Cancer Chemother Pharmacol (2012) 69:439–446
DOI 10.1007/s00280-011-1723-8

ORIGINAL ARTICLE

Phase I study of cediranib in combination with cisplatin plus fluoropyrimidine (S-1 or capecitabine) in Japanese patients with previously untreated advanced gastric cancer

Taroh Satoh · Yasuhide Yamada · Kei Muro · Hidetoshi Hayashi · Yasuhiro Shimada · Daisuke Takahari · Keisei Taku · Takako Eguchi Nakajima · Xiaojin Shi · Kathryn H. Brown · Narikazu Boku

Received: 14 June 2011 / Accepted: 29 July 2011 / Published online: 19 August 2011

Abstract
Purpose The primary objective of this Phase I study was to assess the safety and tolerability of the vascular endothelial growth factor signalling inhibitor cediranib in combination with cisplatin plus an oral fluoropyrimidine, in Japanese patients with previously untreated advanced gastric cancer.
Methods Patients received continuous, once-daily oral doses of cediranib 20 mg in combination with either cisplatin (60 mg/m^2 iv day 1) plus S-1 (40–60 mg bid, days 1–21) every 5 weeks for a maximum of eight cycles [Arm A]; or cisplatin (80 mg/m^2 iv, day 1) plus capecitabine (1,000 mg/m^2 bid, days 1–14) every 3 weeks for a maximum of six cycles [Arm B]. In both arms, the assessment period for dose-limiting toxicities (DLTs) was the first 21 days of cycle 1.
Results Fourteen patients (Arm A, $n = 6$; Arm B, $n = 8$) were enrolled and received at least one dose of cediranib. One patient in each arm experienced a DLT (Arm A; decreased appetite, grade 3; Arm B, decreased appetite, fatigue and hyponatraemia, all grade 3). Overall, the most common adverse events were decreased appetite, fatigue and nausea (all $n = 13$ [92.9%]). Preliminary efficacy evaluation showed one confirmed (Arm A) and three unconfirmed (Arm A, $n = 1$; Arm B, $n = 2$) partial responses that were ongoing at data cut-off.
Conclusions Cediranib 20 mg/day in combination with cisplatin and S-1 or capecitabine was tolerable, with no new toxicities identified, and showed preliminary evidence of antitumour activity.

Keywords Cediranib · VEGF signalling · Phase I · Gastric cancer · Japanese

T. Satoh (✉) · H. Hayashi
Kinki University School of Medicine, Osaka, Japan
e-mail: taroh@cfs.med.osaka-u.ac.jp

Present Address:
T. Satoh
Department of Frontier Science for Cancer and Chemotherapy, Osaka University Graduate School of Medicine, 2-15 Yamadaoka Suita City, Osaka 565-0871, Japan

Y. Yamada · Y. Shimada
National Cancer Centre Hospital, Tokyo, Japan

K. Muro · D. Takahari
Aichi Cancer Centre Hospital, Aichi, Japan

K. Taku
Shizuoka Cancer Centre, Shizuoka, Japan

T. E. Nakajima · N. Boku
St. Marianna University School of Medicine, Kanagawa, Japan

X. Shi
AstraZeneca KK, Osaka, Japan

K. H. Brown
AstraZeneca, Alderley Park, Macclesfield, UK

Introduction

Gastric cancer is the most common malignancy in Japan. GLOBOCAN figures revealed that in 2008, there were 102,040 new cases of gastric cancer, and 50,156 deaths were attributed to this disease in Japan [1]. The only curative treatment is surgery, however, over half of patients present with inoperable tumours. For those patients with unresectable tumours and receiving best supportive care, outcomes are extremely poor with median survival times ranging from 3 to 5 months [2–4].

Combination chemotherapy regimens with platinum-based cisplatin plus an oral fluoropyrimidine are commonly used as first-line treatment for advanced gastric cancer in Japan [5]. This treatment regimen is based on early-phase clinical trials that showed cisplatin in combination with 5-fluorouracil (5-FU) or oral fluoropyrimidines yielded overall response rates of approximately 40% and median survival times of 7–13 months [6–10].

Vascular endothelial growth factor (VEGF) plays an essential role in the formation and maintenance of tumour vasculature [11]. The addition of bevacizumab, an anti-VEGF-A antibody, to standard chemotherapy has demonstrated clinical benefit in patients with advanced colorectal cancer [12–14] and non-small-cell lung cancer [15].

Cediranib is an oral, highly potent VEGF signalling inhibitor with activity against all three VEGF receptors [16, 17]. Initial clinical evaluation of cediranib monotherapy demonstrated that it is suitable for once-daily oral dosing in Japanese [18] and Western [19] patients, with biological activity at doses ≥20 mg/day [19]. Subsequent Phase I studies showed that cediranib 30 mg/day was generally well tolerated in combination with various standard anticancer treatments, with encouraging preliminary evidence of antitumour activity [20–23]. However, when the protocol for the present study was being developed, emerging data from Phase II and III trials indicated that cediranib 20 mg was the highest tolerable dose suitable for chronic once-daily dosing in combination with chemotherapy, with higher doses not considered to be more effective [24, 25]. Consequently, the dose of cediranib selected for this combination study was 20 mg/day. The primary objective of the current Phase I study (ClinicalTrials.gov, number NCT00960349) was to assess the safety and tolerability of cediranib 20 mg/day in combination with capecitabine/cisplatin or S-1/cisplatin in Japanese patients with previously untreated advanced gastric cancer.

Methods

Patients

Japanese patients ≥20 years of age with histologically or cytologically confirmed previously untreated recurrent or metastatic unresectable gastric adenocarcinoma were eligible for inclusion. Patients were required to have a life expectancy ≥12 weeks and a World Health Organization performance status of 0 or 1. The main exclusion criteria were as follows: significant respiratory, cardiac, hepatic or renal dysfunction; unstable brain metastases; poorly controlled hypertension; significant haemorrhage (>30 ml bleeding/episode in the previous 3 months) or haemoptysis (>5 ml fresh blood in the previous 4 weeks); arterial thromboembolic events in the previous 12 months; history of other malignancies within the previous 5 years; any unresolved toxicity according to Common Terminology Criteria for Adverse Events (CTCAE) from prior radiotherapy; recent (<14 days) major thoracic or abdominal surgery; and incomplete recovery from prior surgery. All patients provided written informed consent. The study was approved by the institutional review board at each participating centre and conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and the AstraZeneca policy on Bioethics [26].

Study design

This was a multicentre, open-label, non-randomized, Phase I study. Eligible patients received cediranib 20 mg/day orally (starting on day 2 in cycle 1) in combination with either cisplatin (60 mg/m^2 intravenous [iv], day 1) plus S-1 (40–60 mg orally twice daily, days 1–21) [Arm A] or cisplatin (80 mg/m^2 iv, day 1) plus capecitabine (1,000 mg/m^2 orally twice daily, days 1–14) [Arm B] (Fig. 1). One cycle of treatment in Arm A was 5 weeks, and one cycle of treatment in Arm B was 3 weeks. The rest periods in Arms A (2 weeks) and B (1 week) were consistent with standard clinical practice for administration of S-1 and capecitabine, respectively. The chemotherapy treatments in Arms A and B were continued for a maximum of eight and six cycles, respectively. Thereafter, treatment of cediranib plus S-1/capecitabine could be continued until a discontinuation criterion was met. Patients were initially entered into Arm A. Following enrolment of six patients into Arm A, patients were then entered into Arm B.

The primary study objective was to assess the safety and tolerability of cediranib in combination with S-1/cisplatin or capecitabine/cisplatin. After entry of six evaluable patients in each arm, a safety review committee (SRC) discussed whether the regimen was tolerated. The treatment was considered tolerable if ≤1 of the six patients experienced a DLT. If 2–3 of the six patients experienced a DLT, either the SRC recommended the combination was tolerated or the cohort was expanded to include three further evaluable patients. If ≥4 patients experienced a DLT, the treatment was considered intolerable.

In both arms, a DLT was any toxicity considered related to study drug that commenced within the first 21 days of cycle 1 and met any of the following criteria: hypertension or diarrhoea that required cessation of cediranib treatment; an absolute neutrophil count <500/mm^3 for ≥5 days despite growth factor support; a platelet count <50,000/mm^3 for ≥5 days; a dose delay to starting any chemotherapy agent in cycle 2 for longer than 14 days; dose reductions of cediranib due to cediranib-related toxicity; a single increase from baseline in the QT interval corrected for heart rate

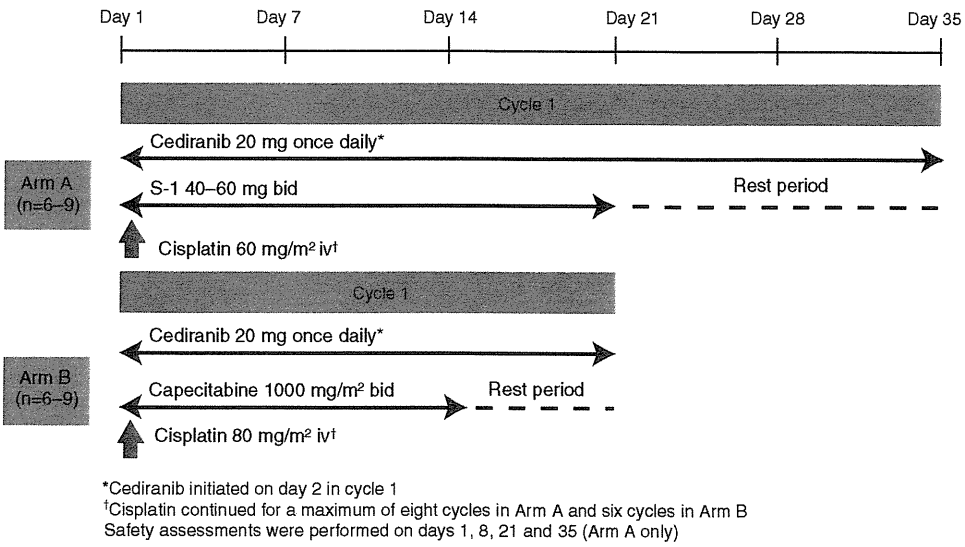

Fig. 1 Study design

*Cediranib initiated on day 2 in cycle 1
†Cisplatin continued for a maximum of eight cycles in Arm A and six cycles in Arm B
Safety assessments were performed on days 1, 8, 21 and 35 (Arm A only)

(QTc) of 60 ms that results in a QTc of at least 460 ms; two QTc measurements >490 ms taken at least 24 h apart; and any other CTCAE grade ≥3 that was, in the opinion of the investigator and the SRC, not clearly related to disease progression, clinically significant and related to the study drug.

Secondary objectives were to determine the steady-state pharmacokinetics (PK) of cediranib alone and in combination with chemotherapy and to investigate the potential effect of cediranib on the PK of the chemotherapy components (cisplatin and S-1/capecitabine [5-FU]). An exploratory objective was to assess the preliminary efficacy of the combination regimens by measurement of tumour response according to the Response Evaluation Criteria In Solid Tumours (RECIST version 1.0) [27].

Assessment of safety and tolerability

After a full physical examination at enrolment, toxicity was monitored throughout the study by the assessment of adverse events (AEs), which were graded according to CTCAE version 3.0. Vital signs (blood pressure [BP], pulse rate and body temperature) were measured, electrocardiograms recorded and samples taken for clinical chemistry, haematology assessment and urinalysis at the screening visit and on days 1, 8 and 21 in both arms; patients in Arm A repeated these assessments on day 35.

Pharmacokinetic assessment

To evaluate steady-state cediranib PK, blood samples were taken immediately before and 1, 2, 4, 6, 8 and 24 h after cediranib treatment on the final day of cycle 1 (cediranib alone) and day 1 of cycle 2 (presence of chemotherapy). To evaluate S-1/capecitabine (5-FU) PK, blood samples were collected immediately before and 0.5, 1, 2, 4, 6 and 8 h after S-1/capecitabine treatment on day 1 of cycle 1 (absence of cediranib) and day 1 of cycle 2 (presence of cediranib). To evaluate cisplatin PK, blood samples were taken pre-dose; 5 min before the end of the 2-h iv infusion; and 2.5, 3, 4, 6, 8 and 24 h post start of infusion on day 1 of cycle 1 (absence of cediranib) and day 1 of cycle 2 (presence of cediranib).

Plasma concentrations of cediranib, capecitabine (5-FU only), S-1 (5-FU only) and cisplatin (total platinum equivalents) were determined using high-performance liquid chromatography with mass spectrometry (LC–MS/MS). PK parameters were calculated using standard non-compartmental analysis.

Assessment of tumour response

Objective tumour assessments determined by RECIST were performed every 12 weeks from the start of treatment until disease progression, death or discontinuation of cediranib due to any other reason.

Results

Patient characteristics

Between August and December 2009, 14 patients were recruited into Arm A ($n = 6$) or Arm B ($n = 8$). Patient demographic and baseline characteristics are summarized in Table 1. At data cut-off (4 January 2010), three patients in Arm A and five patients in Arm B were still receiving cediranib, and one patient in Arm B continued to receive capecitabine and cisplatin. The reasons for discontinuation of cediranib treatment were clinical disease progression

Table 1 Patient demographics and baseline characteristics

Characteristics	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Age, years			
Median	59.5	60.5	60.5
Range	53–71	27–72	27–72
Sex, *n* (%)			
Male	4 (66.7)	5 (62.5)	9 (64.3)
Female	2 (33.3)	3 (37.5)	5 (35.7)
WHO performance status, *n* (%)			
0	3 (50.0)	4 (50.0)	7 (50.0)
1	3 (50.0)	4 (50.0)	7 (50.0)
Number of metastatic sites (%)			
1	1 (16.7)	0	1 (7.1)
>1	5 (83.3)	8 (100.0)	13 (92.9)
Recurrence, *n* (%)	0	1 (12.5)	1 (7.1)
Stage IV, *n* (%)	6 (100)	7 (87.5)	13 (92.9)
Measurable target lesion, *n* (%)	5 (83.3)	6 (75.0)	11 (78.6)
Histology, *n* (%)			
Adenocarcinoma (intestinal)	1 (16.7)	3 (37.5)	4 (28.6)
Adenocarcinoma (diffuse)	1 (16.7)	0	1 (7.1)
Tubular adenocarcinoma	3 (50.0)	2 (25.0)	5 (35.7)
Signet ring carcinoma	1 (16.7)	3 (37.5)	4 (28.6)

WHO World Health Organization

(Arms A and B, $n = 1$), AEs (Arms A and B, $n = 1$) and withdrawal of consent (Arm A, $n = 1$). One patient in Arm B was revealed ineligible at cycle 2 due to a pulmonary embolism at baseline; this patient discontinued study treatment but was included in safety analyses.

Safety and tolerability

All patients received at least one dose of cediranib and were therefore evaluable for safety. The median (range) daily cediranib dose was 16.0 (12.9–20.0) mg in Arm A and 15.9 (13.7–20.0) mg in Arm B, and median (range) duration of actual exposure to cediranib was 72.5 days (13–127) for Arm A and 38.5 days (13–62) for Arm B. The median (range) number of chemotherapy cycles received was 2.5 (1–4) for both arms.

Overall, 12 (86%) [Arm A, $n = 5$; Arm B, $n = 7$] patients experienced one or more cediranib dose interruptions, with one patient from each arm having a dose reduction to 15 mg/day. All six patients in Arm A experienced a dose reduction or interruption of S-1 and seven patients (87.5%) in Arm B experienced a dose reduction or interruption of capecitabine. Five patients in each arm (Arm A, 83.3%; Arm B, 62.5%) had a dose reduction or dose delay of cisplatin. Two patients in Arm A (alopecia, $n = 1$; diarrhoea, stomatitis, fatigue, decreased appetite and hyponatraemia, $n = 1$) and one patient in Arm B (diarrhoea, fatigue, decreased appetite and hypomagnesaemia) experienced AEs that led to permanent discontinuation of cediranib treatment.

Table 2 Most common adverse events (incidence > 30% in total population)

AE, preferred term	All grades, *n* (%)		
	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Decreased appetite	5	8	13 (92.9)
Fatigue	5	8	13 (92.9)
Nausea	5	8	13 (92.9)
Constipation	3	7	10 (71.4)
Diarrhoea	5	5	10 (71.4)
Stomatitis	4	6	10 (71.4)
Hypertension	3	6	9 (64.3)
Weight decreased	5	4	9 (64.3)
Neutropenia	5	3	8 (57.1)
Vomiting	3	5	8 (57.1)
Alopecia	2	4	6 (42.9)
Dysphonia	2	4	6 (42.9)
Hiccups	1	4	5 (35.7)
Leukopenia	3	2	5 (35.7)
Proteinuria	3	2	5 (35.7)

AE adverse event

DLTs were reported in one patient in Arm A (decreased appetite, grade 3) and one patient in Arm B (decreased appetite, fatigue and hyponatraemia; all grade 3). In Arm A, the investigator assessed that decreased appetite was related to S-1 and/or cisplatin. In Arm B, the investigator judged decreased appetite and hyponatraemia related to cediranib, S-1 and cisplatin, and stomatitis related to cediranib and S-1. The SRC decided neither DLT warranted cohort expansion for further evaluation of safety.

The most commonly reported AEs were decreased appetite, fatigue and nausea (all $n = 13$ [92.9%]) [Table 2]. Five (83%) patients in Arm A and six (75%) patients in Arm B experienced AEs grade ≥3 (Table 3). Hypertension was reported as an AE in nine patients (Arm A, $n = 3$; Arm B, $n = 6$), only one (Arm B) of which was

Table 3 Any CTCAE grade ≥3 adverse events

	Grade	Cediranib + S-1 + cisplatin ($n = 6$)	Cediranib + capecitabine + cisplatin ($n = 8$)	Total ($n = 14$)
Neutropenia	3	3	2	5 (35.7)
Hypokalaemia	3	0	3	3 (21.4)
Hyponatraemia	3	1	2	3 (21.4)
Decreased appetite	3	1	1	2 (14.3)
Fatigue	3	0	2	2 (14.3)
Anaemia	3	0	1	1 (7.1)
Diarrhoea	3	1	0	1 (7.1)
Haemoglobin decreased	3	1	0	1 (7.1)
Hyperbilirubinaemia	3	0	1	1 (7.1)
Hyperglycaemia	3	0	1	1 (7.1)
Hypertension	3	0	1	1 (7.1)
Hypomagnesaemia	3	0	1	1 (7.1)
Platelet count decreased	3	1	0	1 (7.1)
Pulmonary embolism	4	0	1	1 (7.1)
Stomatitis	3	1	0	1 (7.1)
Syncope	4	1	0	1 (7.1)
White blood cell count decreased	3	1	0	1 (7.1)
Wound infection	3	1	0	1 (7.1)

grade 3; no action was taken regarding dose adjustment. One patient in Arm A experienced grade 4 transient syncope on day 6, cycle 2. A head computed tomography (CT) scan showed no cerebral haemorrhage and the syncope resolved on the same day it appeared. The investigator considered this event to be related to cediranib, S-1 and cisplatin. One patient from Arm B experienced a grade 4 pulmonary embolism that was identified on day 18, cycle 2 after the patient complained of chest pain. After careful review of the baseline CT scan, the pulmonary embolism was found to be pre-existing at study entry. The investigator judged the event as worsening of the pulmonary embolism related to cediranib, capecitabine and cisplatin. Increases in thyroid stimulating hormone were observed in both arms, but free T4 and T3 remained within normal limits for the majority of these patients. Increases were observed in alanine aminotransferase and aspartate aminotransferase in both arms, but most values were generally within the normal ranges. There were no clinically relevant results related to electrocardiogram, physical findings or other safety observations.

Five serious AEs (SAEs) were reported in three patients in Arm A (decreased appetite, $n = 2$; hyponatraemia, $n = 1$; stomatitis, $n = 1$; syncope, $n = 1$), and in addition to the pulmonary embolism in one patient, three other SAEs were reported in a separate patient in Arm B (decreased appetite, hyponatraemia and fatigue). All SAEs, except for the pulmonary embolism, had resolved by data cut-off. There were no deaths in the period to data cut-off in either arm.

Pharmacokinetics

A summary of PK parameters for cediranib, cisplatin and S-1/capecitabine is shown in Table 4. Only six patients (Arm A, $n = 2$; Arm B, $n = 4$) were evaluable for PK analysis, having completed the planned sampling schedule; therefore, limited data were available for within-patient comparison. In Arm A ($n = 2$), the PK parameters for S-1 in combination with both cediranib and cisplatin were similar to those for S-1 when administered with cisplatin alone, and the PK parameters for cediranib were similar in the presence and absence of chemotherapy; however, there were insufficient data to draw meaningful conclusions on the PK in Arm A. Based on limited data from Arm B ($n = 4$), the cediranib PK parameters were similar in the absence and presence of capecitabine/cisplatin. The PK profile of capecitabine was generally similar in the absence and presence of cediranib; one patient (patient 4 in Table 4) had a higher exposure in the presence of cediranib, but the reason for this is not clear as no interaction would be expected. In all patients (Arms A and B), slight increases in exposure to cisplatin (total platinum equivalents; maximum plasma concentration [C_{max}] and area under plasma concentration–time curve from time zero to 8 h [AUC_{0-8h}]) were observed when cediranib was administered with chemotherapy compared with chemotherapy alone; however, samples collected in the absence of cediranib were obtained following single-dose cisplatin, whereas those collected in the presence of cediranib were obtained following multiple-dose cisplatin.

Efficacy

Seven patients (Arm A, $n = 4$; Arm B, $n = 3$) had a post-baseline scan and were therefore evaluable for efficacy. Tumour shrinkage was observed in five of these patients (Fig. 2); the mean largest change from baseline was −41.8% in Arm A ($n = 4$) and −26.3% in Arm B ($n = 3$). One patient in Arm A had a partial response that was ongoing at data cut-off (duration >79 days). Among the four patients with stable disease ($n = 2$ in each arm), three had unconfirmed partial responses at data cut-off. One patient in each arm had a best response of progressive disease.

Table 4 Summary of pharmacokinetic parameters

Analyte	Patient	Combination	C_{max}, ng/ml	AUC, ng h/ml
Arm A				
Cediranib	Patient 1	Cediranib alone	25.5	378
		Cediranib + S-1 + cisplatin	51.3	598
	Patient 2	Cediranib alone	153	2,640
		Cediranib + S-1 + cisplatin	192	2,780
5-FU	Patient 1 (60 mg S-1)	S-1 + cisplatin	58.6	302
		Cediranib + S-1 + cisplatin	92.1	446
	Patient 2 (50 mg S-1)	S-1 + cisplatin	182	908
		Cediranib + S-1 + cisplatin	130	644
Cisplatin	Patient 1	S-1 + cisplatin	2,740	12,700
		Cediranib + S-1 + cisplatin	3,040	14,100
	Patient 2	S-1 + cisplatin	2,400	10,400
		Cediranib + S-1 + cisplatin	2,790	12,600
Arm B				
Cediranib	All patients ($n = 4$)	Cediranib alone	77.5 (32.9–99.9)	1,180 (479–1,800)
	All patients ($n = 4$)	Cediranib + capecitabine + cisplatin	86.3 (50.2–115)	1,220 (687–1,850)
5-FU	Patient 3 (1,600 mg capecitabine)	Capecitabine + cisplatin	130	283
		Cediranib + capecitabine + cisplatin	284	421
	Patient 4 (1,750 mg capecitabine)	Capecitabine + cisplatin	132	187
		Cediranib + capecitabine + cisplatin	983	889
	Patient 5 (1,450 mg capecitabine)	Capecitabine + cisplatin	167	305
		Cediranib + capecitabine + cisplatin	105[a]	335[a]
	Patient 6 (1,600 mg capecitabine)	Capecitabine + cisplatin	287	518
		Cediranib + capecitabine + cisplatin	392[b]	647[b]
Cisplatin	All patients ($n = 4$)	Capecitabine + cisplatin	3,430 (2,720–3,840)	16,900 (13,500–18,900)
	All patients ($n = 4$)	Cediranib + capecitabine + cisplatin	4,620 (3,230–5,720)	21,700 (16,600–23,600)

AUC_{0-24h} was calculated for cediranib; AUC_{0-4h} for capecitabine (5-FU); and AUC_{0-8h} for cisplatin and S-1 (5-FU)

In Arm B, cediranib and cisplatin parameters are expressed as mean (min–max); all other data are individual patient values as there are insufficient data to summarize by mean value

AUC area under the plasma concentration–time curve, C_{max} maximum plasma (peak) drug concentration

[a] Dose of 1,300 mg capecitabine administered: data dose normalized to 1,450 mg

[b] Dose of 1,200 mg capecitabine administered: data dose normalized to 1,600 mg

Discussion

The impact of conventional chemotherapy on advanced gastric cancer remains modest, with median survival times reaching a plateau of 7–13 months [6–8]. More effective treatment options are needed. In this Phase I study, we evaluated the VEGF signalling inhibitor cediranib in combination with cisplatin and S-1 or capecitabine in Japanese patients with previously untreated locally advanced or metastatic unresectable gastric adenocarcinoma. Treatment was tolerable, with only one patient in each arm experiencing a DLT. Overall, the safety profile of each regimen was consistent with previous studies of the individual agents in patients with advanced cancer [8, 9, 18, 19, 23, 28–30], and no new toxicities were identified. The most commonly reported AEs were decreased appetite, fatigue and nausea. There were no reports of severe hypertension as a SAE, and the overall incidence of hypertension was consistent with that reported in a Phase I study of cediranib monotherapy in Japanese patients [18].

Insufficient PK data preclude any meaningful conclusions relating to Arm A. Based on the limited PK data from Arm B, there was no clear indication of a consistent interaction between cediranib and cisplatin/capecitabine. This is not unexpected as it is considered unlikely that cisplatin, capecitabine or S-1 would affect cediranib routes of metabolism [31]. The slight increases in cisplatin exposure observed in all patients when cediranib was administered with chemotherapy compared to chemotherapy alone may be due to an accumulation of platinum following multiple dosing.

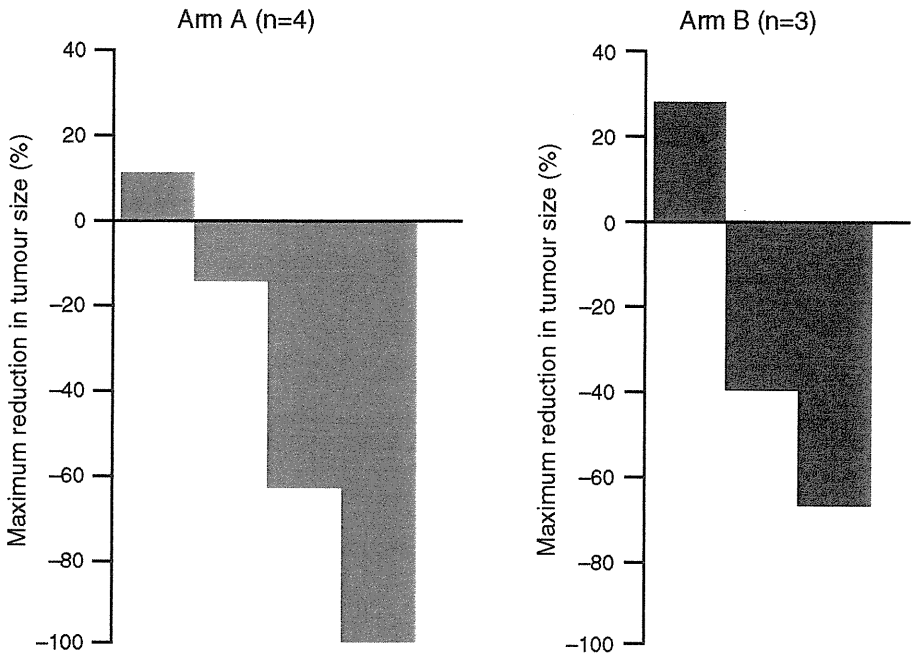

Fig. 2 Waterfall plot for best change in tumour size in each patient

In this small Phase I study, tumour shrinkage was observed in five of seven evaluable patients. This preliminary evidence of antitumour activity is consistent with the efficacy findings observed in an early-phase dose-finding study of sorafenib, a multi-targeted kinase inhibitor with activity versus VEGFR-2 and -3, in combination with capecitabine and cisplatin as a first-line treatment for patients with advanced gastric cancer [32]. However, targeting VEGF signalling with bevacizumab, an anti-VEGF-A monoclonal antibody, in patients with advanced gastric cancer met with disappointing results in the recently reported Phase III AVAGAST study [33]. This first-line study failed to meet its primary endpoint of improved overall survival with the addition of bevacizumab to cisplatin plus capecitabine/5-FU, although an efficacy analysis by geographical region revealed that, for both arms, median overall survival was greatest for patients who enrolled in the Asia/Pacific region. Despite the primary outcome of the AVAGAST study, the bevacizumab regimen showed significant advantages for the secondary efficacy endpoints of progression-free survival and overall response rate, suggesting that anti-VEGF treatment strategies are worthy of continued investigation in advanced gastric cancer.

In conclusion, cediranib 20 mg plus cisplatin and S-1 or capecitabine had a manageable tolerability profile as a first-line treatment in Japanese patients with advanced gastric cancer and showed preliminary evidence of antitumour activity.

Acknowledgments Funding for this study was provided by AstraZeneca. We thank Paul Williams, PhD, from Mudskipper Bioscience, who provided medical writing assistance funded by AstraZeneca.

Conflict of interest X.S. and K.H.B. are employees of AstraZeneca and own stock. T.S., Y.Y, K.M., H.H., Y.S., D.T., K.T., T.E.N. and N.B. declare no conflicts of interest.

References

1. GLOBOCAN statistics. 2008. Available at http://globocan.iarc.fr/
2. Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA, Rausch M (1993) Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. Cancer 72:37–41
3. Glimelius B, Hoffman K, Haglund U, Nyren O, Sjoden PO (1994) Initial or delayed chemotherapy with best supportive care in advanced gastric cancer. Ann Oncol 5:189–190
4. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M (1995) Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. Br J Cancer 71: 587–591
5. Fujii M, Kochi M, Takayama T (2010) Recent advances in chemotherapy for advanced gastric cancer in Japan. Surg Today 40:295–300
6. Ohtsu A, Shimada Y, Yoshida S, Saito H, Seki S, Morise K, Kurihara M (1994) Phase II study of protracted infusional 5-fluorouracil combined with cisplatinum for advanced gastric cancer: report from the Japan clinical oncology group (JCOG). Eur J Cancer 30A:2091–2093
7. Koizumi W, Kurihara M, Sasai T, Yoshida S, Morise K, Imamura A, Akazawa S, Betsuyaku T, Ohkubo S, Takahashi H et al (1993) A phase II study of combination therapy with 5′-deoxy-5-fluorouridine and cisplatin in the treatment of advanced gastric cancer with primary foci. Cancer 72:658–662
8. Koizumi W, Tanabe S, Saigenji K, Ohtsu A, Boku N, Nagashima F, Shirao K, Matsumura Y, Gotoh M (2003) Phase I/II study of S-1 combined with cisplatin in patients with advanced gastric cancer. Br J Cancer 89:2207–2212

9. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M (2008) S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 9:215–221
10. Van Cutsem E, Kang Y, Chung H, Shen L, Sawaki A, Lordick F, Hill J, Lehle M, Feyereislova A, Bang Y (2009) Efficacy results from the ToGA trial: a phase III study of trastuzumab added to standard chemotherapy (CT) in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC). J Clin Oncol 27(18S):abst LBA4509
11. Ferrara N (2002) Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol 29:10–14
12. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
13. Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E, Sarkar S (2005) Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. J Clin Oncol 23: 3706–3712
14. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J (2008) Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 26:2013–2019
15. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542–2550
16. Heckman CA, Holopainen T, Wirzenius M, Keskitalo S, Jeltsch M, Wedge SR, Jürgensmeier JM (2007) Inhibition of VEGF-C-induced VEGFR-3 activity and lymphatic endothelial cell function by the tyrosine kinase inhibitor AZD2171. Proc Am Assoc Cancer Res 48:abst 2999
17. Wedge SR, Kendrew J, Hennequin LF, Valentine PJ, Barry ST, Brave SR, Smith NR, James NH, Dukes M, Curwen JO, Chester R, Jackson JA, Boffey SJ, Kilburn LL, Barnett S, Richmond GH, Wadsworth PF, Walker M, Bigley AL, Taylor ST, Cooper L, Beck S, Jürgensmeier JM, Ogilvie DJ (2005) AZD2171: a highly potent, orally bioavailable, vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for the treatment of cancer. Cancer Res 65:4389–4400
18. Yamamoto N, Tamura T, Yamamoto N, Yamada K, Yamada Y, Nokihara H, Fujiwara Y, Takahashi T, Murakami H, Boku N, Yamazaki K, Puchalski TA, Shin E (2009) Phase I, dose escalation and pharmacokinetic study of cediranib (RECENTIN), a highly potent and selective VEGFR signaling inhibitor, in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 64:1165–1172
19. Drevs J, Siegert P, Medinger M, Mross K, Strecker R, Zirrgiebel U, Harder J, Blum H, Robertson J, Jürgensmeier JM, Puchalski TA, Young H, Saunders O, Unger C (2007) Phase I clinical study of AZD2171, an oral vascular endothelial growth factor signaling inhibitor, in patients with advanced solid tumors. J Clin Oncol 25:3045–3054
20. Laurie SA, Gauthier I, Arnold A, Shepherd FA, Ellis PM, Chen E, Goss G, Powers J, Walsh W, Tu D, Robertson J, Puchalski TA, Seymour L (2008) Phase I and pharmacokinetic study of daily oral AZD2171, an inhibitor of vascular endothelial growth factor tyrosine kinases, in combination with carboplatin and paclitaxel in patients with advanced non-small-cell lung cancer: the National Cancer Institute of Canada clinical trials group. J Clin Oncol 26:1871–1878
21. Chen E, Jonker D, Gauthier I, Maclean M, Wells J, Powers J, Seymour L (2009) Phase I study of cediranib in combination with oxaliplatin and infusional 5-fluorouracil in patients with advanced colorectal cancer. Clin Cancer Res 15:1481–1486
22. Goss G, Shepherd FA, Laurie S, Gauthier I, Leighl N, Chen E, Feld R, Powers J, Seymour L (2009) A phase I and pharmacokinetic study of daily oral cediranib, an inhibitor of vascular endothelial growth factor tyrosine kinases, in combination with cisplatin and gemcitabine in patients with advanced non-small cell lung cancer: a study of the National Cancer Institute of Canada Clinical Trials Group. Eur J Cancer 45:782–788
23. LoRusso P, Shields AF, Gadgeel S, Vaishampayan U, Guthrie, T, Puchalski T, Xu J, Liu Q (2010) Cediranib in combination with various anticancer regimens: results of a phase I multi-cohort study. Invest New Drugs. doi:10.1007/s10637-010-9484-5. Epub ahead of print
24. Goss GD, Arnold A, Shepherd FA, Dediu M, Ciuleanu TE, Fenton D, Zukin M, Walde D, Laberge F, Vincent MD, Ellis PM, Laurie SA, Ding K, Frymire E, Gauthier I, Leighl NB, Ho C, Noble J, Lee CW, Seymour L (2010) Randomized, double-blind trial of carboplatin and paclitaxel with either daily oral cediranib or placebo in advanced non-small-cell lung cancer: NCIC clinical trials group BR24 study. J Clin Oncol 28:49–55
25. Robertson JD, Botwood NA, Rothenberg ML, Schmoll H-J (2009) Phase III trial of FOLFOX plus bevacizumab or cediranib (AZD2171) as first-line treatment of patients with metastatic colorectal cancer: HORIZON III. Clin Colorectal Cancer 8:59–60
26. AstraZeneca (2011) Global policy: bioethics. Available at http://www.astrazeneca.com/Responsibility/Code-policies-standards/Policiesstandards
27. Therasse P (2002) Measuring the clinical response. What does it mean? Eur J Cancer 38:1817–1823
28. vanCruijsen H, Voest EE, van Herpen CML, Hoekman K, Witteveen PO, Tjin-A-Ton ML, Punt CJ, Puchalski T, Milenkova T, Giaccone G (2006) Phase I evaluation of AZD2171, a highly potent, selective VEGFR signaling inhibitor, in combination with gefitinib, in patients with advanced tumors. J Clin Oncol 24(S18):abst 3017
29. Lee SS, Lee JL, Ryu MH, Chang HM, Kim TW, Kang HJ, Kim WK, Lee JS, Kang YK (2007) Combination chemotherapy with capecitabine (X) and Cisplatin (P) as first line treatment in advanced gastric cancer: experience of 223 patients with prognostic factor analysis. Jpn J Clin Oncol 37:30–37
30. Lee JO, Lee KW, Oh DY, Kim JH, Im SA, Kim TY, Bang YJ (2009) Combination chemotherapy with capecitabine and cisplatin for patients with metastatic hepatocellular carcinoma. Ann Oncol 20:1402–1407
31. Schulz-Utermoehl T, Spear M, Pollard CR, Pattison C, Rollison H, Sarda S, Ward M, Bushby N, Jordan A, Harrison M (2010) In vitro hepatic metabolism of cediranib, a potent vascular endothelial growth factor tyrosine kinase inhibitor: interspecies comparison and human enzymology. Drug Metab Dispos 38:1688–1697
32. Kim C, Lee J-L, Choi YH, Kang BW, Ryu M-H, Chang HM, Kim TW, Kang Y-K (2011) Phase I dose-finding study of sorafenib in combination with capecitabine and cisplatin as a first-line treatment in patients with advanced gastric cancer. Invest New Drugs. doi:10.1007/s10637-010-9531-2. Epub ahead of print
33. Kang Y, Ohtsu A, Van Cutsem E, Rha SY, Sawaki A, Park P, Lim H, Wu J, Langer B, Shah MA (2010) AVAGAST: a randomized, double-blind, placebo-controlled, phase III study of first-line capecitabine and cisplatin plus bevacizumab or placebo in patients with advanced gastric cancer (AGC). J Clin Oncol 28(18S):abst LBA4007

ANTICANCER RESEARCH *31*: 3535-3542 (2011)

Docetaxel plus 5-Fluorouracil and Cisplatin (DCF) Induction Chemotherapy for Locally Advanced Borderline-resectable T4 Esophageal Cancer

TOMOYA YOKOTA[1], SHUNZO HATOOKA[2], TAKASHI URA[1], TETSUYA ABE[2], DAISUKE TAKAHARI[1], KOHEI SHITARA[1], MOTOO NOMURA[1], CHIHIRO KONDO[1], AYAKO MIZOTA[1], YASUSHI YATABE[3], MASAYUKI SHINODA[2] and KEI MURO[1]

[1]*Department of Clinical Oncology,* [2]*Department of Thoracic Surgery, and* [3]*Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan*

Abstract. *Background: This study aimed to evaluate the efficacy of docetaxel plus 5-fluorouracil and cisplatin (DCF) induction chemotherapy for locally advanced borderline-resectable T4 esophageal cancer. Patients and Methods: We retrospectively analyzed data regarding thirty patients with borderline-resectable T4 tumor who received either DCF or cisplatin plus 5-fluorouracil (FP) as induction chemotherapy. Results: The overall response rate was significantly better for the DCF group than the FP group. In the DCF group, 6/16 patients achieved a grade 2 histological post-chemotherapeutic effect after treatment, compared to 1/14 in FP group. Except for myelotoxicity, no other significant differences in toxicity were observed during induction chemotherapy between groups. The DCF regimen did not result in increased postoperative complications compared to the FP regimen. Postoperative recurrence or distant metastasis was observed in 7/10 of FP patients and 5/12 of DCF patients. Conclusion: DCF induction chemotherapy may be an option for conversion therapy of initially unresectable, locally advanced esophageal cancer.*

Surgical treatment with three-field lymph node dissection has contributed to improvement in the survival rates of advanced esophageal cancer patients (1, 2). However, analyses of disease recurrence patterns after surgery alone have suggested that surgery alone was insufficient for local control, and have prompted the addition of adjuvant radiotherapy, chemotherapy, or chemoradiotherapy. The introduction of these types of multidisciplinary treatments is thought necessary to improve outcome, especially in advanced esophageal cancer.

Western and Japanese physicians have very different opinions of the roles of chemotherapy and radiotherapy in achieving local control. Based on several clinical trials assessing the effectiveness of neoadjuvant chemoradiotherapy, patients with resectable but advanced squamous cell carcinoma (SCC) of the esophagus usually receive preoperative chemoradiotherapy in Western countries. However, in Japan, there have not been any randomized controlled studies to evaluate the clinical significance of preoperative chemoradiotherapy. After the results of the Japan Clinical Oncology Group (JCOG) 9907 study were reported, neoadjuvant chemotherapy with cisplatin plus 5-fluorouracil (FP) followed by surgery emerged as a new standard treatment for clinical stage II or III esophageal cancer in Japan (3). However, patients with unequivocal T4 disease were excluded from this study, and many Japanese institutions exclude T4 disease as an indication for surgery. In patients with T4 tumors and/or M1 lymph node metastasis, chemoradiotherapy with FP is considered standard treatment (4).

At our institution, we have sometimes seen patients with locally advanced esophageal cancer suspected of invading adjacent organs, but not definitively diagnosed as T4 disease. We called these cases 'borderline-resectable T4' cancer. A recent controlled study at an experienced center demonstrated a 2-year survival of around 52% for patients with locally advanced SCC of the esophagus (T3-T4N0-N1) who received neoadjuvant chemoradiotherapy followed by surgery (5), in contrast to the 40% survival for similar patients receiving chemoradiotherapy alone reported in a multicenter trial by Bedenne and co-workers (6). This survival difference suggests that the addition of surgery to chemoradiotherapy for locally advanced SCC can result in

Correspondence to: Tomoya Yokota, MD, Ph.D., Department of Clinical Oncology, Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Tel: +81527626111, Fax: +81527642963, e-mail: tomoya.yokota@gmail.com

Key Words: DCF, induction chemotherapy, borderline-resectable T4, esophageal cancer, cisplatin, 5-fluorouracil, docetaxel.

improved local control and survival. Therefore, our treatment strategy for such locally advanced cancer includes surgery.

It has been recently reported that patients with SCC of the head and neck who received induction chemotherapy using docetaxel, cisplatin, and 5-fluorouracil (DCF) achieved significantly longer survival than patients who received FP induction chemotherapy (7, 8). DCF chemotherapy also significantly improved overall survival compared with FP in patients with advanced gastric or gastroesophageal adenocarcinoma (9). In addition, it has been reported that a DCF regimen was effective against locally advanced esophageal SCC (10). Therefore, since 2007, we have administered DCF as intensive induction chemotherapy with the aim of curative resection of borderline-resectable T4 tumors. The purpose of this study was to evaluate the efficacy of DCF induction therapy for locally advanced borderline-resectable esophageal cancer by determining the response rates, presence of residual tumor after surgery, histological post-chemotherapeutic effects, safety, and postoperative complications in both FP and DCF regimens. We also investigated postoperative recurrence patterns and survival outcomes.

Patients and Methods

Patients. Data regarding 30 patients with locally advanced borderline-resectable T4 esophageal cancer, at Aichi Cancer Center Hospital between 2001 and 2010, were retrospectively analyzed in this study. Of these, 16 patients received DCF regimen and 14 patients FP regimen as induction chemotherapy, aiming at curative resection. Esophagography, endoscopy, computed tomography (CT) of the chest and abdomen, and/or 18-fluorodeoxyglucose positron-emission tomography (FDG PET)/CT fusion imaging were performed to determine both pretreatment clinical stages and treatment responses. Clinical staging was performed according to the tumor-node-metastasis (TNM) classification of the International Union Against Cancer (UICC), sixth edition (11). A tumor was considered to be borderline resectable T4 if prior induction therapy had not been performed and it also had not been unequivocally determined to be clinical T4. For each patient, the pretreatment tumor depth was estimated, and tumor resectability was determined by the multidisciplinary tumor board of our institution. Written informed consent was obtained from all patients.

Induction chemotherapy. Induction chemotherapy using the FP regimen consisted of intravenous cisplatin (80 mg/m^2) on day 1, and a continuous infusion of 5-fluorouracil (800 mg/m^2/day) for 5 days, given every 4 weeks for two cycles. The DCF regimen was based on our previous phase II study (12), and consisted of intravenous docetaxel (60-70 mg/m^2) and cisplatin (60-70 mg/m^2) on day 1, and a continuous infusion of 5-fluorouracil (750-800 mg/m^2/day) for 5 days, given every 4 weeks for two cycles. Patients in the DCF group were given prophylactic antibiotics. Granulocyte colony-stimulating factor (G-CSF) was used if patients had grade 4 neutropenia or febrile neutropenia, but was not used for prophylaxis. Hematologic and nonhematologic toxicity was assessed according to National Cancer Institute Common Toxicity Criteria (NCICTC) (version 3.0) and the highest grade occurring anytime during induction chemotherapy was reported. Restaging evaluations were typically performed by CT or FDG-PET/CT fusion imaging 1-2 weeks after the completion of chemotherapy. Because few patients had measurable disease as determined by Response Evaluation Criteria in Solid Tumors (RECIST), the treatment response of each primary esophageal lesion was endoscopically evaluated, and categorized as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) (13). PR was defined as obvious morphological change, such as reduction or flattening of tumor or elevated lesion around the ulcer, along with healing of the ulcer floor. If a clinical response was seen, and curative resection was thus considered possible, the patient was scheduled for surgery 4-6 weeks after the last day of chemotherapy.

Surgical procedure and histopathologic response evaluation. All the patients submitted to surgery underwent a subtotal esophagectomy with regional lymphadenectomy through a right thoracotomy and laparotomy, and reconstruction was performed using the stomach via a retrosternal route with cervical anastomosis through a neck incision. Evaluations of residual tumor (R) were classified as follows: R0, no residual tumor; R1, suspicious of residual tumor or microscopic residual tumor; and R2, macroscopic residual tumor. The entire tumor bed was cut into slices containing the entire esophageal wall, and histological therapeutic effects were classified as follows: grade 3, complete disappearance of viable cancer cells in the tumor bed; grade 2, disappearance of greater than two thirds of viable cancer cells; and grade 1, disappearance of less than two thirds of viable cancer cells (14).

Statistical analysis. The Chi-square test, Fischer exact test, and Student's *t*-test were used to analyze the relationship between variables, using SYSTAT 12 software (Systat Software Inc., Richmond, CA USA). Progression-free survival (PFS) was calculated from the date of initial chemotherapy until disease relapse, or censored at last follow-up visit. Overall survival (OS) was calculated from the starting date of first-line chemotherapy until death from any cause, or censored at last follow-up visit. Survival data were analyzed using the Kaplan-Meier method. Comparison of survival curves was carried out using a log-rank test. Two-sided p values <0.05 were considered statistically significant.

Results

Patient characteristics. Of 14 patients treated with FP regimen, 7 patients commenced FP therapy between 2001 and 2006, and the remaining patients between 2007 and 2010. All patients treated with DCF regimen commenced therapy between 2007 and 2010. Patient characteristics are presented in Table I. There were no significant differences in age, gender, or performance status (PS) between the FP and DCF patient groups. Most of the primary tumors were located in the thoracic esophagus. N1 and M1 tumors included either regional or nonregional lymph node metastasis, without distant metastasis. The histological diagnosis of all patient tumors was SCC (Table I). In one patient, although the primary lesion was superficial (T1), swelling of the left recurrent nerve lymph node (No. 106recL) was highly suspicious of invasion into the trachea, and the tumor was therefore considered to be unresectable.

Table I. *Patient characteristics.*

	FP (n=14)	DCF (n=16)
Age, years		
Median (range)	63 (55-72)	63.5 (40-75)
Gender		
Male	13	13
Female	1	3
ECOG PS		
0	0	2
1	14	14
Location of primary tumor		
Ce	1	0
Ut	2	7
Mt	8	7
Lt	3	2
cT†		
1	0	1§
2	0	0
3	0	0
4	14	15
cN†		
0	3	1
1	11	15
cM†		
0	9	11
1a	3	3
1b*	2	2
Histology		
Well-differentiated SCC	1	4
Moderately differentiated SCC	11‡	7
Poorly differentiated SCC	0	1
SCC of unknown differentiation	2	4
Adjacent organs		
Aorta	5	5
Lung	1	1
Jugular vein	0	1
Pulmonary vein	0	2
Bronchus	5	5
Trachea	2	5
Others	3	0

†UICC, sixth edition. ‡One patient's tumor consisted of basaloid carcinoma mixed with moderately differentiated squamous cell carcinoma. *M1b excluding distant metastasis. §One patient's primary lesion was superficial (T1), although swelling of the left recurrent nerve lymph node (No. 106recL) was highly suspicious of invasion into the trachea, and the tumor was therefore considered to be unresectable. FP: Cisplatin plus 5-fluorouracil; DCF: docetaxel plus 5-fluorouracil and cisplatin; ECOG: Eastern Cooperative Oncology Group; PS: performance status; Ce: cervical esophagus; Ut: upper thoracic esophagus; Mt: middle thoracic esophagus; Lt: lower thoracic esophagus; SCC: squamous cell carcinoma.

Table II. *Efficacy of induction chemotherapy.*

	FP (n=14)	DCF (n=16)	*P*-value
Response			
CR	0	1†	
PR	2	9‡	
SD	12	6	
PD	0	0	
CR+PR	2	10	0.0072
Residual tumor (R)			
0	5	10	0.1432
1	1	1	
2	3	1	
NE	5	4	
Histological therapeutic effect			
0	0	0	
1	7	7	
2	1	6	
3	0	0	
NE	6	3	
>Grade 2	1	6	0.0499

†Complete response was achieved in 1 patient, who chose subsequent chemoradiotherapy instead of operation after induction chemotherapy. ‡In 1 patient, the primary lesion showed a partial response, whereas a new lesion occurred in an abdominal lymph node after induction chemotherapy. NE: Patients in whom residual tumor or histological therapeutic effect were not evaluated, included those for whom esophagectomy was not performed even after induction therapy. CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease: NE: not evaluable.

Efficacy outcomes. PR was observed in 2/14 and 9/16 of patients treated with FP and DCF, respectively. The overall response rate was significantly better in the patients undergoing DCF than in those receiving FP (10/16 *vs.* 2/14, p=0.0072).

Of 16 patients treated with the DCF regimen, 4 patients did not go to esophagectomy due to the following reason: upon achievement of CR to DCF therapy in both the primary lesion and lymph nodes, one patient chose subsequent chemoradiotherapy instead of surgery. Subsequent chemoradiotherapy was also performed for another three patients because their clinical response to DCF was insufficient to perform curative resection. Although one male patient treated with DCF achieved PR in the primary lesion, a new lesion occurred in an abdominal lymph node after DCF therapy. Because both the primary lesion and the new lesion in the abdominal lymph node were considered technically resectable, he underwent surgical treatment. Of 14 patients treated with the FP regimen, chemoradiotherapy instead of surgery was chosen by 4 patients because curative resection was not considered possible. Overall, R0 resection was achieved in 10/16 of patients receiving DCF and in 5/14 of patients receiving FP.

The surgical specimens were serially sectioned and examined microscopically. Histological examination of the primary lesion revealed that 6/16 of patients treated with DCF and 1/14 of patients with FP therapy achieved a grade 2 post-chemotherapeutic effect (Table II, p=0.0499).

Adverse events associated with induction chemotherapy. The worst toxicities seen during the treatment periods are listed in Table III. Grade 3 or 4 neutropenia occurred in 10/16 of

Table III. *Summary of toxicity during induction chemotherapy.*

	FP (n=14)		DCF (n=16)	
	Grade 3	Grade 4	Grade 3	Grade 4
Hematologic toxicity				
Leukopenia	1	0	9	1
Neutropenia	0	1	2	8
Febrile neutropenia	0	0	4	0
Anemia	1	0	0	1
Thrombocytopenia	0	0	1	0
Non-hematologic toxicity				
Nausea/vomiting	0	0	1	0
Diarrhea	0	0	0	0
Mucositis	0	0	2	0
Anorexia	0	0	1	0
Renal	0	0	0	0
Infection	1	0	1	0

FP: Cisplatin plus 5-fluorouracil; DCF: docetaxel plus 5-fluorouracil and cisplatin.

Table IV. *Postoperative complications.*

	FP (n=10)	DCF (n=12)
Pneumonia	2	3
Cardiovascular (pulmonary embolism, arrhythmia, venous embolism)	2	1
Laryngeal nerve palsy	1	1
Anastomotic leak	0	2
Wound infection	2	1
Hemorrhage	0	0
Pneumoderma	0	1
Lymphorrhea	0	1
Chylothorax	1	0
Infection	1†	2‡
Pancreatic juice leakage	0	1

†One patient developed cholecystitis after surgery. ‡One patient developed methicillin-resistant Staphylococcus aureus bacteremia and another developed mediastinal abscess after surgery. FP: Cisplatin plus 5-fluorouracil; DCF: docetaxel plus 5-fluorouracil and cisplatin.

patients in the DCF group and in 1/14 of patients in the FP group (p=0.0017). Despite antibiotic prophylaxis, the rate of febrile neutropenia was higher in the DCF group. The percentages of patients with grade 3 or 4 anemia and thrombocytopenia were similar in both groups. Although grade 3 oral mucositis occurred in two patients in the DCF group, there were no major differences in the incidence rates of severe nonhematologic toxicity during induction chemotherapy in the two groups. None of the patients developed treatment-related perforation of the esophageal wall, esophagobronchial fistula, mediastinal fistula, or aortic fistula. There were no treatment-related deaths in either group.

Postoperative complications. The in-hospital mortality rate after surgery was 0% in both of the treatment groups. The postoperative complication rate was 4/10 in the FP group and 6/12 in the DCF group. Details of the postoperative complications are listed in Table IV. Overall, there were no remarkable differences in the postoperative complications among the two study groups (Table IV). Notably, the incidence of overall infections, including pneumonia, wound infection, and other infections, was similar in the two groups.

Survival. PFS was analyzed for 22 patients who underwent induction chemotherapy followed by surgery. The median PFS for the DCF group was 15.7 months, which was longer than that for the FP group (8.4 months); however, the difference was not significant (p=0.740; Figure 1A). OS was analyzed for all patients who underwent induction chemotherapy regardless of surgery. The OS for the DCF group was also longer compared to that of the FP group (35.9 months *vs.* 19.0 months); however, the difference was not significant (p=0.285; Figure 1B). The 1-year survival rate in the DCF group was 90.0%, which was superior to 1-year survival in the FP group (58.3%, Figure 1B).

Patterns of postoperative recurrence. At the time of analysis, the recurrence rates after surgery were 7/10 in the FP group and 5/12 in the DCF group (p=0.1839). There were 7 patients with distant metastases in the FP group. The sites of distant metastases included the bone (N=1), lung (N=2), abdominal lymph node (N=2), and cervical lymph nodes (N=1); and one patient had recurrences in the bone, adrenal gland, and an abdominal lymph node. In another patient, recurrence in an abdominal lymph node was followed by liver metastasis. There were five patients in the DCF group with distant metastasis, and one patient with both locoregional and distant metastasis. The sites of distant metastases included abdominal lymph node (N=1), chest wall (N=1), and muscle (N=1); and, notably, bone metastases (N=5) were observed in all DCF patients who had recurrences.

Discussion

The prognosis of esophageal cancer patients with locally advanced SCC remains poor (15). Because of the high rate of postoperative complications, attention has shifted to neoadjuvant treatment. In the JCOG 9907 study, preoperative chemotherapy with FP was found to be superior to postoperative FP for OS in patients with resectable (non-T4), clinical stage II or III esophageal cancer (3). Based on this result, the standard treatment strategy for unequivocal T3

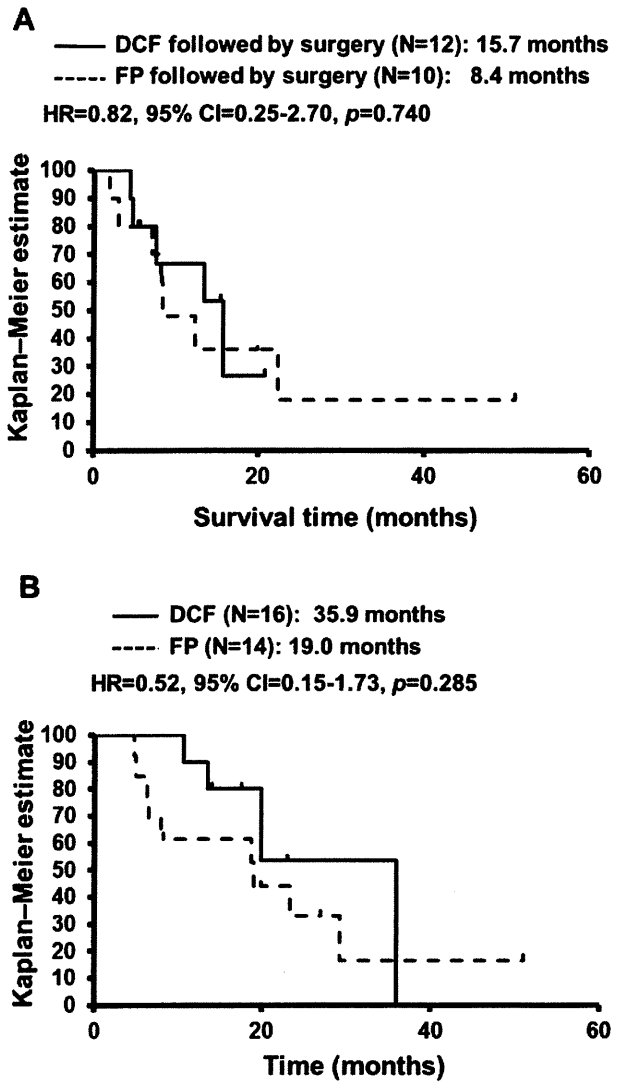

Figure 1. *Kaplan-Meier plot showing progression-free survival (A) and overall survival (B) in the docetaxel plus 5-fluorouracil and cisplatin (DCF) and cisplatin plus 5-fluorouracil (FP) induction chemotherapy groups.*

disease is preoperative chemotherapy with FP followed by radical surgery. However, local recurrence is commonly observed among the patterns of postoperative recurrence in patients receiving preoperative chemotherapy, even after three-field lymphadenectomy. In a meta-analysis of clinical trials of neoadjuvant chemotherapy, Gebski *et al.* demonstrated that there was no significant preoperative chemotherapy effect on all-cause mortality in patients with SCC (hazard ratio 0.88; p=0.12) (16). Furthermore, subgroup analysis of the JCOG 9907 study revealed that the survival benefit of neoadjuvant chemotherapy in stage III disease was less than the benefit in stage II disease. Although development of more intensive preoperative therapy is needed for local tumor control of advanced esophageal cancer in order to improve survival, there is no consensus on whether chemotherapy or chemoradiotherapy should be performed as preoperative treatment.

Preoperative chemoradiotherapy with FP is expected to be a promising, new standard preoperative therapy for esophageal cancer. Indeed, in Western countries, many patients with stage II or III SCC have received neoadjuvant chemoradiotherapy followed by surgery. Stahl *et al.* reported that chemoradiotherapy (40 Gy) followed by surgery improves local tumor control in patients with locally advanced esophageal SCC (17). However, treatment-related mortality was significantly increased in the group undergoing chemoradiotherapy followed by surgery compared to the group undergoing chemoradiotherapy alone (12.8% *vs.* 3.5%, respectively; p=0.03). Thus, there remains concern regarding the potential risks of surgery after chemoradiotherapy. Most randomized controlled studies of neoadjuvant chemoradiotherapy have included surgery alone as the control arm, and these studies failed to demonstrate significant improvement in survival, particularly among patients with histologic subtypes of SCC (18-22).

In this study, we retrospectively investigated if DCF was a more powerful preoperative chemotherapy agent than FP for the treatment of patients with locally advanced esophageal cancer, which were suspected of invading adjacent organs, but were not unequivocal T4 lesions (*i.e.*, borderline-resectable T4 disease). This is a patient subgroup for which we hypothesized that preoperative intensive chemotherapy could contribute to conversion of the lesion to curative resectability, which could lead to improved survival outcomes. Because patients with unequivocal T4 tumors have poor survival outcomes after surgical treatment and are usually treated in the palliative setting with FP or nedaplatin plus 5-fluorouracil with concurrent radiotherapy (4, 23, 24), we excluded unequivocal T4 patients from our analysis. Our results demonstrated that the overall response rate and R0 resection rate were better in patients receiving DCF than in patients receiving FP. One patient treated with DCF achieved complete response.

Histopathological findings in resected specimens revealed more favorable post-chemotherapeutic effects in DCF patients than in FP patients. These findings suggest that DCF induction chemotherapy for advanced esophageal cancer may be a promising preoperative option for local tumor control and may result in a high rate of curative resection. The Medical Research Council Oesophageal Cancer Working Group (MRC) found a 60% R0 resection rate among patients treated with neoadjuvant FP compared with a 54% rate in patients treated with surgery alone, which led to improved overall survival (p<0.0001) (25). Furthermore, it was reported that pathologic response after neoadjuvant therapy is associated with survival in patients with esophageal cancer (26). These findings suggest that pathologic response to neoadjuvant therapy and R0 resection are the major determinants of

survival. Our survival analysis indicated that the 1-year survival rate in the DCF group was 90.0%, which is superior to that seen in the FP group, and this DCF result is also superior to survival in patients with unequivocal T4 disease (4). The addition of docetaxel to cisplatin plus 5-fluorouracil may further improve pathologic response and subsequently improve survival in patients with advanced esophageal cancer.

As expected, the DCF regimen induced more leucopenia and neutropenia than FP, but did not lead to more frequent infectious complications. The myelotoxicity seen in the DCF group was consistent with that seen in other studies (7, 8), and was manageable probably because patients received prophylactic antibiotics. No significant differences in nonhematologic toxicity were observed during induction chemotherapy. Furthermore, the DCF regimen did not increase the risk of postoperative complications compared to the FP regimen. This result suggests that esophagectomy after DCF therapy is as safe as after FP therapy.

However, 5/12 patients receiving DCF followed by surgery experienced distant failure within 24 months after surgery. Therefore, we cannot conclude that preoperative DCF chemotherapy is able to provide local tumor control and also to prevent distant failure. Furthermore, the present analysis lacks the statistical power to demonstrate a significant survival benefit of the DCF regimen, because this is a single-institution retrospective study based on a small patient group and short observation period. To achieve better survival after DCF, it may be necessary to determine the predictive factors for tumor recurrence, in order to prevent the occurrence of distant metastasis, as well as to provide locoregional control.

In conclusion, induction chemotherapy using a DCF regimen may be an effective preoperative treatment that allows subsequent curative surgery for locally advanced borderline-resectable T4 esophageal cancer. However, it is still controversial whether preoperative chemotherapy or chemoradiotherapy should be performed. Our observations should be confirmed by longer follow-up and larger sample size. Therapeutic strategies for controlling distant metastasis, as well as locoregional lesions need additional consideration.

Conflict of Interest Statement

None declared.

References

1 Fujita H, Kakegawa T, Yamana H, Shima I, Toh Y, Tomita Y, Fujii T, Yamasaki K, Higaki K and Noake T: Mortality and morbidity rates, postoperative course, quality of life and prognosis after extended radical lymphadenectomy for esophageal cancer. Ann Surg *222*: 654-662, 1995.

2 Kato H, Watanabe H, Tachimori and Y, Iizuka T: Evaluation of neck lymph node dissection for thoracic esophageal carcinoma. Ann Thorac Surg *51*: 931-935, 1991.

3 Igaki H, Kato H, Ando N, Shinoda M, Shimizu H, Nakamura T, Ozawa S, Yabusaki H, Aoyama N, Kurita A and Fukuda H: A randomized trial of postoperative adjuvant chemotherapy with cisplatin and 5-fluorouracil *versus* neoadjuvant chemotherapy for clinical stage II/III squamous cell carcinoma of the thoracic esophagus (JCOG 9907). J Clin Oncol *26(suppl)*: 215s, abstr 4510, 2008.

4 Ohtsu A, Boku N, Muro K, Chin K, Muto M, Yoshida S, Satake M, Ishikura S, Ogino T, Miyata Y, Seki S, Kaneko K and Nakamura A: Definitive chemoradiotherapy for T4 and/or M1 lymph node squamous cell carcinoma of the esophagus. J Clin Oncol *17*: 2915-2921, 1999.

5 Mariette C, Piessen G, Lamblin A, Mirabel X, Adenis A and Triboulet JP: Impact of preoperative radiochemotherapy on postoperative course and survival in patients with locally advanced squamous cell oesophageal carcinoma. Br J Surg *93*: 1077-1083, 2006.

6 Bedenne L, Michel P, Bouché O, Milan C, Mariette C, Conroy T, Pezet D, Roullet B, Seitz JF, Herr JP, Paillot B, Arveux P, Bonnetain F and Binquet C: Chemoradiation followed by surgery compared with chemoradiation alone in squamous cancer of the esophagus: FFCD 9102. J Clin Oncol *25*: 1160-1168, 2007.

7 Posner MR, Hershock DM, Blajman CR, Mickiewicz E, Winquist E, Gorbounova V, Tjulandin S, Shin DM, Cullen K, Ervin TJ, Murphy BA, Raez LE, Cohen RB, Spaulding M, Tishler RB, Roth B, Viroglio Rdel C, Venkatesan V, Romanov I, Agarwala S, Harter KW, Dugan M, Cmelak A, Markoe AM,Read PW, Steinbrenner L, Colevas AD, Norris CM Jr, Haddad RI; TAX 324 Study Group: Cisplatin and Fluorouracil Alone or with Docetaxel in Head and Neck Cancer. N Engl J Med *357*: 1705-1715, 2007.

8 Vermorken JB, Remenar E, van Herpen C, Gorlia T, Mesia R, Degardin M, Stewart JS, Jelic S, Betka J, Preiss JH, van den Weyngaert D, Awada A, Cupissol D, Kienzer HR, Rey A, Desaunois I, Bernier J, Lefebvre JL; EORTC 24971/TAX 323 Study Group: Cisplatin, fluorouracil, and docetaxel in unresectable head and neck cancer. N Engl J Med *357*: 1695-1704, 2007.

9 Ajani JA, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Marabotti C, Van Cutsem E; V-325 Study Group: Clinical benefit with docetaxel plus fluorouracil and cisplatin compared with cisplatin and fluorouracil in a phase III trial of advanced gastric or gastroesophageal cancer adenocarcinoma: the V-325 Study Group. J Clin Oncol *25*: 3205-3209, 2007.

10 Tanaka Y, Yoshida K, Sanada Y, Osada S, Yamaguchi K and Takahashi T: Biweekly docetaxel, cisplatin, and 5-fluorouracil (DCF) chemotherapy for advanced esophageal squamous cell carcinoma: a phase I dose-escalation study. Cancer Chemother Pharmacol *66*: 1159-1165, 2010.

11 Sobin LH and Wittedkind CH: UICC TNM Classification of Malignant Tumors, 6th edition. New York: Wiley-Liss, Inc., pp. 60-64, 2002.

12 Ura T, Nagase M, Fujii H, Hironaka S, Hatooka S, Hosoya Y, Yokota T, Shitara K, Takahari D, Muro K and Shinoda M: Feasibility study of preoperative docetaxel (D), cisplatin (C), and fluorouracil (F) in esophageal cancer. ASCO Gastrointestinal Cancers Symposium, abstr 81, 2010.

13 Isono K: Guidelines for Clinical and Pathologic Studies on Carcinoma of the Esophagus, ninth edition: Preface, general principles, part I. Esophagus *1*: 61-88, 2004.

14 Japanese Society for Esophageal Diseases: Guidelines for Clinical and Pathologic Studies on Carcinoma of the Esophagus, ninth edition: Preface, general principles, part II. Esophagus *1*: 107-125, 2004.
15 Siewert JR, Stein HJ, Feith M, Bruecher BL, Bartels H and Fink U: Histologic tumor type is an independent prognostic parameter in esophageal cancer: Lessons from more than 1,000 consecutive resections at a single center in the Western world. Ann Surg *234*: 360-369, 2001.
16 Gebski V, Burmeister B, Smithers BM, Foo K, Zalcberg J and Simes J: Australasian Gastro-Intestinal Trials Group: Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol *8*: 226-234, 2007.
17 Stahl M, Stuschke M, Lehmann N, Meyer HJ, Walz MK, Seeber S, Klump B, Budach W, Teichmann R, Schmitt M, Schmitt G, Franke C and Wilke H: Chemoradiation with and without surgery in patients with locally advanced squamous cell carcinoma of the esophagus. J Clin Oncol *23*: 2310-2317, 2005.
18 Walsh TN, Noonan N, Hollywood D, Kelly A, Keeling N and Hennessy TP: A comparison of multimodal therapy and surgery for esophageal adenocarcinoma. N Engl J Med *335*: 462-467, 1996.
19 Urba SG, Orringer MB, Turrisi A, Iannettoni M, Forastiere A and Strawderman M: Randomized trial of preoperative chemoradiation *versus* surgery alone in patients with locoregional esophageal carcinoma. J Clin Oncol *19*: 305-313, 2001.
20 Burmeister BH, Smithers BM, Gebski V, Fitzgerald L, Simes RJ, Devitt P, Ackland S, Gotley DC, Joseph D, Millar J, North J, Walpole ET and Denham JW; Trans-Tasman Radiation Oncology Group; Australasian Gastro-Intestinal Trials Group: Surgery alone *versus* chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: a randomised controlled phase III trial. Lancet Oncol *6*: 659-668, 2005.
21 Bosset JF, Gignoux M, Triboulet JP, Tiret E, Mantion G, Elias D, Lozach P, Ollier JC, Pavy JJ, Mercier M and Sahmoud T: Chemoradiotherapy followed by surgery compared with surgery alone in squamous-cell cancer of the esophagus. N Engl J Med *337*: 161-167, 1997.
22 Lee JL, Park SI, Kim SB, Jung HY, Lee GH, Kim JH, Song HY, Cho KJ, Kim WK, Lee JS, Kim SH and Min YI: A single institutional phase III trial of preoperative chemotherapy with hyperfractionation radiotherapy plus surgery *versus* surgery alone for resectable esophageal squamous cell carcinoma. Ann Oncol *15*: 947-954, 2004.
23 Ishida K, Ando N, Yamamoto S, Ide H and Shinoda M: Phase II study of cisplatin and 5-fluorouracil with concurrent radiotherapy in advanced squamous cell carcinoma of the esophagus: a Japan Esophageal Oncology Group (JEOG)/Japan Clinical Oncology Group trial (JCOG9516). Jpn J Clin Oncol *34*: 615-619, 2004.
24 Ishikura S, Ohtsu A, Shirao K, Muro K, Kagami Y, Nihei K, Mera K, Ito Y, Boku N and Yoshida S: A phase I/II study of nedaplatin and 5-fluorouracil with concurrent radiotherapy in patients with T4 esophageal cancer: Japan Clinical Oncology Group trial (JCOG 9908). Esophagus *2*: 133-137, 2005.
25 Medical Research Council Oesophageal Cancer Working Group: Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet *359*: 1727-1733, 2002.
26 Meredith KL, Weber JM, Turaga KK, Siegel EM, McLoughlin J, Hoffe S, Marcovalerio M, Shah N, Kelley S and Karl R: Pathologic response after neoadjuvant therapy is the major determinant of survival in patients with esophageal cancer. Ann Surg Oncol *17*: 1159-1167, 2010.

Received July 13, 2011
Revised August 17, 2011
Accepted August 18, 2011

British Journal of Cancer (2011) 104, 856–862

www.bjcancer.com

BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer

T Yokota*[,1,2], T Ura[1], N Shibata[2], D Takahari[1], K Shitara[1], M Nomura[1], C Kondo[1], A Mizota[1], S Utsunomiya[3], K Muro[1] and Y Yatabe[2]

[1]Department of Clinical Oncology, Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan; [2]Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan; [3]Department of Gastroenterology, Nagoya Kyoritsu Hospital, Nakagawa-ku, Nagoya 454-0933, Japan

BACKGROUND: Activating mutation of *KRAS* and *BRAF* are focused on as potential prognostic and predictive biomarkers in patients with colorectal cancer (CRC) treated with anti-EGFR therapies. This study investigated the clinicopathological features and prognostic impact of *KRAS/BRAF* mutation in advanced and recurrent CRC patients.

METHOD: Patients with advanced and recurrent CRC treated with systemic chemotherapy ($n=229$) were analysed for *KRAS/BRAF* genotypes by cycleave PCR. Prognostic factors associated with survival were identified by univariate and multivariate analyses using the Cox proportional hazards model.

RESULTS: *KRAS* and *BRAF* mutations were present in 34.5% and 6.5% of patients, respectively. *BRAF* mutated tumours were more likely to develop on the right of the colon, and to be of the poorly differentiated adenocarcinoma or mucinous carcinoma, and peritoneal metastasis. The median overall survival (OS) for *BRAF* mutation-positive and *KRAS* 13 mutation-positive patients was 11.0 and 27.7 months, respectively, which was significantly worse than that for patients with wild-type (wt) *KRAS* and *BRAF* (40.6 months) (*BRAF*; HR = 4.25, $P<0.001$, *KRAS13*; HR = 2.03, $P=0.024$). After adjustment for significant features by multivariate Cox regression analysis, *BRAF* mutation was associated with poor OS (HR = 4.23, $P=0.019$).

CONCLUSION: Presence of mutated *BRAF* is one of the most powerful prognostic factors for advanced and recurrent CRC. The *KRAS13* mutation showed a trend towards poor OS in patients with advanced and recurrent CRC.

British Journal of Cancer (2011) **104,** 856–862. doi:10.1038/bjc.2011.19 www.bjcancer.com
Published online 1 February 2011

Keywords: *BRAF*; *KRAS*; prognostic marker; colorectal cancer; chemotherapy

Although the epidermal growth factor receptor (EGFR) has important roles in cell differentiation and proliferation in normal cells, activation of EGFR signalling is frequently observed in colorectal cancer (CRC) cells, resulting in cell proliferation, migration and metastasis, evasion of apoptosis, or angiogenesis (Fang and Richardson, 2005). Indeed, ~35% of CRC tissues carry a mutation in codons 12 or 13 of *KRAS* that leads to the constitutive activation of downstream pathways, including the Ras/Raf/MAP/MEK/ERK and/or PTEN/PI3K/Akt pathways (Kinzler and Vogelstein, 1999; Wan *et al*, 2004; Benvenuti *et al*, 2007; Di Nicolantonio *et al*, 2008; Souglakos *et al*, 2009). *BRAF* is a downstream molecule of *KRAS*. Although more than 40 somatic mutations in the *BRAF* kinase domain have been described, the most common mutation across various cancers is the classic GTG→GAG substitution at the position 1799 of exon 15, which results in the V600E amino acid change, and the subsequent constitutive activation of the EGFR signalling pathway. Recent studies from Western countries have suggested that *BRAF* mutations occur in 10–20% of patients with sporadic disease (Jass, 2007; Benvenuti *et al*, 2007; Di Nicolantonio *et al*, 2008; Souglakos *et al*, 2009; Fariña-Sarasqueta *et al*, 2010), whereas other reports have revealed that tumours harbouring *BRAF* mutations have different clinical and histopathological features compared with tumours that harbour *KRAS* mutations (Kim *et al*, 2006; Deng *et al*, 2008; Zlobec *et al*, 2010). However, the frequency and clinicopathological features of *KRAS/BRAF* mutation in Japanese CRC patients remain unknown.

Information on *KRAS/BRAF* genotype is extremely useful in systemic chemotherapy for advanced and recurrent CRC patients, not just for predicting the therapeutic efficiency of anti-EGFR therapy, but also for identifying patients with poor prognoses. Therefore, both *KRAS* and *BRAF* are currently being focused on as potential prognostic and predictive biomarkers in patients with metastatic disease treated with anti-EGFR therapies, such as panitumumab and cetuximab (Karapetis *et al*, 2008; Bokemeyer *et al*, 2009; Tol *et al*, 2009; Van Cutsem *et al*, 2009). A number of retrospective analyses have revealed that patients with *KRAS* mutations do not benefit from cetuximab treatment, suggesting that *KRAS* genotype is a useful predictive marker for cetuximab therapy in CRC (Karapetis *et al*, 2008; Bokemeyer *et al*, 2009; Van Cutsem *et al*, 2009). It has also been reported that wild-type (wt) *BRAF* is required for a successful response to panitumumab or cetuximab therapies in metastatic CRC (Di Nicolantonio *et al*, 2008; Laurent-Puig *et al*, 2009; Souglakos *et al*, 2009; De Roock

*Correspondence: Dr T Yokota; E-mail: tomoya.yokota@gmail.com
Received 29 October 2010; revised 21 December 2010; accepted 10 January 2011; published online 1 February 2011

et al, 2010). In contrast, the prognostic relevance of *KRAS* genotype in CRC has been controversial despite a number of multi-institutional investigations dating from the 1990s (Andreyev *et al*, 1998; French *et al*, 2008; Kakar *et al*, 2008; Ogino *et al*, 2009; Roth *et al*, 2010). Although few studies have investigated the impact of *KRAS12* and *KRAS13* mutations on CRC prognosis, a series of recent studies have highlighted the potential adverse prognostic impact of *BRAF* mutations, using both patients with stage II and III disease and patients across all disease stages (Ogino *et al*, 2009; Fariña-Sarasqueta *et al*, 2010). Although Tol *et al* (2009) analysed *BRAF* genotypes in 520 metastatic CRC patients, all the patients were treated with chemotherapy plus bevacizumab with or without cetuximab. Furthermore, *BRAF* genotypes were analysed in a large subgroup of 845 metastatic CRC treated with FOLFIRI and FOLFOX chemotherapy with or without cetuximab as the first-line treatment in the CRYSTAL and OPUS studies, respectively (Bokemeyer *et al*, 2010). Thus, although the prognostic value of *BRAF* has been analysed in CRC patients treated with specific chemotherapy regimens, it remains unclear what impact the *KRAS12, KRAS13,* and *BRAF* mutations have on clinical outcomes of all patients with advanced or recurrent CRC treated with systemic treatments.

We have previously introduced the cycleave PCR technique as applicable to the routine screening of *KRAS/BRAF* mutations in CRC from pathological specimens, such as surgical and biopsy specimens (Yokota *et al*, 2010). Cycleave PCR utilises chimeric DNA-RNA-DNA probes labelled with a fluorescent dye and quencher, and the accuracy of cycleave PCR in detecting *KRAS/BRAF* mutations has been confirmed by assessment of the concordance between cycleave PCR and reverse transcriptase PCR-coupled direct sequencing (Yatabe *et al*, 2006; Yokota *et al*, 2010).

The aim of this study was to evaluate the *KRAS/BRAF* genotypes of advanced and recurrent CRC patients and to assess the effects of these genotypes on clinical outcome. To this end, we analysed the frequencies of the *KRAS12, KRAS13* and *BRAF* mutations, and correlated these results with the clinicopathological features of 229 Japanese CRC patients.

PATIENTS AND METHODS

Patients and tissues

Analysis of the genes encoding *KRAS* and *BRAF* was performed on surgically resected or biopsied specimens from CRC patients at our institution from 2002 to 2010. Hematoxylin and eosin (H and E)-stained slides were retrospectively collected and histologic subtypes were reviewed by an experienced gastrointestinal pathologist. Clinicopathological and survival analyses were subsequently performed on all patients with advanced and recurrent CRC who underwent systemic chemotherapy. Clinical data, including patient age at diagnosis, tumour location, and metastatic sites, were retrieved from patient records. Right-sided cancers included tumours from the caecum to transverse colon, left-sided included tumours from the splenic flexure to the rectosigmoid junction. Specimens used for *KRAS/BRAF* genotyping were either frozen or paraffin embedded tissues. For the *KRAS/BRAF* genotyping, appropriate approvals were obtained from the institutional review committee and written informed consent was obtained from all patients.

DNA extraction

DNA was extracted from surgical or biopsy specimens. Briefly, tumour cell-rich areas in H and E-stained sections were marked under a microscope, and tissues scratched from the same areas were sequentially deparaffinised and unstained. Recovered tissues were incubated in 1X PCR buffer containing 100 $\mu g\,ml^{-1}$ proteinase K for 1 h at 54 °C. After heat inactivation at 95 °C for 3 min, samples were used directly as template DNA for PCR assay.

KRAS/BRAF genotyping by cycleave PCR

To detect point mutations at *KRAS* codons 12, 13 and 61, we used the cycleave PCR technique (Yatabe *et al*, 2006; Sakamoto *et al*, 2007; Yokota *et al*, 2010). Each chimeric DNA-RNA-DNA probe was labelled with a fluorescent dye and quencher at each end that targeted the G12D, G12V, G12R, G12C, G12S, or G12A mutations in codon 12, the G13D or G13C mutations in codon 13, or the G61H, G61L, G61E, or G61K mutations in codon 61 of *KRAS*. We also designed probes that targeted the V600E mutation in *BRAF*. The PCR reactions were performed using a cycleave PCR core kit (TAKARA, Co. Ltd, Ohtsu, Japan). Fluorescent signals were quantified using the Smart Cycler system (SC-100; Cepheid, Sunnyvale, CA, USA).

Statistical analysis

The χ^2, Fischer's exact tests and Student's *t*-tests were used to analyse the relationship between variables using SYSTAT software (SYSTAT Software Inc., Richmond, CA, USA). The *KRAS* wt/*BRAF* wt (wild/wild), *KRAS12* mutant (G12X), *KRAS13* mutant (G13X), and *BRAF* mutant (V600E) groups were analysed separately. Overall survival (OS) was calculated from the starting date of the first-line chemotherapy until death from any cause, or censored at last follow-up visit. Survival data were analysed using the Kaplan–Meier product-limit method. Comparison of survival curves was carried out using the log-rank test. We first performed a univariate comparison of survival functions for factors that could potentially affect the survival time using the log-rank test, and then a multivariate analysis using the Cox proportional hazards model. *P*-values <0.05 were considered statistically significant, and all *P*-values represent two-sided significance tests.

RESULTS

Frequency of *KRAS* and *BRAF* gene mutations in CRC patients

According to our previous investigation on the spectrum of *KRAS* genotypes in our database of CRC cases, the most frequent mutations at *KRAS* codon 12 were the G12D, G12V, G12R, G12C, G12S and G12A mutations, which accounted for more than 95% of the codon 12 mutations. Similarly, the G13D and G13C mutations at codon 13, and the G61H, G61L, G61E, and G61K mutations at codon 61 were also found to be the most common at each site (Yokota *et al*, 2010). All the *KRAS* mutations we located have been previously described as oncogenically active and were present in the COSMIC (catalogue of somatic mutations in cancer) database (Sanger Institute, Cambridge, UK). Therefore, a series of specific probes targeting the common mutations in *KRAS* codons 12, 13 and 61 were designed for subsequent analysis of *KRAS* mutation frequency in our population of CRC patients. Because the most common mutation in *BRAF* is a valine to glutamate transition at position 600 of the protein (V600E), we designed probes targeting the V600E mutation in *BRAF*.

We initially analysed the *KRAS* genotypes of 349 CRC patients at our institution for which pathological specimens were available by cycleave PCR. The *KRAS* mutations were present in 35.7% ($n=126$) of patients tested, including 24.4% ($n=86$) that exhibited codon 12 mutations and 11.3% ($n=40$) that exhibited codon 13 mutations. However, only 4.7% ($n=15$) of the patients tested were positive for the *BRAF* V600E mutation ($n=319$). None of the *KRAS*-mutated samples carried a concomitant *BRAF* mutation. Approximately 2–3% of the surgical specimens could

Table 1 Spectrum of *KRAS/BRAF* mutations in CRC

	KRAS			
BRAF	**Wild type**	**G12**	**G13**	**61**
Wild type	135	53	26	0
V600E	15	0	0	0

Abbreviation: CRC = colorectal cancer. $n = 229$.

not be evaluated by cycleave PCR, probably due to over-fixation by formalin, as we reported previously (Yokota *et al*, 2010).

For the subsequent clinicopathological and survival analysis, we picked out 229 patients with advanced and recurrent CRC for which we could access complete clinicopathological information. The *KRAS* mutations were present in 34.5% ($n = 79$) of advanced and recurrent CRC patients, including 23.1% ($n = 53$) with codon 12 mutations and 11.4% ($n = 26$) with codon 13 mutations. The *BRAF* mutation was found in 6.6% ($n = 15$) of this population (Table 1).

Association of *BRAF/KRAS* mutations with clinicopathological features

We then correlated the *KRAS* and *BRAF* genotypes with clinicopathological features of CRC, including primary tumour location, histological findings, and sites of metastases. We categorised the population into four subtypes; those with wt *KRAS* and *BRAF* (wild/wild), *KRAS12* mutations (G12X), *KRAS13* mutations (G13X), and *BRAF* mutations (V600E).

For disease status, recurrent disease was more frequent in the *KRAS12* and *KRAS13* mutant groups than in the wild/wild group. There was no association between *KRAS/BRAF* genotype and age, gender or PS. Primary tumours were located at the rectum in almost half of the wild/wild and G12X populations. However, right-side tumour location was more frequent (60%) in patients with *BRAF* mutation in all subtypes ($P = 0.0391$) (Table 2). Furthermore, 46.2% (12 out of 26) of the primary tumours with *KRAS13* mutations were located on the right side whereas the frequencies of right-side location were 20.7% (28 out of 135) and 26.4% (14 out of 53), for the wild/wild and G12X groups, respectively (Table 2). The *BRAF* and *KRAS13* mutations were present in 14.3% (9 out of 63) and 19.0% (17 out of 63) of right-sided CRC, respectively. These results suggested that the *BRAF* and *KRAS* codon 13 mutations were associated with a right-sided tumour location.

Analysis with respect to histology showed that the frequencies of poorly differentiated adenocarcinoma (por), mucinous carcinoma (muc) and signet-ring cell carcinoma (sig) were <10.9% in patients with wt *BRAF*, which supported previous reports that such histologies are rare in CRC (Ogino *et al*, 2006; Catalano *et al*, 2009). However, 60.0% (9 out of 15) of CRC cases with *BRAF* mutation were of the por or muc subtypes, although no signet-ring cell carcinomas were observed. The *BRAF* mutations were present in 36.0% (9 out of 25) of patients with por/muc histology. Furthermore, 60.0% (9 out of 15) of CRCs with *BRAF* mutation metastasised to the peritoneum, compared with ~15% of CRCs with other subtypes ($P = 0.0062$) (Table 2). However, Fisher's exact test indicated no statistically significant correlation between tumour histology and peritoneal metastasis in *BRAF* mutant patients. No other significant differences or trends in metastatic patterns with respect to *KRAS/BRAF* genotypes were observed.

Details of the first line chemotherapy regimens used are shown in Table 2. In all, 66.4% of patients were treated with oxaliplatin-based regimens, 14.4% with irinotecan-based regimens, and 19.2% with fluoropyrimidine-based chemotherapy without oxaliplatin or irinotecan. There were no significant differences in treatment regimens between *KRAS/BRAF* genotypes. A total of 86 (63.7%) patients with wild/wild tumours and five (33.3%) patients with *BRAF* mutation-positive tumours received anti-EGFR therapy, whereas few patients with *KRAS12* or *KRAS13* mutations received anti-EGFR therapy (1.9% and 3.8%, respectively).

Survival

The median OS for *BRAF* mutation-positive patients was 11.0 months, which was significantly worse than for patients with wt *KRAS* and *BRAF* (40.6 months) (HR = 4.25, 95% CI 2.08–8.67, $P < 0.001$; Figure 1). The median OS for all *KRAS* mutation-positive patients, including those with *KRAS12* or *KRAS13* mutations, was not statistically different to that of wt *KRAS* and *BRAF* patients (HR = 1.51, 95% CI 0.97–2.36, $P = 0.071$). However, if OS for *KRAS13* mutation-positive patients was analysed separately from *KRAS12* mutation-positive patients, then the median OS for *KRAS13* mutation-positive patients was significantly worse than that for wt *KRAS* and *BRAF* patients (27.7 months *vs* 40.6 months, HR = 2.03, 95% CI 1.10–3.74, $P = 0.024$; Figure 1). In contrast, the median OS for *KRAS12* mutation-positive patients was 38.8 months, similar to that for wt *KRAS* and *BRAF* patients (HR = 1.28, 95% CI 0.74–2.19, $P = 0.376$; Figure 1). Univariate analysis showed that two other variables were also significantly associated with poor survival, PS ECOG ≥ 2 and gender (Table 3). *KRAS13* mutation was not statistically associated with poor survival by univariate analysis. This was because we compared OS for *KRAS13* mutation-positive patients with that for wt *KRAS13* patients, which included *KRAS12* and *BRAF* mutation-positive patients as well as wt *KRAS* and *BRAF* patients. The por/sig/muc histology and lung metastasis showed a trend towards poor OS ($P = 0.066$ and $P = 0.061$, respectively).

To correct for significant prognostic factors, a Cox proportional hazards model that included age, gender, PS, *KRAS* status, *BRAF* status, pathological finding, number of metastasis and metastatic sites, was used. As two variables, WBC and ALP, had missing data, they were not included in the multivariate analysis. *BRAF* mutation and PS ECOG ≥ 2 were confirmed as poor prognostic factors. Specifically, the relative risk of death for patients with *BRAF* mutation was 4.23 (95% CI 1.76–10.2) compared with patients with wt *BRAF* tumours ($P = 0.001$) (Table 3). Multivariate analysis also found that por/sig/muc histology, age > 65, and liver metastasis were negative independent prognostic factors. However, *KRAS13* mutation was not found to be an independent prognostic factor.

DISCUSSION

In this study, we examined the incidence of *KRAS* and *BRAF* mutations in advanced and recurrent CRC patients, and clarified the relationship between *KRAS/BRAF* genotypes and clinicopathological features, including survival. Up to now, estimates of *KRAS* gene mutation frequency in metastatic CRCs have been based on selective clinical studies or drug admission trials with variable inclusion criteria. To our knowledge, the present report is the first to provide data on the frequency and type of *KRAS/BRAF* mutations from a large Japanese population of advanced and recurrent CRC patients tested in a routine setting.

Our results showed that *KRAS* mutation was observed in around 35% of CRC cases, which included 25% of patients with mutations at codon 12 and 10% of patients with mutations at codon 13. This observation agreed well with previous studies on selected cohorts that reported frequencies in the range of 30–42% (Table 1). The cycleave PCR technique was simultaneously applied to the detection of *BRAF* mutation, thought to be an adverse prognostic marker as well as a predictive marker for anti-EGFR therapy. Our analysis demonstrated that the *BRAF* V600E mutation was observed in ~5% of CRC patients, which appeared to be lower

Table 2 Association of *BRAF* and *KRAS* mutational status with clinicopathological features in colorectal cancer

KRAS/BRAF status	**Wild/wild**	**KRAS mutant**			**BRAF mutant**		
Clinicopathological features	***n* = 135**	**G12X *n* = 53**	**G13X *n* = 26**	**Total (G12X+G13X) *n* = 79**	**V600E *n* = 15**	***P*-value**	**Overall *n* = 229**
Age at diagnosis (median)	62 (27–83)	62 (40–85)	68 (41–79)	63 (40–85)	62 (30–80)		
Gender							
Female	47 (34.8%)	27 (50.9%)	13 (50.0%)	40 (50.6%)	8 (53.3%)	0.1082	95
Male	88 (65.2%)	26 (49.1%)	13 (50.0%)	39 (49.4%)	7 (46.7%)		134
ECOG PS							
0–1	115 (85.2%)	46 (86.8%)	22 (84.6%)	68 (86.1%)	13 (86.7%)	0.7898	196
>2	9 (6.7%)	4 (7.5%)	3 (11.5%)	7 (8.9%)	2 (13.3%)		18
Unknown	11 (8.1%)	3 (5.7%)	1 (3.8%)	4 (5.1%)	0 (0.0%)		15
Tumour location							
Right sided	28 (20.7%)	14 (26.4%)	12 (46.2%)	26 (32.9%)	9 (60.0%)	0.0391	63
Left sided	41 (30.4%)	13 (24.5%)	3 (11.5%)	16 (20.3%)	3 (20.0%)		60
Rectum	64 (47.4%)	25 (47.2%)	11 (42.3%)	36 (45.6%)	3 (20.0%)		103
Other	2 (1.5%)	1 (1.9%)	0 (0.0%)	1 (1.3%)	0 (0.0%)		3
Disease status							
Advanced	82 (60.7%)	26 (49.1%)	11 (42.3%)	37 (46.8%)	9 (60.0%)	0.2269	128
Recurrence	53 (39.3%)	27 (50.9 %)	15 (57.7%)	42 (53.2%)	6 (40.0%)		101
Histological subtype							
Well	28 (20.7%)	8 (15.1%)	7 (26.9%)	15 (19.0%)	1 (6.7%)	<0.0001	44
Mod	91 (67.4%)	37 (69.8%)	18 (69.2%)	55 (69.6%)	5 (33.3%)		151
por/sig/muc	10 (7.4%)	5 (9.4%)	1 (3.8%)	6 (7.6%)	9 (60.0%)		25
Other	1 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0.0%)		1
Unknown	5 (3.7%)	3 (5.7%)	0 (0.0%)	3 (3.8%)	0 (0.0%)		8
Metastatic sites							
Liver	90 (66.7%)	31 (58.5%)	15 (57.7 %)	46 (58.2%)	10 (66.7%)	0.6595	146
Peritoneum	30 (22.2%)	11 (20.8%)	4 (15.4%)	15 (20.0%)	9 (60.0%)	0.0062	54
Lung	42 (31.1%)	21 (39.6%)	10 (38.5%)	31 (39.2%)	5 (33.3%)	0.6867	78
CNS	1 (0.7%)	0 (0.0%)	1 (3.8%)	1 (1.3%)	0 (0.0%)	0.3503	2
Bone	9 (6.7%)	3 (5.7%)	2 (7.7%)	5 (6.3%)	2 (13.3%)	0.7736	16
Number of metastatic sites							
>2	64 (47.4%)	23 (43.4%)	14 (53.8%)	37 (46.8%)	10 (66.7%)	0.4078	111
<1	71 (52.6%)	30 (56.6%)	12 (46.2%)	42 (53.2%)	5 (33.3%)		118
WBC							
WBC>10000	9 (6.7%)	4 (7.5%)	2 (7.7%)	6 (7.6%)	0 (0.0%)	0.7622	15
WNL	100 (74.1%)	38 (71.7%)	20 (76.9%)	58 (73.4%)	14 (93.3%)		172
Unknown	26 (19.3%)	11 (20.8%)	4 (15.4%)	15 (20.2%)	1 (6.7%)		42
ALP							
ALP>300	59 (43.7%)	18 (34.0%)	12 (46.2%)	30 (38.0%)	6 (40.0%)	0.6635	95
WNL	49 (36.3%)	24 (45.3%)	10 (38.5%)	34 (43.0%)	8 (53.3%)		91
Unknown	27 (20.0%)	11 (20.8%)	4 (15.4%)	15 (20.0%)	1 (6.7%)		43
First-line regimen							
IRI-based	24 (17.8%)	6 (11.3%)	2 (7.7%)	8 (10.1%)	1 (6.7%)	0.4062	33
OXA-based	85 (63.0%)	37 (69.8%)	17 (65.4%)	54 (68.4%)	13 (86.7%)		152
Others	26 (19.3%)	10 (18.9%)	7 (26.9%)	17 (21.5%)	1 (6.7%)		44
Anti-EGFR treatment							
Yes	86 (63.7%)	1 (1.9%)	1 (3.8%)	2 (2.5%)	5 (33.3%)	<0.0001	93
No	44 (32.6%)	52 (98.1%)	25 (96.2%)	77 (97.5%)	10 (66.7%)		131
Unknown	5 (3.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)		5

Abbreviations: CNS = central nervous system; ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; PS = performance status; well = well-differentiated adenocarcinoma; mod = moderately differentiated adenocarcinoma; por = poorly differentiated adenocarcinoma; muc = mucinous carcinoma; sig = signet-ring cell carcinoma; CNS = central nervous system; IRI = irinotecan; OXA = oxaliplatin, ALP = alkaline phosphatase; WNL = within normal range; WBC = white blood cells. Patients with both wild-type *KRAS* and wild-type *BRAF* were designated as wild/wild. All patients with *KRAS* mutations ($n = 79$) either in codon 12 (G12X) or in codon 13 (G13X) are shown as total (G12X+G13X). **P*-values calculated between wild-type *KRAS* and *BRAF* (wild/wild), *KRAS12* mutant (G12X), *KRAS13* mutant (G13X), and *BRAF* mutant (V600E) groups.

than that previously reported from Western countries. None of the CRC patients in our study carried both *KRAS* and *BRAF* mutations, supporting the hypothesis that *KRAS* and *BRAF* mutations occur in a mutually exclusive manner (Rajagopalan *et al*, 2002; Frattini *et al*, 2004; Ahlquist *et al*, 2008). One possible explanation for the comparatively low frequency of *BRAF* mutation might be the

different ethnic group. Indeed, several studies have reported that the mutation rates of DNA mismatch repair (MMR) genes, such as *hMSH2* and *hMLH1*, in hereditary non-polyposis colorectal cancer, is variable between countries. Therefore, geographical variation may account for differences in the mutation spectrum of *BRAF*, as observed for MMR genes (Wei *et al*, 2003; Lee *et al*, 2005; Goldberg *et al*, 2008).

We also investigated the clinicopathological characteristics of CRC patients with respect to *KRAS12*, *KRAS13* and *BRAF* mutations. In accordance with previous reports (Kim *et al*, 2006; Deng *et al*, 2008; Zlobec *et al*, 2010), *BRAF* mutation occurred more frequently in right-sided tumour locations. We also found that 60.0% of the *BRAF* mutation-positive specimens were of the poorly differentiated adenocarcinoma or mucinous carcinoma subtypes. It was recently reported that mucinous histology predicts a poor response to oxaliplatin- and/or irinotecan-based chemotherapies and is correlated with poor OS (Catalano *et al*, 2009). As *BRAF* mutation was more frequent in mucinous groups than non-mucinous carcinoma, as demonstrated by the present study and others (Ogino *et al*, 2006), the poor prognosis associated with mucinous histology may be at least partially explained by *BRAF* gene mutation. These specific clinicopathological features support the hypothesis that the *BRAF* mutation-mediated carcinogenesis in CRC is initiated by altered *BRAF* function as an early step in the serrated pathway (Bennecke *et al*, 2010), leading to activation of RAF-MEK-ERK-MAP signalling.

In contrast to *BRAF* mutation, no significant differences in clinicopathological parameters were observed according to *KRAS* genotype. However, our analysis did suggest that *KRAS13* mutations were also associated with right-sided tumour location. This result raises the possibility that *KRAS13* may have a distinct phenotype from that of other *KRAS* genotypes.

Using a representative cohort of 229 sporadic CRCs, we identified the *BRAF* V600E mutation as an independent prognostic factor for survival in patients with advanced and recurrent CRC. The presence of the *BRAF* mutation is associated with a significantly higher risk of dying of cancer-related causes, independently of other factors such as age, gender, PS, *KRAS* status, pathological finding, number of metastasis and metastatic sites, in agreement with other recent studies (Ogino *et al*, 2009; Tol *et al*, 2009; Bokemeyer *et al*, 2010; Fariña-Sarasqueta *et al*, 2010). For example, analysis of stage II and stage III CRC patients (Fariña-Sarasqueta *et al*, 2010) was consistent with the finding that 44% of our population included recurrent disease. The *BRAF* mutation was correlated with survival in a heterogeneous group of CRC patients that included all disease stages (Ogino *et al*, 2009). Furthermore, a positive correlation between *BRAF* mutation and shorter survival was demonstrated in a homogeneous group of metastatic CRC patients treated with a specific chemotherapy regimen with or without cetuximab (Tol *et al*, 2009; Bokemeyer *et al*, 2010). However, our study focused on the advanced and recurrent group who received systemic chemotherapy, including fluoropyrimidines, in combination with oxaliplatin, irinotecan, bevacizumab and anti-EGFR antibody in several lines. Even though all of the patients in our study received systemic chemotherapy, a positive correlation between *BRAF* mutation and shorter survival was still demonstrated, independent of treatment arm.

The prognostic value of *KRAS* mutations in CRC remains controversial, even though *KRAS* mutations have been associated with a poor response to anti-EGFR antibody therapy in metastatic CRC (Karapetis *et al*, 2008; Bokemeyer *et al*, 2009; Van Cutsem *et al*, 2009). Despite a number of studies investigating a prognostic role for *KRAS* mutations, no definitive conclusions can be drawn (Castagnola and Giaretti, 2005). This may be due to differences

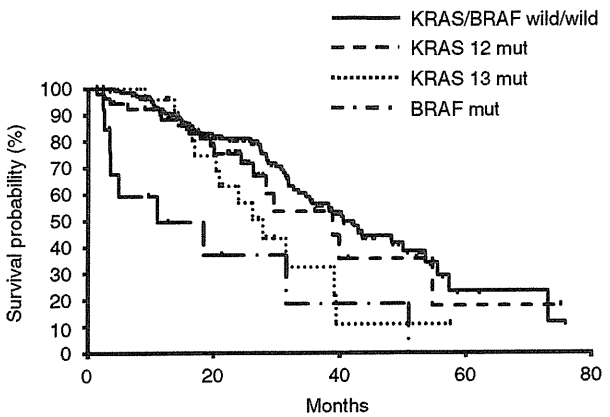

Figure 1 Kaplan–Meier plot showing overall survival in metastatic and recurrent colon cancer patients according to *KRAS* and *BRAF* V600E mutational status ($n = 229$). mut, mutated.

Table 3 Factors associated with overall survival in univariate and multivariate analyses

	Univariate analysis		Multivariate analysis	
Variable	**Hazard ratio (95% CI)**	***P*-value**	**Hazard ratio (95% CI)**	***P*-value**
Age >65	0.74 (0.48–1.13)	0.157	0.55 (0.34–0.90)	0.018
Female	1.59 (1.06–2.37)	0.025	1.35 (0.85–2.12)	0.201
PS (ECOG) ⩾2	6.14 (3.15–12.0)	<0.001	7.66 (3.68–16.0)	<0.001
BRAF mutant	3.78 (1.89–7.54)	<0.001	4.23 (1.76–10.2)	0.001
KRAS 12 mutant	1.03 (0.62–1.74)	0.897	1.57 (0.88–2.81)	0.128
KRAS 13 mutant	1.67 (0.93–3.02)	0.086	1.51 (0.76–2.98)	0.239
Pathology, por/sig/muc	1.74 (0.96–3.14)	0.066	2.38 (1.16–4.90)	0.018
Number of metastasis ⩾2	0.93 (0.63–1.40)	0.738	1.12 (0.61–2.05)	0.714
Liver metastasis	1.36 (0.88–2.11)	0.162	1.72 (1.02–2.90)	0.042
Lung metastasis	0.66 (0.42–1.02)	0.061	0.59 (0.32–1.11)	0.100
Peritoneal metastasis	1.21 (0.76–1.93)	0.417	1.56 (0.85–2.88)	0.154
WBC ⩾10000	1.27 (0.51–3.15)	0.605	—	—
ALP ⩾300	1.21 (0.78–1.88)	0.395	—	—
Anti-EGFR treatment	0.80 (0.53–1.20)	0.277	—	—

Abbreviations: ALP = alkaline phosphatase; PS = performance status; ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor; por = poorly differentiated adenocarcinoma; muc = mucinous carcinoma; sig = signet-ring cell carcinoma; CI = confidence interval; WBC = white blood cells.