with reduced toxicity compared with conventional cytotoxic agents, and therefore lend themselves to use in combination regimens. Promising results have been observed in phase I/II studies of erlotinib in combination with bevacizumab [23] and gemcitabine [5]. In NSCLC first-line erlotinib or gefitinib in combination with standard chemotherapy did not improve survival [9, 10, 12, 13], but in a phase III, randomized trial in pancreatic cancer, erlotinib plus gemcitabine significantly prolonged survival and progression-free survival compared with gemcitabine alone [25]. Capecitabine has been used successfully in combination therapy regimens [30, 40], and preclinical studies have demonstrated supra-additive activity with combination regimens comprising capecitabine and any one of a number of anticancer therapies, including gefitinib [21], docetaxel and paclitaxel [8, 38], cyclophosphamide [6] and radiotherapy [39]. In addition, at least additive antitumor activity has been demonstrated with capecitabine and trastuzumab, an agent specifically targeting the HER2 receptor [7].

The current study examined the antitumor activity and tolerability of erlotinib in combination with capecitabine in human colorectal, breast and epidermal cancer xenograft models. Further aims of the study were to examine the effects of single-agent erlotinib therapy on tumor growth, and on TP and DPD levels in tumor tissue.

## Materials and methods

### Test agents

Erlotinib (F. Hoffmann-La Roche, Nutley, NJ, USA) was provided as a fine powder. After suspension in vehicle (0.2% (w/v) carboxymethylcellulose containing 0.1% (v/v) Tween 80), erlotinib was sonicated for 5 min and homogenized for 7 min prior to administration. Capecitabine (F. Hoffmann-La Roche, Nutley, NJ, USA) was provided as a powder and suspended in vehicle (40 mM citrate buffer containing 5% (w/v) gum arabic, pH 6.0).

### Animals

Male and female, 4–6-week old BALB/c nu/nu mice were obtained from Nippon Clea (Tokyo, Japan). All animals were allowed to acclimatize and recover from shipping-related stress for 1 week prior to the study. The health of the mice was monitored daily by observation. Chlorinated water and irradiated food were provided ad libitum, and the animals were kept in a 12-h light and dark cycle. All animal experiments were in accordance with the Guidelines for the Care and Use of Laboratory Animals in the Nippon Roche Research Center (Kamakura, Japan).

### Cell lines and culture conditions

LoVo (human colon cancer) cells (American Type Culture Collection [ATCC], Rockville, MD, USA) were maintained in Ham's F-12 medium supplemented with 20% (v/v) fetal bovine serum (FBS). HT-29 (human colon cancer) cells (ATCC) were maintained in McCoy's 5a medium supplemented with 10% (v/v) FBS. A-431 (human epidermal cancer) cells (ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) nutrient mixture containing 4 mM L-glutamine, 18 mM sodium bicarbonate, 23 mM glucose and 10% (v/v) FBS. KPL-4 (human inflammatory breast cancer) cells [20] were kindly provided by Dr. J. Kurebayashi (Kawasaki Medical School, Kurashiki, Japan), and were maintained in DMEM containing 10% (v/v) FBS. MAXF401 (human breast cancer) cells were kindly provided by Dr. Prof. H. H. Fiebig (University of Freiburg, Freiburg, Germany), and were maintained in BALB/c nu/nu mice by inoculation subcutaneously (s.c.).

### HER1/EGFR protein levels in tumor tissues

The HER1/EGFR protein was measured by sandwich ELISA (Oncogene, Cat.# QIA35). Samples were prepared homogenized on ice in 10 mM Tris-HCl buffer containing 1.5 mM EDTA, 10% glycerol, 0.1% sodium

azide and protease inhibitors (CALBIOCHEM, cocktail set). The membrane fraction was extracted in accordance with the protocol for the HER1/EGFR ELISA. Protein quantity was measured after the extraction operation.

#### Tumor-growth inhibition studies in vivo

Suspensions of LoVo ( $5 \times 10^6$  cells/mouse), HT-29 ( $5 \times 10^6$  cells/mouse) and A-431 ( $8 \times 10^6$  cells/mouse) cells were inoculated s.c. into the right flank of the mice. A piece of MAXF401 was transplanted s.c. into the right flank of female mice. A suspension of KPL-4 cells ( $1 \times 10^7$  cells/mouse) was orthotopically transplanted into the second mammary fat pad of female mice. Several weeks after tumor inoculation, mice bearing a tumor of approximately 100–300 mm<sup>3</sup> in volume were selected and randomized into control and treatment groups. To evaluate the antitumor activity and tolerability of erlotinib and capecitabine, tumor volume and body weight were assessed twice a week. The tumor volumes were estimated using the equation  $V = ab^2/2$ , where  $a$  and  $b$  are tumor length and width, respectively. Tumor-growth inhibition (%) was calculated as:  $1 - (\text{the tumor volume change of treatment group} / \text{the tumor volume change of control group})$ . Gastrointestinal toxicity was estimated by observing the feces and by detecting fecal occult blood using a test kit (Shionogi Pharma Co., Osaka, Japan). Peripheral blood leukocyte counts were performed to evaluate bone marrow toxicity.

#### Treatment of animals

In the dose-response study, randomized groups of mice ( $n = 5$ ) bearing LoVo tumors were treated with vehicle control or erlotinib at doses of 50, 75, 100 or 125 mg/kg once daily for 2 weeks. The MTD of erlotinib for this study was determined in a separate experiment. For this purpose, the MTD was defined as half of the lowest toxic dose. Cohorts of mice bearing LoVo, HT-29, KPL-4, MAXF401 or A-431 tumors were each randomized to groups of five or six mice and treated with erlotinib 100 mg/kg or vehicle control once daily for 2 weeks. In the combination studies, mice bearing LoVo or A-431 tumors were each randomized into groups of six mice and treated with erlotinib 100 mg/kg [80% maximum tolerated dose (MTD)], capecitabine 359 mg/kg (66% MTD) or their respective vehicle controls, or a combination of erlotinib 100 mg/kg and capecitabine 359 mg/kg or combined vehicle control, once daily for 2 weeks. Mice bearing KPL-4 tumors were randomized into groups of five and treated with erlotinib 100 mg/kg (80% MTD), capecitabine 90 mg/kg (66% MTD in this model) or their respective vehicle controls, or a combination of erlotinib 100 mg/kg and capecitabine 90 mg/kg or combined vehicle control, once daily for 2 weeks. In all animals, erlotinib, capecitabine and

vehicle controls were administered orally, using a 1 ml syringe and a gavage needle for mice.

#### TP and DPD protein levels in tumor tissues

Tumor samples were collected on the day following the final treatment and immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  prior to assay. Tumor tissues were homogenized in phosphate buffered saline (PBS) and centrifuged at 10,000 g for 20 min at  $4^\circ\text{C}$ . The protein concentration of the supernatant was determined using a DC Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA). The levels of TP and DPD were measured by sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific to human TP and DPD, as described previously [26, 28].

In the LoVo model, TP and DPD upregulation was confirmed immunohistochemically, using a polyclonal anti-human TP antibody number 6 (prepared at the Nippon Roche Research Center) and anti-DPD monoclonal antibody (2H9-1b, Roche Diagnostics Ltd, Nutley, NJ, USA) [19]. Polyclonal anti-human TP antibody number 6, obtained by rabbit immunization, was used for immunohistochemical analysis. Antigen was purified from recombinant TP protein. Formalin-fixed, paraffin-embedded specimen sections were dewaxed in xylene and dehydrated by passage through a graded ethanol series to tap water. Antigen retrieval was achieved with steaming in 10 mM citrate buffer (pH 6.0) for 20 min followed by 20 min cooling down at room temperature. After blocking with 0.3% hydrogen peroxide in methanol, the sections were further blocked for 30 min with 3% skimmed milk. The sections were incubated overnight ( $4^\circ\text{C}$ ) with anti-TP polyclonal antibody number 6. Sections were subsequently incubated with biotinylated rat anti-rabbit immunoglobulins followed by avidin-peroxidase reagent. Reactivity was visualized using 3,3'-diaminobenzidine as the substrate.

#### Statistical analysis

The Mann-Whitney  $U$  test was used to determine differences in tumor volume, body weight and tumor TP and DPD concentrations between the groups.

## Results

#### HER1/EGFR expression in tumors

The levels of HER1/EGFR expression in tumor tissues were examined. The membrane fraction was prepared and measured with ELISA. The levels in HT-29, LoVo, KPL-4, MAXF401 and A-431 tumors were 2.93, 1.43,  $< 1.0$ , 2.48, and 14.7 pg/mg protein, respectively.

## Effects of erlotinib on established human tumor xenografts

### Dose response study in LoVo

Erlotinib demonstrated dose-dependent antitumor activity against LoVo tumors at doses ranging from 50–125 mg/kg/day (Fig. 1). On day 15, after 14 days' treatment with erlotinib, tumor inhibition rates of 52, 62 and 85% were observed with erlotinib doses of 75, 100 and 125 mg/kg, respectively. The 50 mg/kg dose of erlotinib did not show significant antitumor activity. Substantial weight loss ( $\geq 20\%$  of the body weight at start of treatment) was not observed at any of the doses tested. No toxic deaths were observed.

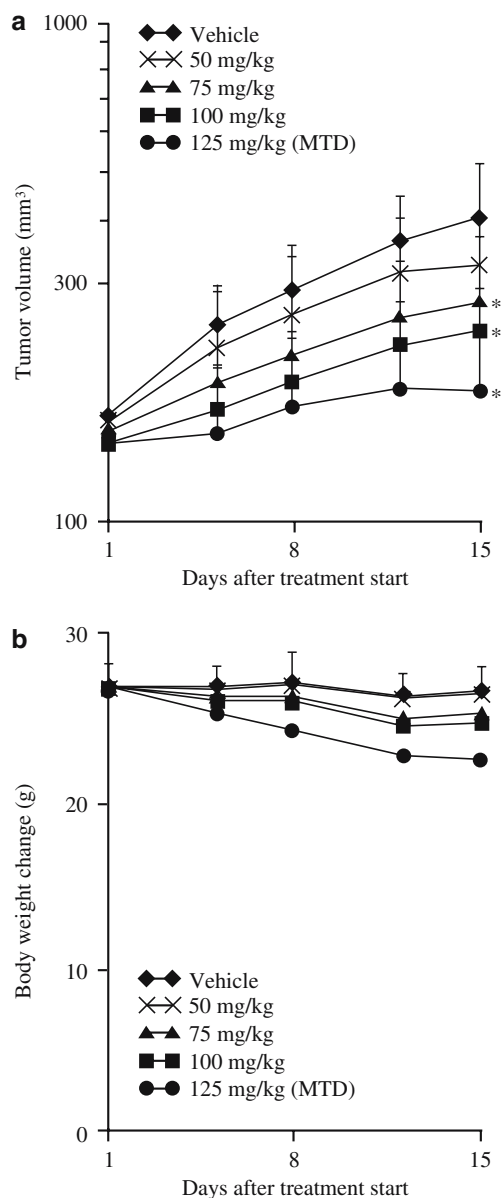
In a separate experiment, erlotinib 250 mg/kg/day was shown to be lethal in mice bearing LoVo tumors and in mice bearing A-431 tumors (data not shown). The MTD was defined as half of the lowest toxic dose and was therefore confirmed to be erlotinib 125 mg/kg/day formulated in carboxymethylcellulose/Tween 80.

### Antitumor activity as a single agent in five xenograft models

Erlotinib at a dose of 100 mg/kg/day (80% of MTD) was administered to mice bearing established HT-29, LoVo, KPL-4, MAXF401 or A-431 tumors. Significant tumor-growth inhibition was observed in the LoVo, KPL-4 and A-431 models (74, 71, and 85%, respectively; Fig. 2). In the HT-29 model, erlotinib showed less marked, but statistically significant antitumor activity (46%,  $P < 0.05$ ). Weak inhibition of MAXF401 tumor growth (20%) was observed after 15 days; this was not statistically significant.

### Combination activity of erlotinib and capecitabine in colon, breast and epidermal tumor xenografts

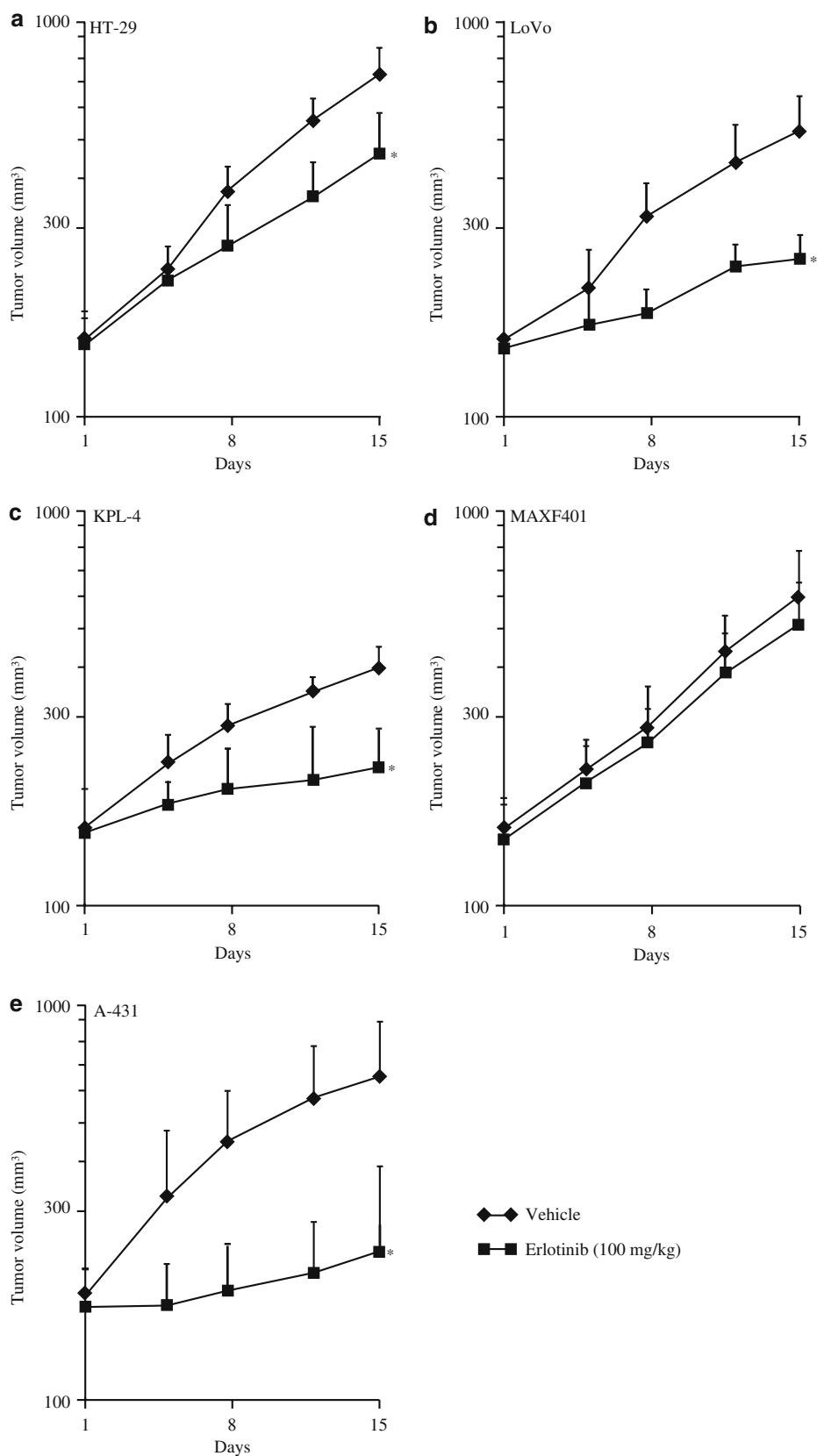
Combination therapy with erlotinib and capecitabine was examined in the three erlotinib-sensitive tumor models: LoVo, KPL-4 and A-431. In the LoVo CRC model, 100 mg/kg/day of erlotinib (80% of MTD) and 359 mg/kg of capecitabine (67% of MTD [14]) were administered separately and in combination, once daily for 14 days starting on day 17 following tumor inoculation. Combined treatment with erlotinib and capecitabine achieved significantly increased tumor inhibition compared with either capecitabine or erlotinib administered as single agents (Fig. 3;  $P < 0.05$ ). On day 15 after treatment started, inhibition of tumor growth was 43, 74 and 95% in the capecitabine, erlotinib and combination treatment groups, respectively. Furthermore, the anti-tumor activity of the combination treatment was greater than that of capecitabine at the MTD (539 mg/kg, 56%).



**Fig. 1** Dose-dependent antitumor activity of erlotinib in the human colon cancer xenograft model (LoVo). Mean values  $\pm$  standard deviation (SD) of tumor volume (cm<sup>3</sup>). Vehicle (diamonds), erlotinib at 50 (crosses), 75 (triangles), 100 (squares) or 125 (circles) mg/kg. \*  $P < 0.05$  versus vehicle control

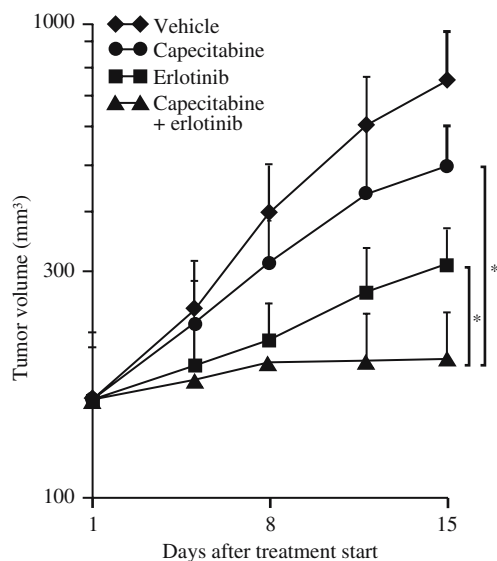
In the KPL-4 breast cancer model, 100 mg/kg/day of erlotinib (80% of MTD) and 90 mg/kg of capecitabine (67% of MTD for this model only [37]) were administered once daily for 14 days, commencing on day 16 following tumor inoculation. Combined treatment with erlotinib and capecitabine achieved significantly increased tumor inhibition compared with capecitabine administered as a single agent (Fig. 4;  $P < 0.05$ ). Compared with erlotinib alone, the combination achieved more marked inhibition of tumor growth (but the difference was not statistically significant). Tumor-growth inhibition on day 15 was 36, 71 and 88%, in the capecitabine, erlotinib and the combination groups,

**Fig. 2** Antitumor activity of erlotinib (100 mg/kg/day) as a single agent in five human cancer xenograft models (HT-29, LoVo, KPL-4, MAXF401 and A-431). Mean values  $\pm$  SD of tumor volume ( $\text{cm}^3$ ). Vehicle (diamonds) and erlotinib (squares). \*  $P < 0.05$  versus vehicle control



respectively. A combination regimen comprising 75 mg/kg of erlotinib (60% of MTD) and 90 mg of capecitabine (66% of MTD) achieved 82% inhibition of

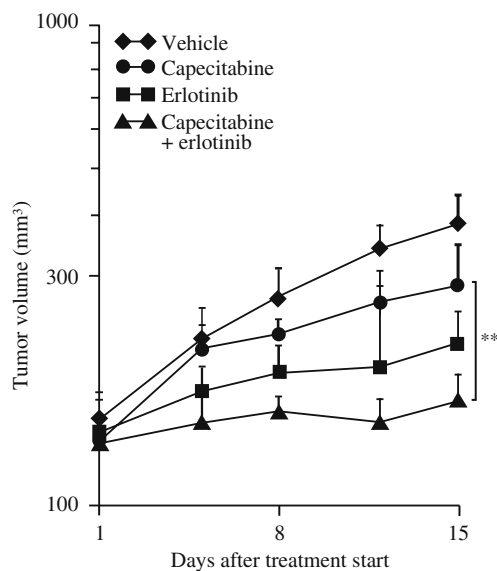
tumor growth, which was significantly increased compared with single-agent erlotinib (45%; data not shown) or capecitabine. A lower dose of capecitabine was used



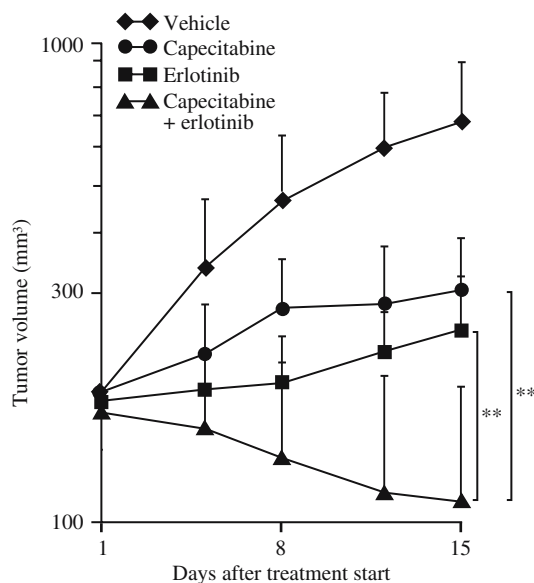
**Fig. 3** Effect of erlotinib (100 mg/kg/day) in combination with capecitabine (359 mg/kg/day) on tumor volume in LoVo human tumor xenograft models. Mean values  $\pm$  SD of tumor volume ( $\text{cm}^3$ ). Vehicle (diamonds), capecitabine alone (circles), erlotinib alone (squares) and capecitabine in combination with erlotinib (triangles). \*  $P < 0.05$

in the KPL-4 model, because this cell line is known to induce cachexia [20].

In the A-431 epidermal cancer model, 100 mg/kg/day of erlotinib (80% of MTD) and 359 mg/kg of capecitabine (66% of MTD) were administered once daily for 14 days, commencing 12 days after tumor inoculation (Fig. 5). At day 15, a highly significant increase in tumor-growth inhibition was observed with the combination



**Fig. 4** Effect of erlotinib (100 mg/kg/day) in combination with capecitabine (90 mg/kg/day) on tumor volume in KPL-4 human tumor xenograft models. Mean values  $\pm$  SD of tumor volume ( $\text{cm}^3$ ). Vehicle (diamonds), capecitabine alone (circles), erlotinib alone (squares) and capecitabine in combination with erlotinib (triangles). \*\*  $P < 0.01$



**Fig. 5** Effect of erlotinib (100 mg/kg/day) in combination with capecitabine (359 mg/kg/day) on tumor volume in A-431 human tumor xenograft models. Mean values  $\pm$  SD of tumor volume ( $\text{cm}^3$ ). Vehicle (diamonds), capecitabine alone (circles), erlotinib alone (squares) and capecitabine in combination with erlotinib (triangles). \*\*  $P < 0.01$

compared with single-agent therapies, with tumor-growth inhibition of 76, 85 and 112% documented in the capecitabine, erlotinib and combination groups, respectively. As for the LoVo model, the antitumor activity of the combination was more potent than that of capecitabine at the MTD dose (539 mg/kg, 105%) in the A-431 model.

In all of the models tested, combination treatment with erlotinib and capecitabine was not associated with significant toxicity, and there was no mean body weight loss greater than 20% of pretreatment body weight. Intestinal toxicity (diarrhea) and myelotoxicity (peripheral blood leukocyte count) were further evaluated in a separate experiment in mice bearing LoVo tumors. These mice were treated with erlotinib 100 mg/kg/day and 359 mg/kg of capecitabine once daily for 14 days. On day 15, no intestinal toxicity (assessed by fecal-form observation and occult blood test) and no significant differences between the peripheral blood leukocyte counts were observed in any group compared with the control values (Table 1).

**Table 1** Erlotinib with capecitabine: toxicities in the LoVo human colon cancer model (mean values  $\pm$  SD)

Group	<i>n</i>	WBC count ( $\times 10^6$ cells/ml)	Body weight (g)
Control	5	4.5 $\pm$ 0.6	24.7 $\pm$ 1.0
Erlotinib	5	5.0 $\pm$ 0.5	25.0 $\pm$ 1.0
Capecitabine	5	3.9 $\pm$ 0.5	25.1 $\pm$ 0.4
Combination	5	3.9 $\pm$ 0.4	21.2 $\pm$ 3.1

## Effects of erlotinib on tumor enzyme levels

The effect of erlotinib treatment on TP and DPD levels in tumor tissue was examined. Tumor TP and DPD levels are summarized in Table 2. In the LoVo model, upregulation of TP and DPD was observed at 100 mg erlotinib/kg/day (Table 2). In this model, TP upregulation was also confirmed immunohistochemically. Immunohistochemical staining of TP was very strong in LoVo tumors removed from mice treated with erlotinib, whereas tumor samples from vehicle-treated animals did not express TP (Fig. 6). The immunohistochemistry of DPD in the LoVo model was not different between the control and erlotinib treated groups.

In the MAXF401 and HT-29 models, significant ( $P < 0.05$ ) differences in TP levels were observed in tumors from animals treated with erlotinib 100 mg/kg compared with vehicle control, but there were no significant differences in DPD levels in these models. No significant differences were observed in TP or DPD levels in the KPL-4 model. In the A-431 model, TP expression was significantly upregulated by the administration of erlotinib 100 mg/kg, but DPD was unaffected. In the A-431 model, the TP:DPD ratio was therefore increased (1.7-fold increase,  $P < 0.01$ ).

## Discussion

Human tumor xenograft models are commonly used for the evaluation of anticancer agents. In the current study, five human tumor xenograft models were used to evaluate the activity and tolerability of erlotinib, administered both alone and in combination with capecitabine.

Erlotinib demonstrated a dose-dependent antitumor effect when administered to mice bearing LoVo colon tumors at doses ranging from 50–125 mg/kg/day. At the lowest dose of erlotinib showing a significant response (75 mg/kg/day), tumor inhibition of approximately 50% was observed. Under the conditions of this experiment, erlotinib was well tolerated at these doses, with no deaths recorded and mean body weight maintained ( $\geq 80\%$ ) in all groups, after 2 weeks of treatment. Additional data showed that erlotinib 250 mg/kg/day was lethal in this model (data not shown), indicating that the MTD for erlotinib is 125 mg/kg/day, when

formulated as a suspension in carboxymethylcellulose/Tween 80. Further long-term studies of this combination are recommended to identify optimal doses for prolonged administration.

In mice bearing human colon (LoVo), breast (KPL-4), or epidermal (A-431) tumors, erlotinib monotherapy resulted in significant inhibition of tumor growth compared with vehicle control. The antitumor activity of erlotinib in these tumor xenograft models is encouraging. Further clinical investigation of erlotinib is warranted in tumors expressing HER1/EGFR, such as breast [18], colon [22], head and neck [11] and NSCLC [36] cancers.

It has been postulated that HER1/EGFR inhibition may potentiate the activity of anticancer agents by inhibiting the ability of cells to repair chemo- or radiotherapy-induced damage [17, 47]. This could enhance the effects of conventional chemotherapies, allowing the use of lower doses, and thereby reducing the incidence and/or intensity of the adverse events associated with these therapies. In the current study, erlotinib and capecitabine were investigated at doses corresponding to 80 and 67% of their respective MTDs, and the combination was well tolerated, with no significant increase in toxicity compared with the constitutive single agents. In the A-431 and LoVo models, the combination of agents showed more potent antitumor activity than the single agent capecitabine alone at the MTD. Such results are suggestive that the combination of erlotinib with capecitabine may show clinical benefit in patients. Although the combination of erlotinib with concurrent platinum doublets for NSCLC in the first-line setting did not show improved survival in the overall study population in phase III trials [9, 13], in a phase III, randomized trial in pancreatic cancer, erlotinib plus gemcitabine significantly prolonged survival and progression-free survival compared with gemcitabine alone [25]. Other investigations are underway to optimize the use of erlotinib as a targeted cancer therapy. Furthermore, the oral administration schedules for both erlotinib and capecitabine offer the potential for significantly improved patient convenience and quality of life compared with conventional intravenous chemotherapy-based regimens.

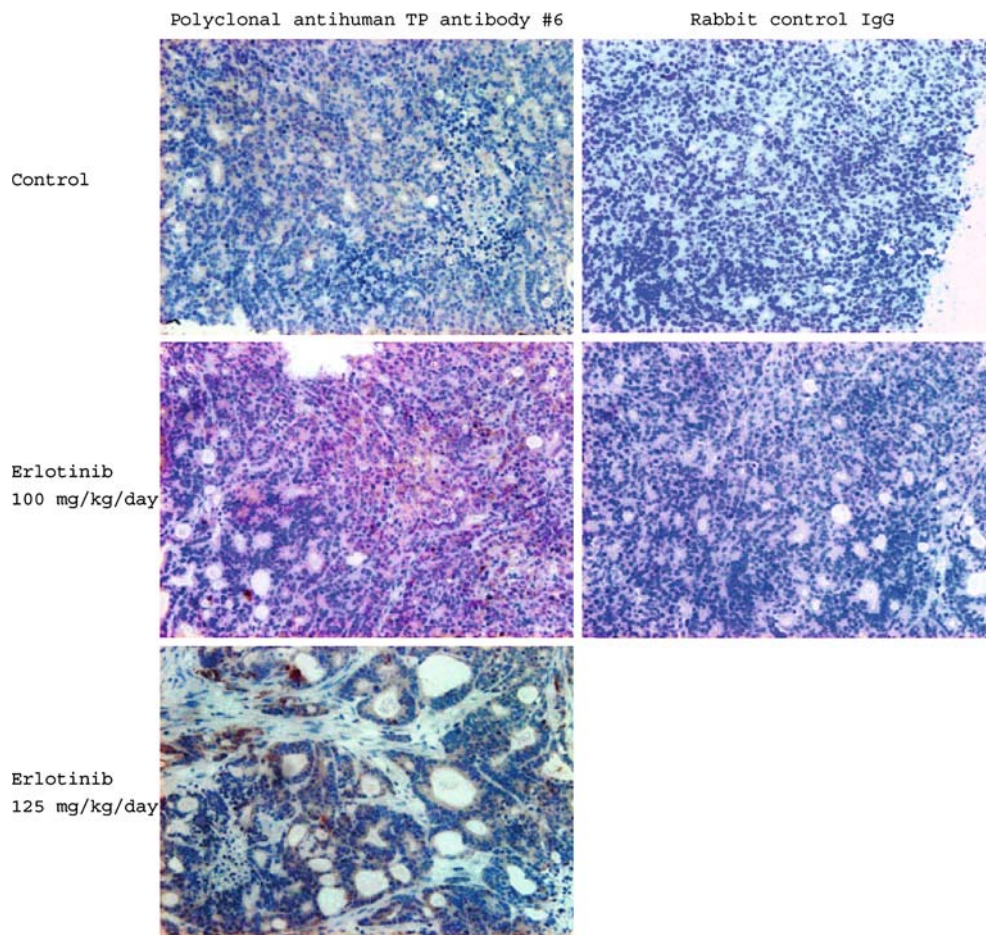
In accordance with the established clinical efficacy of capecitabine in the management of colorectal and breast cancer [1, 2, 44], capecitabine administered as a single

**Table 2** Effect of erlotinib (100 mg/kg/day) on tumor TP and DPD expression in five human cancer xenograft models

Tumor model	n	TP		DPD	
		Control	Erlotinib	Control	Erlotinib
A-431	5	408 ± 76	670 ± 126*	71.8 ± 10.6	70.8 ± 12.8
LoVo	5	13.5 ± 2.5	24.0 ± 7.3*	10.4 ± 3.3	24.0 ± 6.7*
HT-29	6	0.95 ± 0.11	1.16 ± 0.11*	21.9 ± 3.6	25.0 ± 3.3
KPL-4	5	45.8 ± 8.5	36.5 ± 10.8	< 2.0	< 2.0
MAXF401	5	11.0 ± 1.7	12.9 ± 1.1*	3.36 ± 1.12	2.99 ± 0.28

Values are means ± SD of enzyme specific activity (units/mg total protein). A unit of activity is defined as the generation of 1  $\mu$ g 5-FU/h for TP or the catabolism of 1 pmol 5-FU/min for DPP\*  $P < 0.05$  versus control group

**Fig. 6** Effect of erlotinib (100 mg/kg/day) on tumor TP expression in LoVo model, as assessed by immunohistochemical analysis



agent significantly inhibited tumor growth in breast and colorectal tumor xenograft models in the current study. Additionally, capecitabine yielded good activity against A-431 tumors. The combination of erlotinib and capecitabine afforded at least additive activity against tumor growth in colon, breast and epidermal models.

The antitumor effects of chemotherapy are mediated through reversible G1 cell cycle arrest and a rapid apoptotic response [17]. It has been postulated that inhibition of HER1/EGFR-mediated proliferation and survival signals may lead to increased apoptosis in response to DNA damage in these tumors [27]. In this way, the synchronous administration of erlotinib may potentiate the cytotoxic effects of capecitabine through increased apoptosis in the LoVo, KPL-4 and A-431 epithelial-derived human tumor models, and may therefore achieve supra-additive tumor inhibition compared with the constitutive single agents.

Anticancer agents, such as gefitinib, docetaxel, paclitaxel, cyclophosphamide and radiotherapy, have been shown to augment the effects of capecitabine through upregulation of tumor TP concentrations [8, 15, 21, 38, 39]. TP plays a key role in the conversion of capecitabine to 5-FU in tumors, and the TP:DPD ratio has been shown to predict for capecitabine antitumor activity in human tumor xenograft models [14]. Erlotinib treatment

affected the TP upregulation in four out of the five models, whereas DPD levels were only increased in one of the tumor models in this study. Therefore, it is possible that erlotinib, similar to other anticancer treatments, may enhance the effects of capecitabine through positive effects on TP upregulation and TP:DPD ratios.

For clarification of TP and DPD upregulation, immunohistochemical analysis was explored. The level of TP staining in tissue samples from erlotinib-treated mice was clearly stronger than in samples from vehicle-control animals, whereas the staining of DPD was similar between groups. Immunohistochemical methods are able to more clearly identify TP upregulation compared with an ELISA. The reason for this distinction is that TP expression in tumor tissues is heterogeneous and, therefore, is more adequately studied using immunohistochemistry, as ELISA methods assess enzyme levels in whole tissue.

In the A-431 model, the TP:DPD ratio increased 1.7-fold, suggesting that higher levels of 5-FU could be generated in the tumor, which may lead to an even greater increase in tumor-growth inhibition with no potentiation of body weight loss. Ishikawa et al (1998) [14] reported increased sensitivity to capecitabine when the TP:DPD ratio was increased. However, in the LoVo model, where levels of both enzymes increased,



significant tumor-growth inhibition was observed with combination treatment compared with capecitabine alone. The fact that the increase in DPD was not confirmed by IHC, further limits the interpretation of these results. In the KPL-4 model, there was no change in the TP:DPD ratio, but combination treatment appeared to be at least additive. Moreover, the degree of TP upregulation in this study was modest compared with that seen with other agents [38, 39]. It may be that the level of TP upregulation seen here with erlotinib was not sufficient to exert marked effects on tumor inhibition. Given these limitations, it is not possible to draw firm conclusions about the relationship between erlotinib-induced TP upregulation and capecitabine sensitivity in these tumor models.

While most of the clinical trial data for erlotinib relate to its use in NSCLC, results from phase I/II studies have demonstrated promising activity for erlotinib and capecitabine/erlotinib combination therapy in patients with a wide range of human solid tumor types, including CRC [31] and MBC [16]. Positive phase III data with erlotinib in combination with gemcitabine in pancreatic cancer highlights the potential of erlotinib combination regimens in the treatment of different tumor types. The current preclinical data confirm the significant antitumor activity of erlotinib against breast and colorectal tumor cells and demonstrate that the addition of capecitabine yields at least additive activity compared with the constitutive single-agent activities. The findings of this study therefore support clinical evaluation of erlotinib, both as a single agent and in combination with capecitabine, for the treatment of advanced CRC and MBC.

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