Prediction of the effect of capecitabine in gastric cancer by immunohistochemical staining of thymidine phosphorylase and dihydropyrimidine dehydrogenase

Wasaburo Koizumi[a], Isao Okayasu[b], Ichinosuke Hyodo[c], Junichi Sakamoto[d], Hiroshi Kojima[e] and the Clinical Study Group of Capecitabine

The objective of this study was to investigate the relationship between the expression of thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) and the response to capecitabine in patients with advanced/recurrent gastric cancer. TP and DPD expression in paraffin-embedded specimens of primary lesions, obtained from 25 patients before capecitabine chemotherapy, were evaluated by immunohistochemical staining with anti-TP and anti-DPD monoclonal antibodies. The patients (19 male and six female) had a median age of 65 years (range 37–74). All had a good performance status [Eastern Cooperative Oncology Group (ECOG) 0 or 1]. Overall response rate to capecitabine therapy was 32%. TP was positive in 19 tumors (76%) and DPD was positive in 13 tumors (52%). The response rate (RR) was significantly higher (Fisher's exact test, $P=0.028$) in patients with TP-positive and DPD-negative tumors (RR=60%, 6/10) than in the remaining patients (RR=13%, 2/15). TP and DPD expression profiles are useful for predicting a response to capecitabine. *Anti-Cancer Drugs* **19:819–824**

Anti-Cancer Drugs 2008, **19**:819–824

Keywords: capecitabine, dihydropyrimidine dehydrogenase, gastric cancer, thymidine phoshorylase

[a]Department of Gastroenterology, East Hospital, [b]Department of Pathology, Kitasato University School of Medicine, Sagamihara, [c]Department of Gastroenterology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, [d]Young Leaders' Program of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya and [e]Department of Gastrointestinal Surgery, Aichi Cancer Center Aichi Hospital, Okazaki, Japan

Correspondence to Dr Wasaburo Koizumi, MD, PhD, Associate Professor of Internal Medicine, Department of Gastroenterology, East Hospital, Kitasato University School of Medicine, 2-1-1, Asamizodai, Sagamihara City, Kanagawa, 228-8520, Japan
Tel: +81 42 748 9111; fax: +81 42 748 5120;
e-mail: koizumi@med.kitasato-u.ac.jp

Received 5 February 2008 **Revised form accepted** 19 May 2008

Introduction

Gastric cancer is the commonest form of cancer in Japan [1]. Although 5-fluorouracil (5-FU) remains the most effective single agent in patients with advanced gastric cancer, it is associated with response rates of less than 20%, which tend to be partial and short-lived [2]. Administration of 5-FU by continuous infusion is thought to be more active and better tolerated than intravenous bolus administration, but is associated with added complications, discomfort, and inconvenience of central venous access [3,4].

Capecitabine (Xeloda) is an oral fluoropyrimidine carbamate that generates 5-FU preferentially in tumor tissue. The drug is rapidly and extensively absorbed through the gut as an intact molecule, and then metabolized to 5-FU in three steps [5,6]. First, capecitabine is converted to 5′-deoxy-5-fluorocytidine (5′-DFCR) by carboxylesterase in the liver. Second, 5′-DFCR is converted to 5′-deoxy-5-fluorouridine (5′-DFUR) by cytidine deaminase in the liver and tumor tissues. Finally, 5′-DFUR is converted to 5-FU by thymidine phosphorylase (TP), which is significantly more active in most human tumor tissues than in adjacent healthy tissue [7]. The increasing specificity for tumor cells which occurs with each successive conversion step potentially reduces systemic 5-FU exposure while increasing the dose of 5-FU actually delivered to tumor tissue [5,6,8]. It is hypothesized that this provides an improved therapeutic ratio for capecitabine.

Capecitabine is already replacing intravenous 5-FU/leucovorin in patients with advanced colorectal cancer as a result of its superior response rates, equivalent time to disease progression and overall survival, and improved tolerability profile compared with 5-FU/leucovorin [3,9]. Capecitabine has also shown clear antitumor activity and good tolerability in patients with advanced/metastatic gastric cancer as a single agent [10], as well as in combination with cisplatin [11] and docetaxel [12]. Two phase III trials in the first-line treatment of advanced/metastatic gastric cancer have recently been completed [13,14]. The first, a Korean study, demonstrated the noninferiority of capecitabine plus cisplatin, with a trend towards superiority, compared with 5-FU plus cisplatin [13]. The second, a UK 2×2 factorial trial (REAL2), again demonstrated the noninferiority of capecitabine

0959-4973 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e3283094b5d

versus 5-FU when given as part of a triple combination in the first-line treatment of advanced esophago-gastric cancer [14].

TP, thymidylate synthase (TS), and dihydropyrimidine dehydrogenase (DPD) have been studied as predictive factors for treatment with 5-FU and its derivatives [15–20]. The efficacy of capecitabine has been shown to correlate with the ratio of TP to DPD activities in xenograft models of various human cancer cell lines [21]. TP/DPD expression score has been suggested to be a more favorable factor of the efficacy of capecitabine than TP/TS expression score in patients with breast cancer [22]. We have reported a correlation between the protein expression of TP in tumor tissue and the response to 5′-DFUR treatment in patients with gastric cancer [23]. Nishina *et al.* [24] have also investigated the potential correlation between the ratio of TP to DPD protein expression (measured by ELISA) and response to 5′-DFUR in patients with metastatic gastric cancer, although it is unclear whether TP and DPD expression levels influence the effect of capecitabine in gastric cancer patients.

In this study, we evaluated TP and DPD protein levels by newly established immunohistochemical staining techniques with anti-TP and anti-DPD monoclonal antibodies, and investigated the relationship between the levels of expression of these enzymes and the response to capecitabine in patients with advanced/recurrent gastric cancer in a phase II study.

Methods

Patients

A total of 60 patients with previously untreated advanced or recurrent gastric cancer were entered into a phase II study conducted in Japan [25]. Of these, 25 patients who consented to this additional analysis had baseline archived, formalin-fixed, paraffin-embedded specimens of the primary lesion available for immunohistochemical staining. These patients fulfilled the following eligibility criteria for the phase II study: age 20–74 years; Eastern Cooperative Oncology Group (ECOG)/Zubrod performance status 0–2; and adequate bone marrow, hepatic, and renal function. The institutional review board of each participating center approved the study. The demographic characteristics of the patients are summarized in Table 1.

Table 1 Baseline patient characteristics

	No. of patients analyzed (%)	Total sample (%)
Total number of patients	25	60
Median age, years (range)	65 (37–74)	64 (28–74)
Sex (male/female)	19 (76.0)/6 (24.0)	49 (81.6)/11 (18.3)
ECOG performance status		
0	20 (80.0)	44 (73.3)
1	5 (20.0)	15 (25.0)
2	0 (0)	1 (1.7)
Histological type[a]		
Well differentiated (papillary adenocarcinoma, tubular adenocarcinoma1)	3 (12.0)	7 (11.7)
Moderately differentiated (tubular adenocarcinoma2)	9 (36.0)	24 (40.0)
Poorly differentiated (poorly differentiated adenocarcinoma)	10 (40.0)	22 (36.7)
Undifferentiated (signet-ring cell carcinoma)	3 (12.0)	7 (11.7)
Recurrence		
Yes	12 (20.0)	5 (20.0)
No	48 (80.0)	20 (80.0)
Measurable or nonmeasurable lesion		
Primary	13 (52.0)	23 (38.3)
Liver	12 (48.0)	27 (45.0)
Lymph node	16 (64.0)	41 (68.3)
Lung	1 (4.0)	2 (3.3)

ECOG, Eastern Cooperative Oncology Group.
[a]According to Japanese classification of gastric carcinoma [26].

Immunohistochemical staining and staining assessment

Expression levels of TP and DPD were determined using recombinant monoclonal antibodies specific for human TP and DPD (Roche Applied Science, Mannheim, Germany). For negative controls, the primary antibody was replaced by mouse immunoglobulin G (TP) and rat immunoglobulin G (DPD). We obtained between five and 21 sections (approximately 10) per sample. Staining procedure was followed according to the manual for each antibody kit, with the exception of antibody dilutions and staining assessment. The thicknesses of sections varied between institutions (approximately 3–5 μm) but were determined to be representative of the tumor by hematoxylin-eosin staining image.

The paraffin sections were dewaxed in xylene and dehydrated with graded ethanol. Antigen retrieval was achieved by steaming for 20 min followed by cooling for 20 min at room temperature. Endogenous peroxidase was blocked by incubating the preparations with 0.3% hydrogen peroxide in methanol for 5 min. After washing in phosphate-buffered saline (PBS), nonspecific binding was blocked by preincubation with 20% goat serum in antibody dilution buffer (Roche Applied Science, Mannheim, Germany) for 20 min at room temperature. All sections were incubated overnight at 4°C with the primary antibodies in dilution buffer at the following concentrations: anti-TP monoclonal antibody 1 : 800 (0.125 μg/ml); anti-DPD monoclonal antibody 1 : 400 (0.3125 μg/ml). For the anti-DPD monoclonal antibody, the preparations were washed three times with PBS and incubated with rabbit antirat immunoglobulins in antibody dilution buffer (1 : 12800) for 45 min at room temperature. The slides were then washed three times with PBS and incubated with EnVision + (DAKO, Kyoto, Japan) for 30 min at room temperature. After three more washings with PBS, the preparations were incubated with LiquidDAB + (DAKO)

for 20 min at room temperature. The specimens were rinsed with distilled water, counterstained with Meyer's hematoxylin, and mounted.

Two investigators (W.K. and I.O.), who were blinded with respect to the patient's clinicopathological characteristics, assessed the slides (one slide for each antibody staining). Immunostaining was considered positive for TP and DPD if apparent staining of the cytoplasm and/or nuclear compartment was seen in tumor cells, regardless of the number of cells stained. If no staining was observed, the sample was classified as negative. Moreover, evaluation was not influenced even if the stromal cells were stained.

Chemotherapy

Capecitabine was administered orally at a dosage of 828 mg/m^2 twice daily for 3 weeks, followed by 1 week of no treatment. Chemotherapy was continued until disease progression. Tumor response was evaluated according to Criteria for Evaluating the Efficacy of Chemotherapy/Radiation Therapy in the Treatment of Gastric Cancer, issued by the Japanese Research Society for Gastric Cancer [26]. These criteria differ slightly from the World Health Organization criteria as there are five evaluations of response: complete response (CR); partial response (PR); minor response (MR); no change (NC); and progressive disease (PD). However, in the current study MR and NC were grouped together as stable disease so that the tumor response could be compared with other studies using the World Health Organization criteria. Responders (CR + PR) to capecitabine were confirmed by extramural review by an independent response evaluation committee.

Statistical methods

Statistical analyses were performed with Statistical Analysis System (SAS version 8.2; SAS Institute, Cary, North Carolina, USA). The relationship between the expression of TP, DPD and response to chemotherapy was evaluated by Fisher's exact test. Statistical significance was defined as $P < 0.05$.

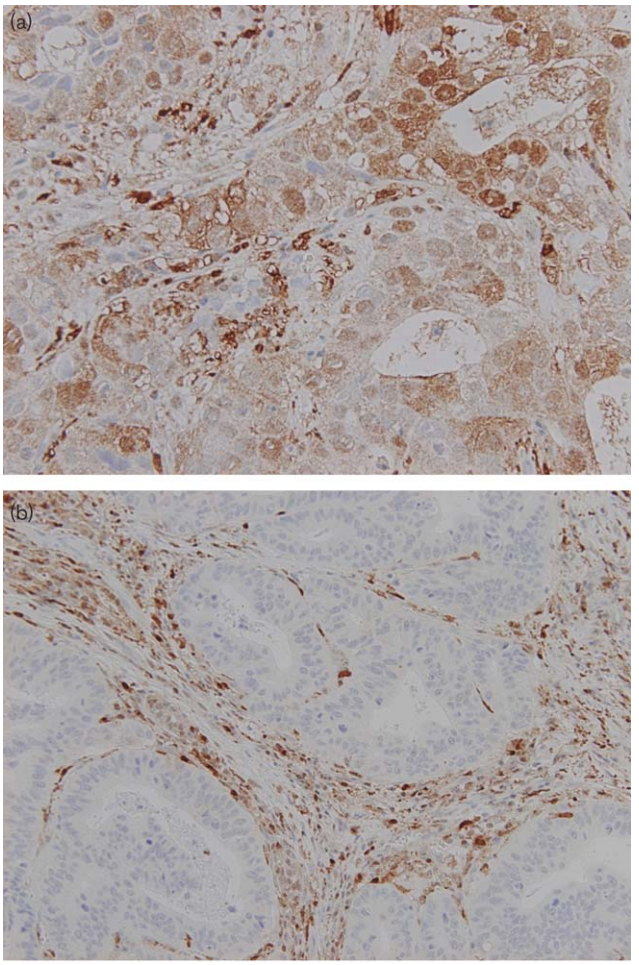
Results

Thymidine phosphorylase and dihydropyrimidine dehydrogenase immunohistochemical staining

TP protein expression in tumor cells was detected in 19 of 25 (76%) specimens. However, TP protein expression in stromal cells was seen in tumor samples from all patients. An example of the pattern of TP expression in both tumor and stromal cells is shown in Fig. 1a and in stromal cells alone in Fig. 1b. Figure 1a also shows that although tumor cells were stained homogeneously, there was some intersite variation evident in the staining.

Expression of DPD protein in tumor cells was detected in 13 of 25 (52%) samples. The pattern of DPD + and DPD – expression is shown in Fig. 2a and b, respectively.

Fig. 1



(a) Immunohistochemical staining of TP expression in both tumor and stromal cells. (b) Immunohistochemical staining of TP expression only in stromal cells. TP, thymidine phosphorylase.

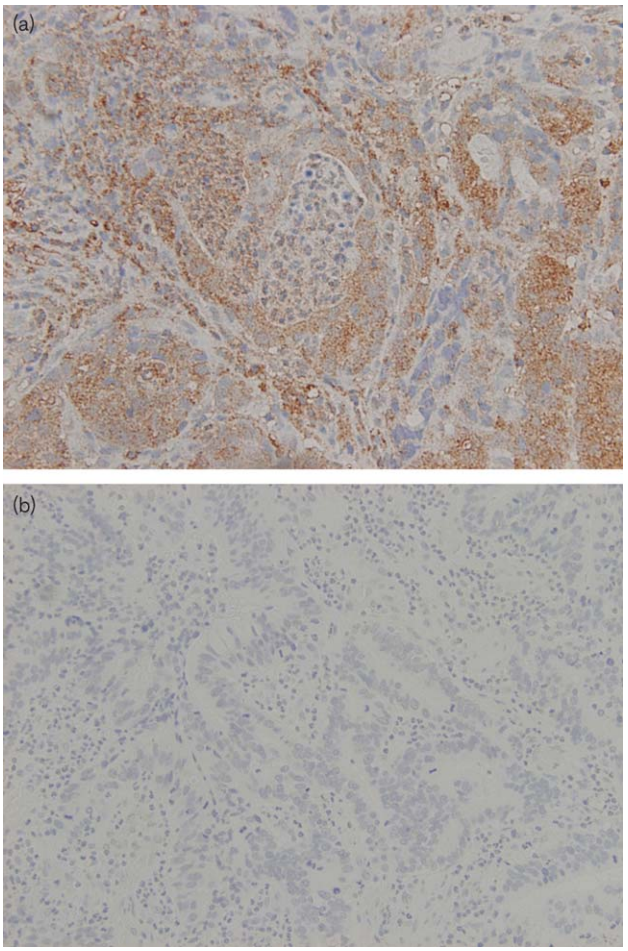
Response to chemotherapy and correlation with immunohistochemical staining

The overall response rate in the patients for whom primary lesion specimens were available was 32% (8/25). The relationships between response rates and the expression levels of each enzyme are detailed in Table 2. The response rate was numerically but not statistically higher (42%) in the 19 patients with TP + tumors compared with 0% in the six patients with TP – tumors ($P = 0.129$); corresponding response rates in patients with DPD + and DPD – tumors were 15% (2/13) and 50% (6/12) ($P = 0.097$), respectively. Response rates were significantly higher (60%) in the 10 patients with TP + and DPD – tumors compared with a 13% response rate in the remaining patients ($P = 0.028$).

Discussion

This study investigated the correlation between TP and DPD expression in the primary tumors of patients with

Fig. 2



(a) Immunohistochemical staining of DPD expression in tumor and stromal cells. (b) Immunohistochemical staining of DPD-negative tumor cells. DPD, dihydropyrimidine dehydrogenase.

Table 2 Relationship between immunohistochemical protein expression levels and patient response

	No. of patients					
	CR	PR	SD	PD	NE	Response rate (%)
TP immunoreactivity						
Positive ($n=19$)	3	5	5	5	1	8 (42)*
Negative ($n=6$)	0	0	2	3	1	0 (0)
DPD immunoreactivity						
Positive ($n=13$)	2	0	5	6	0	2 (15)
Negative ($n=12$)	1	5	2	2	2	6 (50)**
TP and DPD immunoreactivity						
TP+/DPD− ($n=10$)	1	5	2	1	1	6 (60)***
TP+/DPD+ ($n=9$)	2	0	3	4	0	2 (22)
TP−/DPD+ ($n=4$)	0	0	2	2	0	0 (0)
TP−/DPD− ($n=2$)	0	0	0	1	1	0 (0)
Overall response rate	3	5	7	8	2	8 (32)

CR (complete response) = disappearance of evidence of cancer within 4 weeks; DPD, dihydropyrimidine dehydrogenase; NE, not evaluable; PD (progressive disease) $\geqslant$ 25% increase in more than one lesion or the appearance of new lesion(s); PR (partial response) $\geqslant$ 50% reduction in the sum of the products of the perpendicular diameters of all lesions for 4 weeks or more, with no evidence of new lesions or progression of any lesions; SD (stable disease) $\leqslant$ 50% reduction or $<$25% increase in the sum of the products of perpendicular diameters of all lesions, with no evidence of new lesions; TP, thymidine phosphorylase.
*$P=0.129$ (Fisher's exact test).
**$P=0.097$ (Fisher's exact test).
***$P=0.028$ (Fisher's exact test), TP+/DPD− versus other.

advanced/recurrent gastric cancer and the subsequent response to treatment with capecitabine. Other biomarkers including TS are planned to be investigated collectively. However, for the limited specimen we were resigned to selecting those biomarkers in this study.

The response rate of 32% in this subset of patients is 50% higher than in the whole patient population ($n=60$) enrolled in the phase II trial [25]. The difference in response rate is assumed to be the influence of favorable PS.

In our study, 76% and 52% of patients had TP+ or DPD+ tumors, respectively. These rates of expression of pyrimidine-metabolizing enzymes are comparable with previously reported frequencies for TP+ (51–90%) and DPD+ (44%) cells [23,27–30].

The findings of the current study suggest that patients with TP+ and DPD− protein expression in gastric tumors may respond better to capecitabine. With the prior knowledge that TP and DPD are key enzymes in the metabolism of capecitabine to 5-FU and its degradation, there appears to be a correlation between overall response rate and TP/DPD immunostaining in the primary lesion.

Cancer cells expressing high levels of TP are very sensitive to capecitabine and related prodrugs such as 5′-DFUR [31], and TP expression in primary or metastatic tumor tissue has been shown to be associated with response to capecitabine plus irinotecan in patients with colorectal cancer [32]. Tumors with high levels of DPD have been demonstrated to be resistant to 5-FU [33] and capecitabine [34]. The finding that TP+ patients and DPD− patients are more likely responders is in line with these reports.

Combined TP and DPD values was also reported by Nishina *et al.* [24]. They demonstrated that TP/DPD ratios in 5′-DFUR-responding patients were statistically higher than in nonresponder patients. Also human colorectal cancer xenografts showed susceptibility to capecitabine when expressing a high TP/DPD ratio, which reflects high TP and low DPD expression [21].

It is, however, notable that we observed two CRs among patients with TP+ and DPD+ tumors, and yet there

were no responders among those with TP– tumors. Although the difference in response rate between patients with TP+ and TP– tumors was not statistically significant ($P = 0.129$), this result suggests that TP may be the more important predictor of response to capecitabine. This observation is supported by Meropol *et al.* [32] who found that TP protein and gene expression was associated with response to capecitabine plus irinotecan in a slightly larger sample of patients with colorectal cancer ($n = 58$), whereas DPD protein and gene expression was not.

Finding factors that predict sensitivity to anticancer drugs should increase the effectiveness of chemotherapy in the future by identifying potential responders before therapy and by minimizing toxicity in nonresponding patients. Chemopredictive results of TP and DPD biomarker analyses have the potential to offer tailored chemotherapy for patients with gastric cancer. The results of this study support the hypothesis that biomarker profiles may predict a favorable response to capecitabine, although this hypothesis needs to be validated by a larger trial.

Acknowledgements

The authors are grateful to the following investigators who provided specimens from the primary tumor and assisted in this study: Y. Kondo (Sapporo Kousei Hospital), M. Terashima (Fukushima Medical University), T. Akiya (Gunma Cancer Center), T. Ichikura (National Defense Medical College), T. Suzuki (Chiba University School of Medicine), K. Chin (Cancer Institute Hospital), Y. Yamamura (Aichi Cancer Center), K. Kondo (Nagoya National Hospital), H. Takiuchi (Osaka Medical College), and M. Higashino (Osaka City General Hospital).

References

1 Ajiki W, Tsukuma H, Oshima A. Research Group for Population-based Cancer Registration in Japan: Cancer incidence and incidence rates in Japan in 1999: estimates based on data from 11 population-based cancer registries. *Jpn J Clin Oncol* 2004; **34**:352–356.

2 Janunger KG, Hafstrom L, Nygren P, Glimelius B. SBU-group, Swedish Council of Technology Assessment in Health Care: a systematic overview of chemotherapy effects in gastric cancer. *Acta Oncol* 2001; **40**:309–326.

3 Cassidy J, Twelves C, Van Cutsem E, Hoff P, Bajetta E, Boyer M, *et al.* Capecitabine Colorectal Cancer Study Group: first-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. *Ann Oncol* 2002; **13**:566–575.

4 Verso M, Agnelli G. Venous thromboembolism associated with long-term use of central venous catheters in cancer patients. *J Clin Oncol* 2003; **21**:3665–3675.

5 Miwa M, Ura M, Nishida M, Sawada N, Ishikawa T, Mori K, *et al.* 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* 1998; **34**:1274–1281.

6 Schüller J, Cassidy J, Dumont E, Roos B, Durston S, Banken L, *et al.* Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients. *Cancer Chemother Pharmacol* 2000; **45**:291–297.

7 Kono A, Hara Y, Sugata S, Karube Y, Matsushima Y, Ishitsuka H. Activation of 5′-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chem Pharm Bull (Tokyo)* 1983; **31**:175–178.

8 Reigner B, Blesch K, Weidekamm E. Clinical pharmacokinetics of capecitabine. *Clin Pharmacokinet* 2001; **40**:85–104.

9 Van Cutsem E, Hoff PM, Harper P, Bukowski RM, Cunningham D, Dufour P, *et al.* Oral capecitabine vs intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomized, phase III trials. *Br J Cancer* 2004; **90**:1190–1197.

10 Koizumi W, Saigenji K, Ujiie S, Terashima M, Sakata Y, Taguchi T, *et al.* A pilot phase II study of capecitabine in advanced or recurrent gastric cancer. *Oncology* 2003; **64**:232–236.

11 Kim TW, Kang YK, Ahn JH, Chang HM, Yook JH, Oh ST, *et al.* Phase II study of capecitabine plus cisplatin as first-line chemotherapy in advanced gastric cancer. *Ann Oncol* 2002; **13**:1893–1898.

12 Park YH, Ryoo BY, Choi SJ, Kim HT. A phase II study of capecitabine and docetaxel combination chemotherapy in patients with advanced gastric cancer. *Br J Cancer* 2004; **90**:1329–1333.

13 Kang Y, Kang WK, Shin DB, Chen J, Xiong J, Wang J, *et al.* Randomized phase III trial of capecitabine/cisplatin (XP) vs. continuous infusion of 5-FU/cisplatin (FP) as first-line therapy in patients (pts) with advanced gastric cancer (AGC): efficacy and safety results. *J Clin Oncol* 2006; **24** (**Suppl 18**):(abstract LBA4018).

14 Cunningham D, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, *et al.* Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**:36–46.

15 Napieralski R, Ott K, Kremer M, Specht K, Vogelsang H, Becker K, *et al.* Combined GADD45A and thymidine phosphorylase expression levels predict response and survival of neoadjuvant-treated gastric cancer patients. *Clin Cancer Res* 2005; **11**:3025–3031.

16 Takiguchi N, Ishii R, Koda K, Oda K, Miyazaki M. Thymidine phosphorylase expression correlates with malignant potential and anti-tumor effect of doxifluridine on gastric cancer: multivariate analysis for adjuvant chemotherapy doxifluridine vs. 5-fluorouracil. *Oncol Rep* 2003; **10**:1105–1111.

17 Terashima M, Fujiwara H, Takagane A, Abe K, Araya M, Irinoda T, *et al.* Role of thymidine phosphorylase and dihydropyrimidine dehydrogenase in tumour progression and sensitivity to doxifluridine in gastric cancer patients. *Eur J Cancer* 2002; **38**:2375–2381.

18 Lenz HJ, Leichman CG, Danenberg KD, Danenberg PV, Groshen S, Cohen H, *et al.* Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. *J Clin Oncol* 1996; **14**:176–182.

19 Boku N, Chin K, Hosokawa K, Ohtsu A, Tajiri H, Yoshida S, *et al.* Biological markers as a predictor for response and prognosis of unresectable gastric cancer patients treated with 5-fluorouracil and cis-platinum. *Clin Cancer Res* 1998; **4**:1469–1474.

20 Ishikawa Y, Kubota T, Otani Y, Watanabe M, Teramoto T, Kumai K, *et al.* Dihydropyrimidine dehydrogenase and messenger RNA levels in gastric cancer: possible predictor for sensitivity to 5-fluorouracil. *Jpn J Cancer Res* 2000; **91**:105–112.

21 Ishikawa T, Sekiguchi F, Fukase Y, Sawada N, Ishitsuka H. Positive correlation between the efficacy of capecitabine and doxifluridine and the ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase activities in tumors in human cancer xenografts. *Cancer Res* 1998; **58**:685–690.

22 Honda J, Sasa M, Moriya T, Bando Y, Hirose T, Takahashi M, *et al.* Thymidine phosphorylase and dihydropyrimidine dehydrogenase are predictive factors of therapeutic efficacy of capecitabine monotherapy for breast cancer-preliminary results. *J Med Invest* 2008; **55**:54–60.

23 Koizumi W, Saigenji K, Nakamaru N, Okayasu I, Kurihara M. Prediction of response to 5′-deoxy-5-fluorouridine (5′-DFUR) in patients with inoperable advanced gastric cancer by immunostaining of thymidine phosphorylase/platelet-derived endothelial cell growth factor. *Oncology* 1999; **56**:215–222.

24 Nishina T, Hyodo I, Miyaike J, Inaba T, Suzuki S, Shiratori Y. The ratio of thymidine phosphorylase to dihydropyrimidine dehydrogenase in tumour tissues of patients with metastatic gastric cancer is predictive of the clinical response to 5′-deoxy-5-fuorouridine. *Eur J Cancer* 2004; **40**:1566–1571.

25 Sakamoto J, Chin K, Kondo K, Kojima H, Terashima M, Yamamura Y, *et al.* Phase II study of a 4-week capecitabine regimen in advanced or recurrent gastric cancer. *Anticancer Drugs* 2006; **17**:231–236.

26 Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma-2nd English edition-response assessment of chemotherapy and radiotherapy for gastric carcinoma: clinical criteria. *Gastric Cancer* 2001; **4**:1–8.

27 Kikuyama S, Inada T, Shimizu K, Miyakita M. Thymidine phosphorylase expression in gastric cancer in association with proliferative activity and angiogenesis. *Anticancer Res* 2000; **20**:2081–1086.

28 Kimura H, Konishi K, Nukui T, Kaji M, Maeda K, Yabushita K, *et al*. Prognostic significance of expression of thymidine phosphorylase and vascular endothelial growth factor in human gastric carcinoma. *J Surg Oncol* 2001; **76**:31–36.
29 Kikuyama S, Inada T, Shimizu K, Miyakita M, Ogata Y. p53, bcl-2 and thymidine phosphorylase as predictive markers of chemotherapy in patients with advanced and recurrent gastric cancer. *Anticancer Res* 2001; **21**:2149–2153.
30 Miyamoto S, Boku N, Ohtsu A, Yoshida S, Ochiai A, Okabe H, *et al*. Clinical implications of immunoreactivity of thymidylate synthase and dihydropyrimidine dehydrogenase in gastric cancer treated with oral fluoropyrimidine (S-1). Study Group of S-1 for Gastric Cancer. *Int J Oncol* 2000; **17**:653–658.
31 Toi M, Ohashi T, Takatsuka Y, Shin E, Inaji H, Yamamoto Y, *et al*. Selective effect of adjuvant 5′-deoxy-5-fluorouridine treatment for thymidine phosphorylase positive tumors in primary breast cancer. *Proc Am Soc Clin Oncol* 1997; **16**:136a (abstract 481).
32 Meropol NJ, Gold PJ, Diasio RB, Andria M, Dhami M, Godfrey T, *et al*. Thymidine phosphorylase expression is associated with response to capecitabine plus irinotecan in patients with metastatic colorectal cancer. *J Clin Oncol* 2006; **24**:4069–4077.
33 Diasio RB. The role of dihydropyrimidine dehydrogenase (DPD) modulation in 5-FU pharmacology. *Oncology* 1998; **12** (**10 Suppl 7**):23–27.
34 Vallböhmer D, Yang DY, Kuramochi H, Shimizu D, Danenberg KD, Lindebjerg J, *et al*. DPD is a molecular determinant of capecitabine efficacy in colorectal cancer. *Int J Oncol* 2007; **31**:413–418.